

**NF VALIDATION**  
**Validation of alternative analysis methods**  
**Application to the food industry**

**Summary report**  
**according to the standard EN ISO 16140-2:2016**

Quantitative method

**REBECCA+EB**  
**Certificate # AES 10/07-01/08**  
**for the enumeration of *Enterobacteriaceae***  
**in food and feed products**

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## Preamble

- Protocols of validation :

- EN ISO 16140-1 and NF EN ISO 16140-2 (September 2016): Microbiology of the food chain — Method validation

Part 1: Vocabulary.

Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

- Requirements regarding comparison and interlaboratory studies for implementation of the standard EN ISO 16140-2 (version 6).

- Reference method:

- **EN ISO 21528-2 (July 2017):** Microbiology of the food chain – Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count technique

- Application scope:

- **All human food products** by a validation testing of a broad range of foods, including:
  - meat products,
  - dairy and egg products,
  - seafood products,
  - vegetal products,
  - ready-to-eat and ready-to-reheat products,
- **Feeds.**

- Certification body:

- **AFNOR Certification** (<https://nf-validation.afnor.org/>).

## Definitions

- **Method comparison study**

The method comparison study is the part of the validation process that is performed in the expert laboratory. It consists of four parts:

- A comparative study of the results of the reference method to the results of the alternative method in a variety of different items (naturally and/or artificially) contaminated samples (so-called relative trueness study).
- A comparative study of the results of the reference method to the results of the alternative method in artificially contaminated samples using replicates of a single item per category. The data are analyzed using the accuracy profile (AP) approach (so-called AP study).
- An inclusivity/exclusivity study of the alternative method.

- **Relative trueness study**

The relative trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method.

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.

- **Accuracy profile study**

The accuracy profile study is a comparative study between the results obtained by the reference method and the results of the alternative method.

The accuracy profile is the 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.

- **Inclusivity and exclusivity study**

The inclusivity study is a study involving pure target strains to be detected or enumerated by the alternative method.

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

- **Interlaboratory study**

The interlaboratory study is a study performed by multiple laboratories testing identical samples at the same time, the results of which are used to estimate alternative-method performance parameters.

The aim of an interlaboratory study is to determine the variability of the results obtained in different laboratories using identical samples.

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## **Appendices**

Appendix A: Protocol of the alternative method

Appendix B: Protocol of the reference method

Appendix C: Artificial contaminations

Appendix D: Relative trueness study - Raw results

Appendix E: Relative trueness study - Statistical calculations

Appendix F: Accuracy profile study - Raw results

Appendix G: Selectivity - Raw results

Appendix H: Interlaboratory study - Raw results

## 1. Introduction

The REBECCA™ + EB method is validated by AFNOR Certification under the NF VALIDATION mark with the certification number AES 10/07-01/08 according to the standard ISO 16140/A1:2011. The method is intended for all human food products and feed products since its initial validation.

Table 1 summarizes the different steps of the validation that occurred since the initial validation.

*Table 1: Steps of the validation AFNOR certification*

<b>Study</b>	<b>Date</b>	<b>Standards</b>	<b>Expert Laboratory</b>	<b>Observation</b>
Initial validation	2008	ISO 16140:2003 ISO 21528-2:2004	Institut Scientifique d'hygiène et d'Analyse	/
First renewal	October 2011	ISO 16140/A1:2011 ISO 21528-2:2004	Institut Scientifique d'hygiène et d'Analyse	/
Second renewal	October 2015	ISO 16140/A1:2011 ISO 21528-2:2004	Institut Scientifique d'hygiène et d'Analyse	/
Third renewal	July 2019	ISO 16140-2:2016 ISO 21528-2:2017	Microsept	Additional tests to fulfill the updated validation standard and reinterpretation

The present summary report introduces all the results for the AFNOR Certification validation of the REBECCA™ + EB method according to the standard ISO 16140-2:2016 for a broad range of foods and for feeds.

A part of the results set out in this report were produced during validation tests carried out by Institut Scientifique d'Hygiène et d'Analyse as part of NF Validation, in accordance with prevailing requirements.

The remaining part of the results is constituted by the analyses performed by the Laboratory Microsept as part of the requirements of the updated validation standard.

## 2. Protocols of the methods

### 2.1. Alternative method

#### 2.1.1. Principle of the method

REBECCA is a selective medium for the enumeration of  $\beta$ -D-glucuronidase-positive *Escherichia coli* and Enterobacteriaceae (non *E. coli*) in food products.

This medium is a chromogenic medium for the direct enumeration without confirmation in products for human and animal consumptions of:

- $\beta$ -D-glucuronidase-positive *E. coli*,
- $\beta$ -D-glucuronidase-positive *E. coli* and Enterobacteriaceae (non *E. coli*).

The enumeration of *E. coli* is done by the detection of  $\beta$ -D-glucuronidase coloring the colonies in blue with or without a blue halo.

The screening of other Enterobacteriaceae (non *E. coli*) is done by the addition to REBECCA™ base of a specific supplement that colors the colonies in pink to red.

The mixture of selective agents inhibits the growth of the interfering flora.

#### 2.1.2. Protocol of the method

The diagram summarizing the method is shown in appendix A.

From an initial suspension realized according to the prescriptions of the ISO 6887 standard, or directly from a liquid sample, REBECCA+EB plates are inoculated as described below:

- For surface inoculation: inoculate by spreading 0.1 mL of the primary dilution and of its decimal dilutions onto the surface of a 90 mm REBECCA™ +EB plate dried beforehand in an incubator. In the case of the estimation of small numbers, it is possible to spread 1 mL of inoculum either onto the surface of a 140 mm Petri plate or onto the surface of three 90 mm Petri plates.
- For inoculation by pour-plate: place 1 mL of initial suspension or decimal dilutions in a Petri dish. Use one plate per dilution. Add approximately 15 mL of molten REBECCA™ +EB medium (maintained at +44 to +47°C). Mix well and leave to cool and set on a flat horizontal surface.

The inoculated plates are incubated at 37±1°C for 24±2 h.

After incubation, observe the microbial growth and the appearance of the colonies:

- $\beta$ -D-glucuronidase-positive *E. coli* grow as blue colonies with or without halo.
- Enterobacteriaceae (non *E. coli*) grow as pink to red colonies.

Following the period of incubation, count the number of typical colonies for each dish containing, if possible, more than 10 and less than 150 typical colonies, but no more than 300 colonies (typical or atypical).

#### 2.1.3. Restrictions

There are no restrictions on use for the REBECCA+EB method.

## 2.2. Reference method

The EN ISO 21528-2:2017 standard, Horizontal method for the detection and enumeration of Enterobacteriaceae – Colony count technique, was used for the renewal study.

It was the version of 2004 that had been used for the first and the second renewal studies. The modifications applied at the 2017 version are considered as minor.

The workflow of the reference method is presented in Appendix B.



### 3. Methods comparison study

#### 3.1. Relative trueness study

##### 3.1.1. Number and nature of the samples

Considering all the steps of the validation:

- 70 samples were analyzed during the initial validation study,
- 91 samples were analyzed during the third renewal study.

Overall, 161 samples were analyzed giving 104 exploitable results for the surface spreading technique modality and 109 exploitable results for the pour plate modality,

The distribution of the samples per category, type and inoculation technique is given in table 2.

*Table 2: number and nature of the samples analyzed for the relative trueness study (IVS: initial validation study, TRS: third renewal study)*

Category	Type	Surface spreading				Pour plates				
		Analyzed		Kept for the statistical analysis		Analyzed		Kept for the statistical analysis		
		IVS	TRS	IVS	TRS	IVS	TRS	IVS	TRS	
Meats products	a	Raw products	8	4	5	4	8	4	5	4
	b	Ready-to-reheat products	1	7	0	5	1	7	0	5
	c	Smoked and cured products	3	10	3	3	3	10	3	3
		<b>Total</b>	<b>12</b>	<b>21</b>	<b>8</b>	<b>12</b>	<b>12</b>	<b>21</b>	<b>8</b>	<b>12</b>
Dairy & egg products	a	Pasteurized and dehydrated products	9	8	4	3	9	8	4	3
	b	Raw milk products	6	0	5	0	6	0	5	0
	c	Desserts and egg products	0	8	0	6	0	8	0	6
		<b>Total</b>	<b>15</b>	<b>16</b>	<b>9</b>	<b>9</b>	<b>15</b>	<b>16</b>	<b>9</b>	<b>9</b>
Seafood products	a	Raw products	1	6	1	5	1	6	1	5
	b	Marinated and smoked products	1	6	1	4	1	6	1	4
	c	Ready-to-reheat products	9	0	6	0	9	0	6	0
		<b>Total</b>	<b>11</b>	<b>12</b>	<b>8</b>	<b>9</b>	<b>11</b>	<b>12</b>	<b>8</b>	<b>9</b>
Vegetal products	a	Raw products	0	5	0	5	0	5	0	5
	b	Pre-cut and pre-cooked products	0	6	0	5	0	6	0	5
	c	Processed products	10	0	5	0	10	0	7	0
		<b>Total</b>	<b>10</b>	<b>11</b>	<b>5</b>	<b>10</b>	<b>10</b>	<b>11</b>	<b>7</b>	<b>10</b>
Feed products	a	Pet food	6	0	5	0	6	0	6	0
	b	Livestock food	4	4	1	4	4	4	1	4
	c	Ingredients	2	4	2	4	2	4	2	4
		<b>Total</b>	<b>12</b>	<b>8</b>	<b>8</b>	<b>8</b>	<b>12</b>	<b>8</b>	<b>9</b>	<b>8</b>
Ready-to-eat & ready-to-reheat products	a	Ready-to-eat products	10	0	7	0	10	0	8	0
	b	Ready-to-reheat products	0	14	0	6	0	14	0	7
	c	Smoked and marinated products	0	9	0	5	0	9	0	5
		<b>Total</b>	<b>10</b>	<b>23</b>	<b>7</b>	<b>11</b>	<b>10</b>	<b>23</b>	<b>8</b>	<b>12</b>
<b>Total</b>			<b>70</b>	<b>91</b>	<b>45</b>	<b>59</b>	<b>70</b>	<b>91</b>	<b>49</b>	<b>60</b>
			<b>161</b>		<b>104</b>		<b>161</b>		<b>109</b>	

### 3.1.2. Artificial contaminations

Artificial contaminations were performed using spiking and seeding protocols. The strains used, and the contamination protocols are presented in Appendix C.

~~Naturally contaminated~~ Not inoculated samples were also used: 118 of them were analyzed that led to the obtention of 71 interpretable results. Naturally contaminated samples represent thus 65.1% of the results.

### 3.1.3. Protocols used during the study

The two modalities of enumeration of the alternative method were tested: surface spreading and pour plates. The minimum incubation times of the Petri dishes was applied, namely 22 hours at  $37\pm 1^{\circ}\text{C}$ .

### 3.1.4. Results

Samples were analyzed by the reference and the alternative method so as to obtain at least 15 interpretable results per category and at least 5 per type.

As results were available in duplicates concerning the initial validation study, only the first replicate was considered as part of the calculations of the EN ISO 16140-2:2016 standard.

Raw results are shown in appendix D.

Three kinds of results are not considered as part of the statistical calculations:

- Those expressed with less than 4 colonies per Petri dish for at least one method or inoculation modality,
- those lower or higher than the quantification limits,
- Undetermined results.

All results are presented in scatter plots per category and modality of inoculation in figures below:

- Figure 1: surface spreading technique plots for each category,
- Figure 2: pour plates method plots for each category,
- Figures 3 and 4: scatter plots for all categories per plating technique.

On scatter plots:

- Each type of food is differentiated per type on individual category scatter plots: blue circle: type a / orange diamond: type b / green triangle: type c
- Results expressed with less than 4 colonies per Petri dish for at least one method are indicated by a yellow square,
- Results lower or higher than the quantification limits for one method are indicated by a red square. The value of these results is corrected according to the EN ISO 16140-2:2016 requirements.

Figure 1: Two-dimensional plots per category, using the surface spreading inoculation

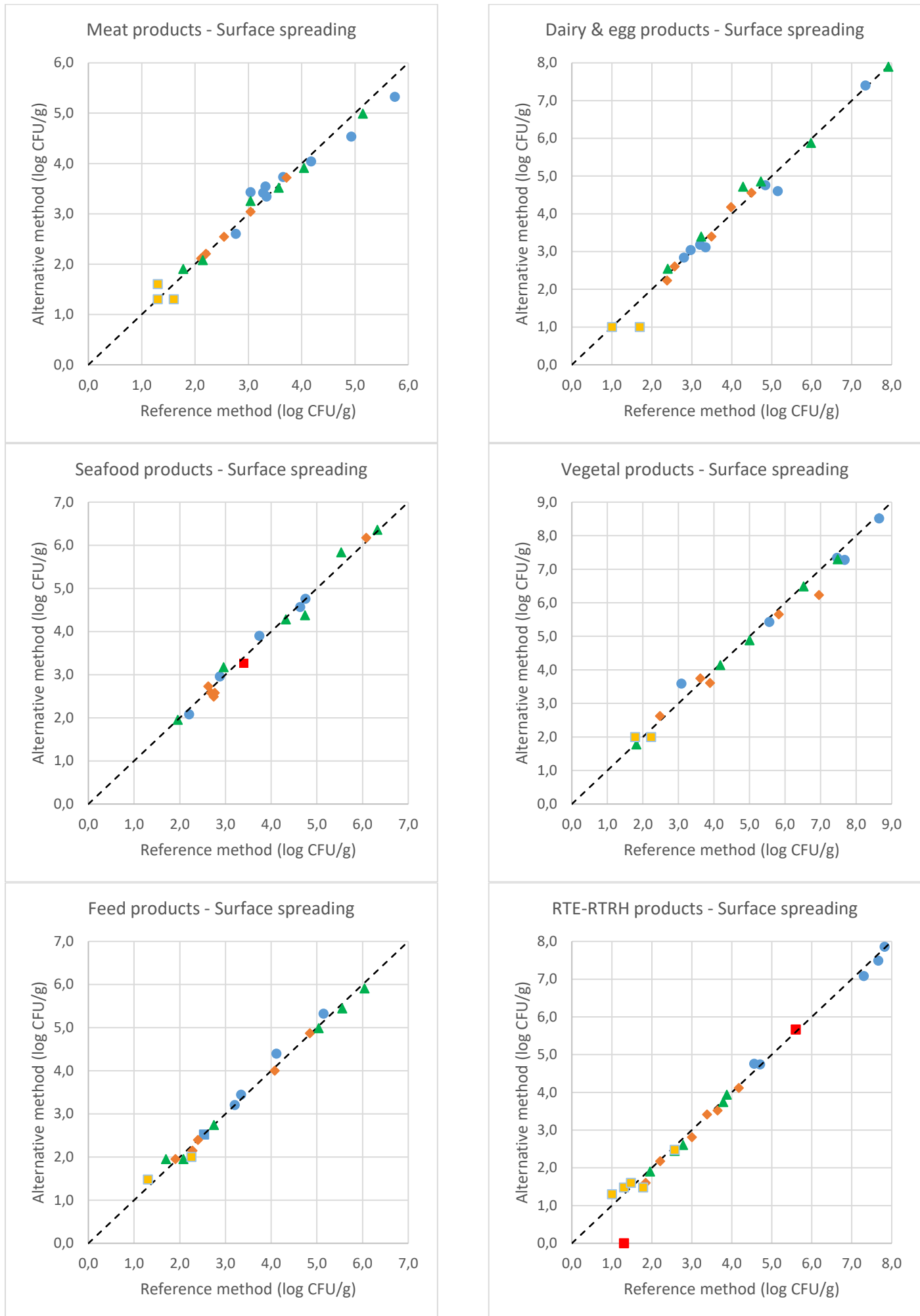


Figure 2: Two-dimensional plots per category, using the pour plate inoculation

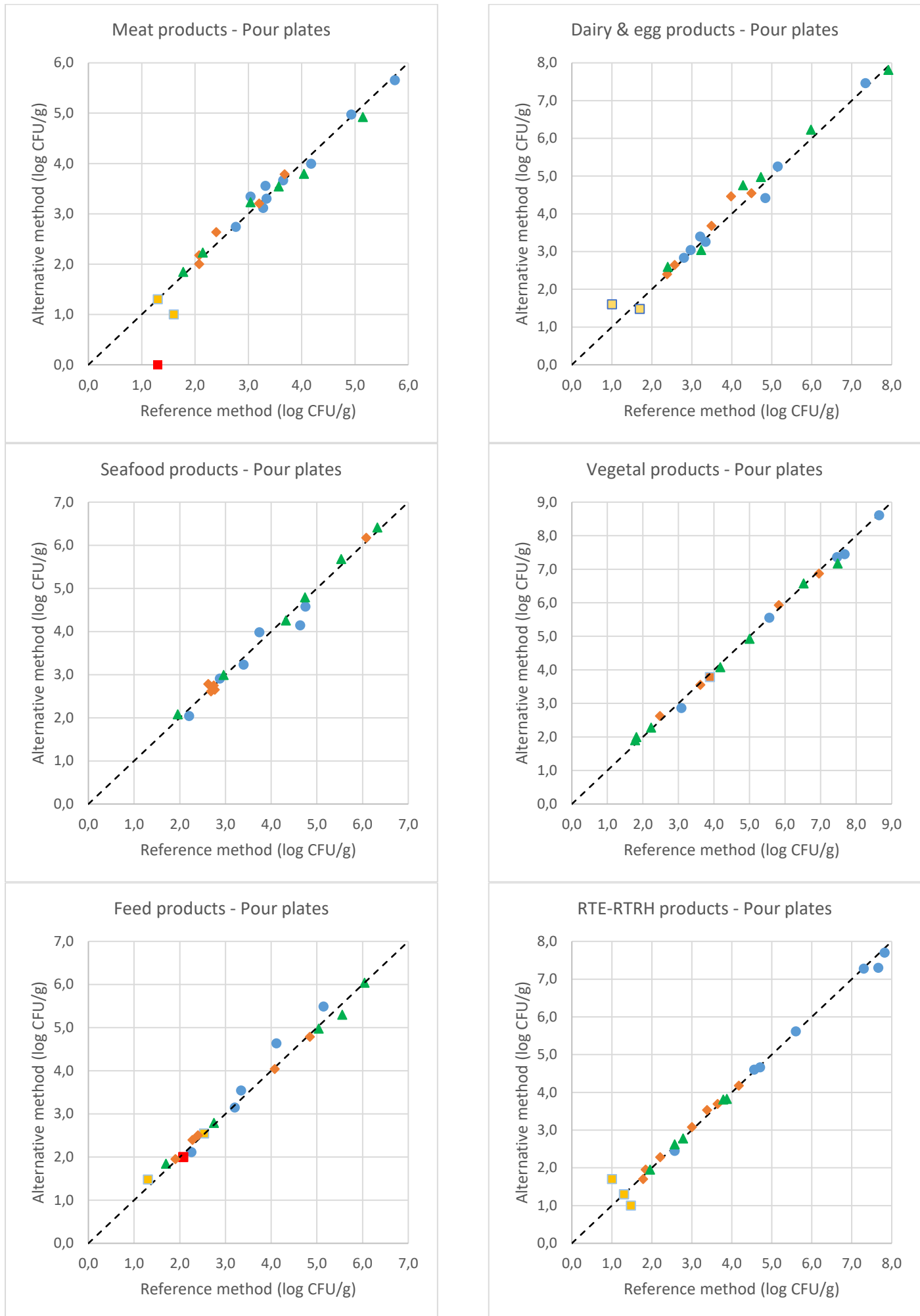


Figure 3: Two-dimensional plots for all categories using the surface spreading inoculation

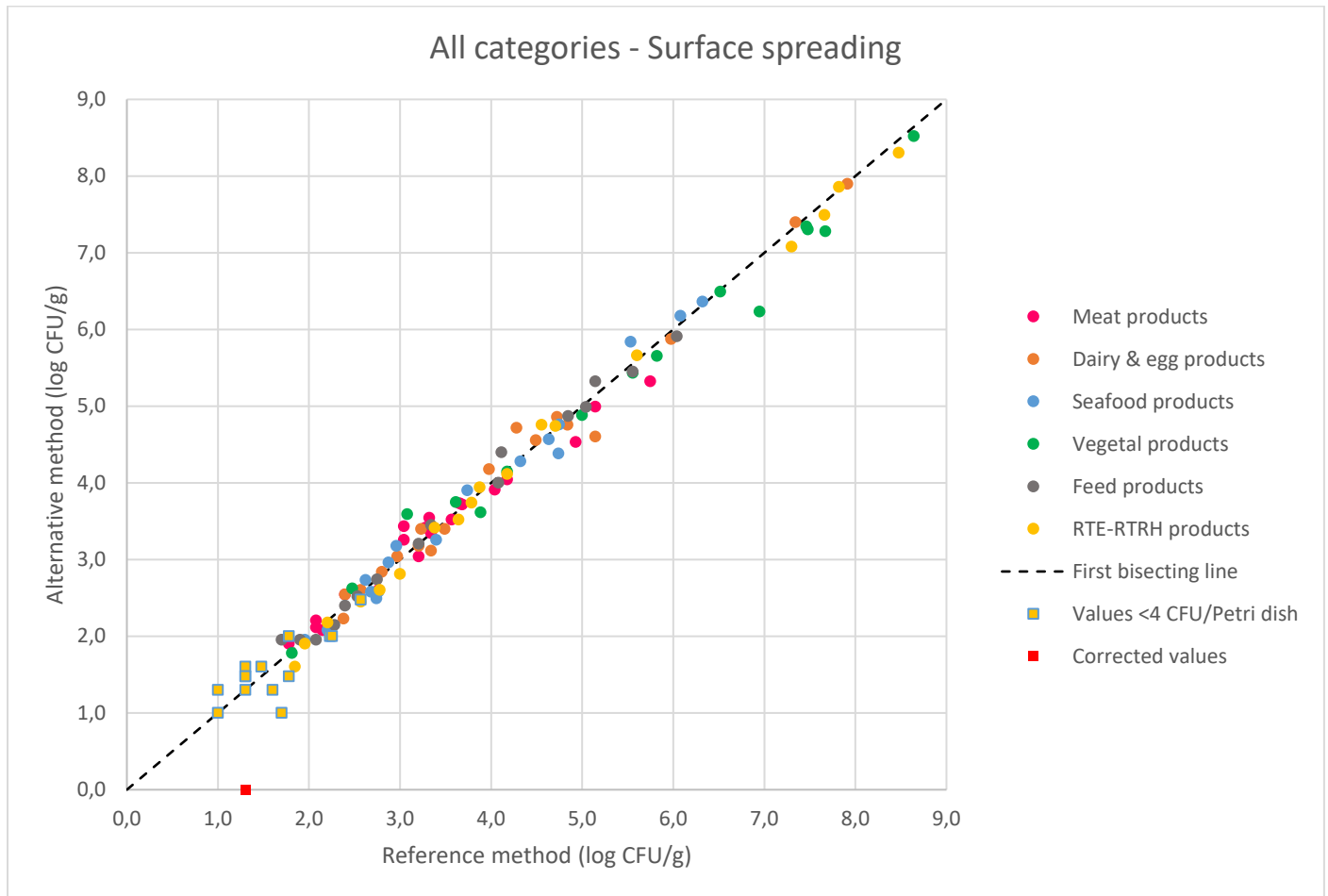
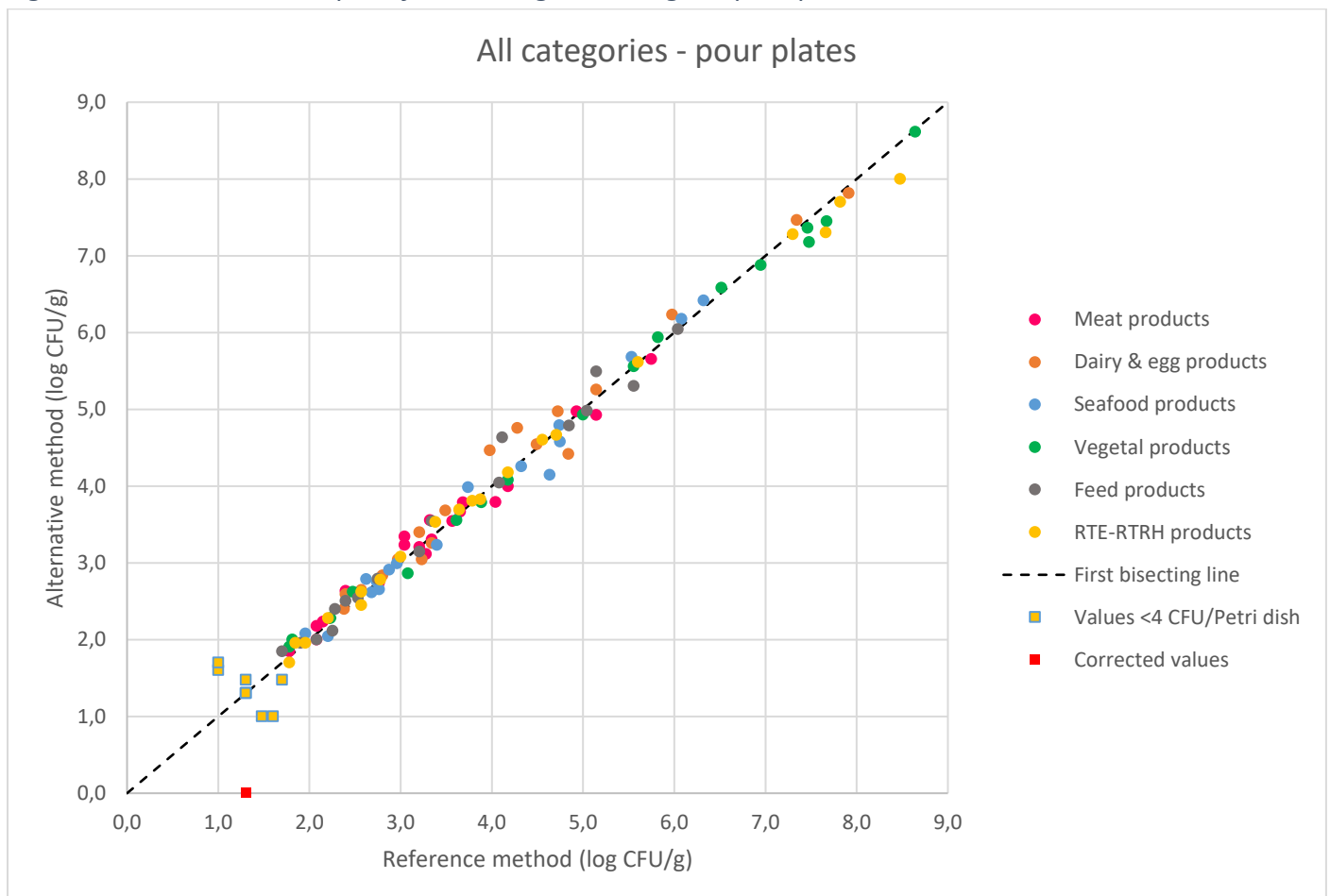


Figure 4: Two-dimensional plots for all categories using the pour plate inoculation



### 3.1.5. Calculation and interpretation of relative trueness study

The results obtained are analyzed using the Bland-Altman method.

Statistical calculations are presented in Appendix E, as well as the results excluded from the statistical analysis per category, type and modality of inoculation.

Table 3 presents the summary of the average differences and standard deviation differences per method, category and for all categories.

Table 3: values for the Bland-Altman difference plot

Inoculation	Category	n	Average difference	Standard deviation differences	Bias	Lower Confidence Limit	Upper Confidence Limit
Surface spreading	MP	20	-0.01	0.20	/	/	/
	DP	18	0.01	0.20	/	/	/
	SP	17	-0.01	0.17	/	/	/
	VP	15	-0.10	0.27	/	/	/
	FP	16	0.02	0.13	/	/	/
	RTE	18	-0.07	0.12	/	/	/
	<b>All cat.</b>	<b>104</b>	<b>-0.03</b>	<b>0.19</b>	<b>-0.03</b>	<b>-0.40</b>	<b>0.35</b>
Pour plate	MP	20	0.01	0.15	/	/	/
	DP	18	0.10	0.22	/	/	/
	SP	17	-0.01	0.17	/	/	/
	VP	17	-0.03	0.14	/	/	/
	FP	17	0.05	0.18	/	/	/
	RTE	20	-0.03	0.15	/	/	/
	<b>All cat.</b>	<b>109</b>	<b>0.01</b>	<b>0.17</b>	<b>0.01</b>	<b>-0.33</b>	<b>0.36</b>

Overall, the average difference is equal to -0,03 (surface spreading method) and 0,01 (pour plate method), showing no bias between the REBECCA+EB method and the reference method.

The average difference varies from -0.10 log CFU/g (vegetal products) to 0.02 CFU/g (feed products) for the surface spreading method and from -0.03 log CFU/g (vegetal products and ready-to-eat, ready-to-reheat products) to 0.10 log CFU/g (dairy & egg products) for the pour plate technique,

Upper and lower 95% confidence interval limits are lower than 0.5 log showing a good correlation between the two methods.

The Bland-Altman difference plots are presented for all categories in figures 5 and 6 for the surface spreading method and the pour plate technique.

As on scatter plots:

- Each category is differentiated by a specific colour,
- Results expressed with less than 4 colonies per Petri dish for at least one method are indicated by a yellow square,
- Results lower or higher than the quantification limits for one method are indicated by a red square. The value of these results is corrected according to the EN ISO 16140-2:2016 requirements.

Figure 5: Bland-Altman difference plot for all categories with the surface spreading method

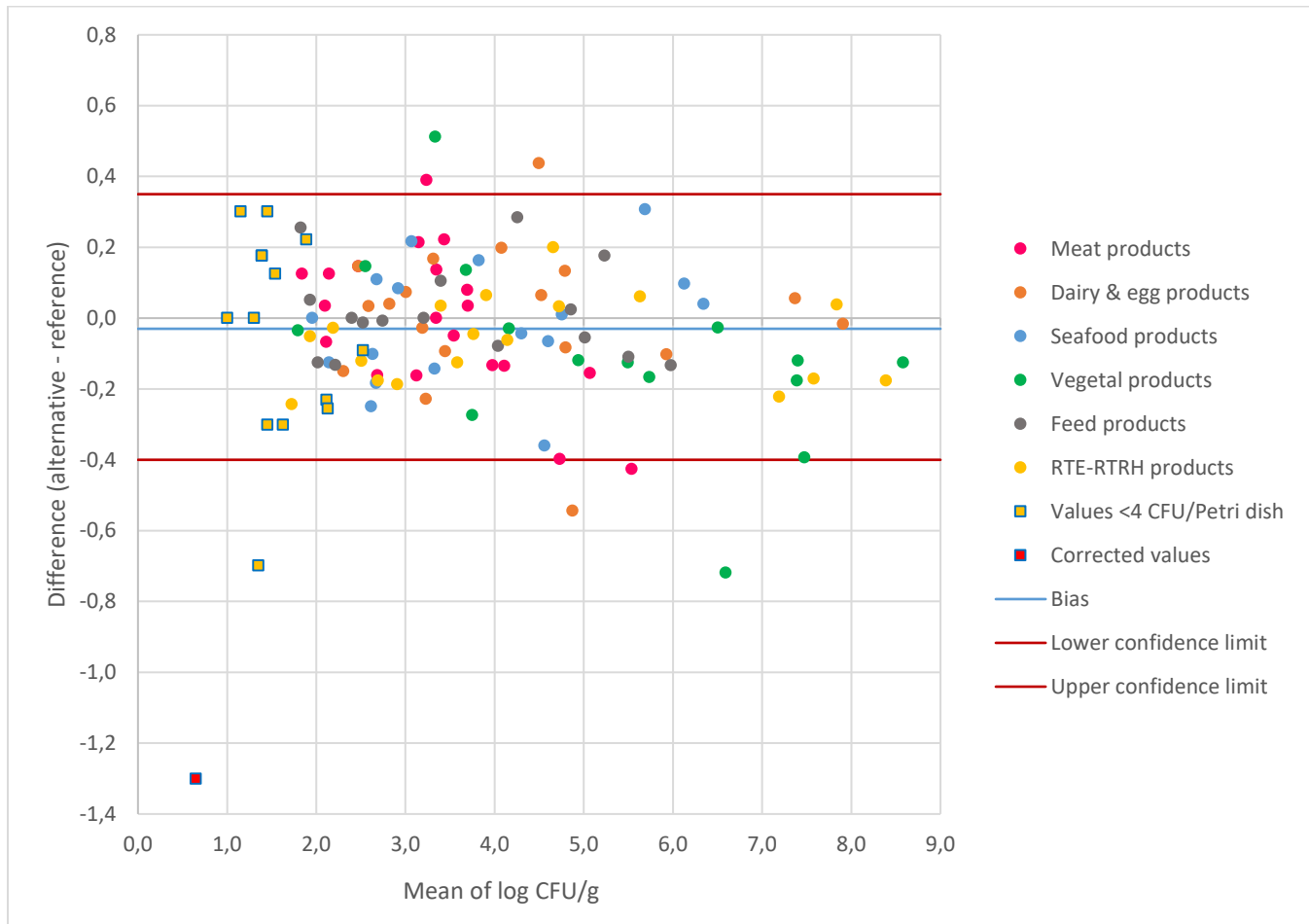
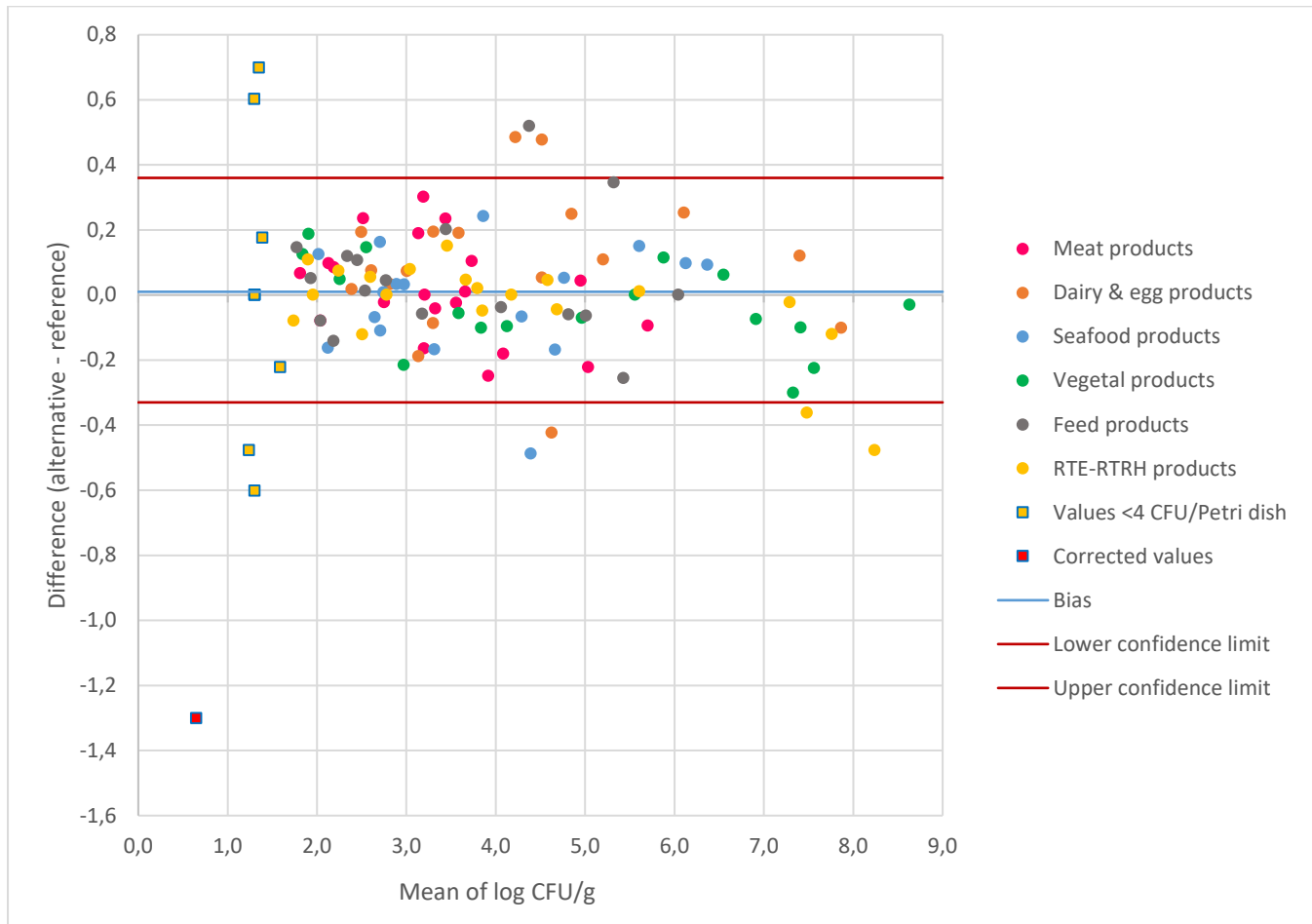


Figure 6: Bland-Altman difference plot for all categories with the pour plate technique



- **Observations:**

Samples for which the average difference is lower or higher than the confidence limits are listed in table 4.

Table 4: values outside the confidence limits on the Bland-Altman difference plot (green cases: values <4 CFU/Petri dish, yellow cases: values lower and higher than the quantification limits, blue: values higher than the confidence limits, red: values lower than the quantification limits)

Inoculation	Category	Type	Sample #	Reference Method value (log)	Alternative Method value (log)	Mean	Difference
<b>Surface spreading:</b>  <b>LCL: -0,40</b>  <b>UCL: +0,35</b>	MP	a	V 7798	5,75	5,32	5,54	-0,43
		a	1691980	3,04	3,43	3,24	0,39
	DP	a	RD 1283	5,15	4,60	4,87	-0,54
		c	1691975	4,28	4,72	4,50	0,44
		a	1692054	1,70	1,00	1,35	-0,70
	VP	a	1714582	3,08	3,59	3,34	0,51
		b	1692026	6,95	6,23	6,59	-0,72
RTE	c	1698386	1,30	0,00	0,65	-1,30	
<b>Pour plate:</b>  <b>LCL: -0,33</b>  <b>UCL: +0,36</b>	MP	b	1692057	1,60	1,00	1,30	-0,60
		c	1692065	1,30	0,00	0,65	-1,30
	DP	a	1691976	4,84	4,41	4,63	-0,42
		b	RD 1282	3,98	4,46	4,22	0,48
		c	1691975	4,28	4,76	4,52	0,48
		c	1691984	1,00	1,60	1,30	0,60
	SP	a	1691997	4,63	4,15	4,39	-0,49
	FP	a	RD 1276	4,11	4,63	4,37	0,52
	RTE	a	S 9939	7,66	7,30	7,48	-0,36
		a	S 9942	8,48	8,00	8,24	-0,48
b		1692061	1,00	1,70	1,35	0,70	
b		1692068	1,48	1,00	1,24	-0,48	

- **Surface spreading method:**

Eight samples are outside the confidence limits: 2 concern corrected values or samples with less than 4 CFU/Petri dish, 3 are higher than the upper confidence limit and 3 are lower than the lower confidence limit.

- **Pour plate technique:**

Twelve samples are outside the confidence limits: 5 concern corrected values or samples with less than 4 CFU/Petri dish, 3 are higher than the upper confidence limit and 4 are lower than the lower confidence limit.

### 3.1.6. Conclusion

The relative trueness study of the alternative method is satisfactory.



## 3.2. Accuracy profile study

### 3.2.1. Protocols

Six matrix-strain couples were tested by both methods. Two batches of a matrix, representative of each category, were inoculated with an Enterobacteriaceae strain at three levels (low, medium and high). For each sample, 5 replicates, represented by 5 different test portions, were tested by each method. This represents a total of 30 analyses per method.

The matrix-strain couples are presented in table 5.

Table 5: matrix-strain couples for the RLoD study

Category	Matrix	Strain	Strain code	Origin of the strain	Target Contamination level (CFU/g)
Meat products	Ground beef	<i>Citrobacter youngae</i>	RAX819A	Ground beef	300
Dairy and egg products	Raw milk cheese	<i>Hafnia alvei</i>	BEY899	Milk	
Seafood products	Raw fish fillet	<i>Klebsiella oxytoca</i>	CGR888	Composite food	
Vegetal products	Frozen vegetables pan	<i>Serratia liquefaciens</i>	AGL470	Vegetal extract	30 000
Feed products	Cat kibbles	<i>Enterobacter cloacae</i>	EBJ453	Mechanically separated chicken meat	1 000 000
Ready-to-eat and ready-to-reheat products	Quiche lorraine	<i>Escherichia coli</i>	UBS981	Ham croissant	

### 3.2.2. Results

Raw data are provided in appendix F.

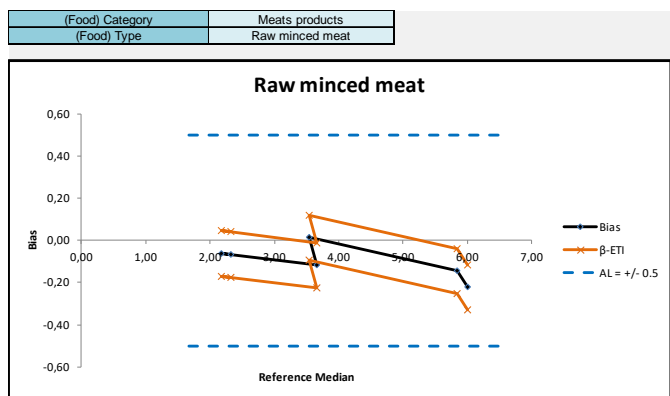
The statistical data and the accuracy profiles are shown:

- in figure 7 for the surface spreading method,
- in figure 8 for the pour plate technique.

Statistical calculations were done according to the Excel spreadsheet named AP calculation tool MCS (clause 6-1-3-3 Calculation and interpretation of accuracy profile study) ver 27-01-2015.xlsx available at <http://standards.iso.org/iso/16140>.

The probability for the tolerance interval is set at 80% and the central value is the median. The acceptability limit is set at  $AL = 0.5 \log_{10}$  CFU/g or ml.

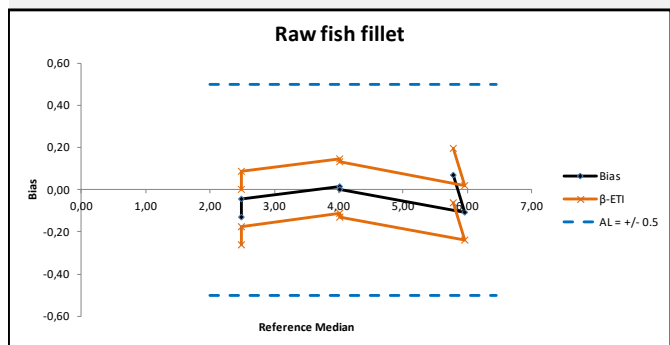
Figure 7: Accuracy profiles per category, using the surface spreading inoculation



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1665805-1665809	2.32	-0,067	-0,174	0,040	YES	YES
1665810-1665814	2.18	-0,062	-0,169	0,045	YES	YES
1665815-1665819	3.66	-0,119	-0,226	-0,012	YES	YES
1665820-1665824	3.53	0,013	-0,094	0,119	YES	YES
1665825-1665829	5.85	-0,146	-0,253	-0,039	YES	YES
1665830-1665834	6.00	-0,222	-0,329	-0,115	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,085	0,074	YES	$\pm 0.500$

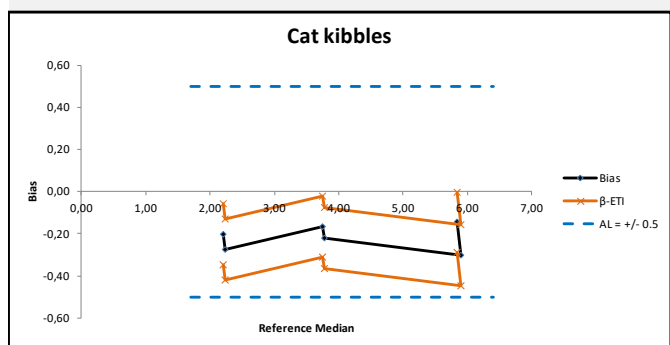
(Food) Category	Seafood products
(Food) Type	Raw fish fillet



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1690868-1690872	2.49	-0,130	-0,260	0,000	YES	YES
1690873-1690877	2.49	-0,044	-0,174	0,086	YES	YES
1690878-1690882	4.02	0,016	-0,114	0,146	YES	YES
1690883-1690887	4.01	0,000	-0,130	0,130	YES	YES
1690888-1690892	5.95	-0,109	-0,239	0,021	YES	YES
1690893-1690897	5.78	0,067	-0,063	0,197	YES	YES

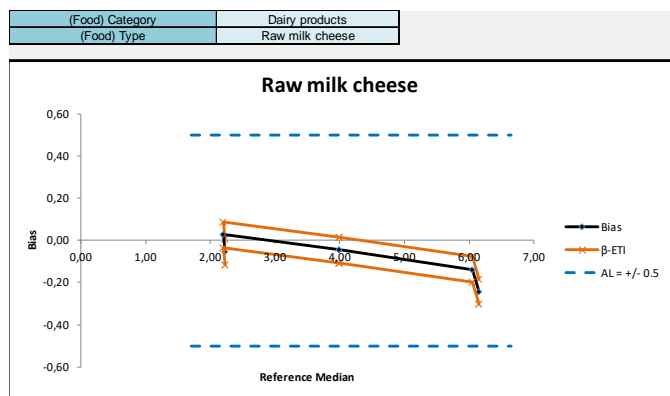
	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,076	0,090	YES	$\pm 0.500$

(Food) Category	Animal feed
(Food) Type	Cat kibbles



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1663784-1663788	2.20	-0,204	-0,348	-0,060	YES	YES
1663789-1663793	2.23	-0,276	-0,421	-0,132	YES	YES
1663794-1663798	3.75	-0,168	-0,313	-0,024	YES	YES
1663799-1663803	3.78	-0,222	-0,366	-0,077	YES	YES
1663804-1663808	5.90	-0,301	-0,445	-0,157	YES	YES
1663809-1665744	5.85	-0,146	-0,290	-0,002	YES	YES

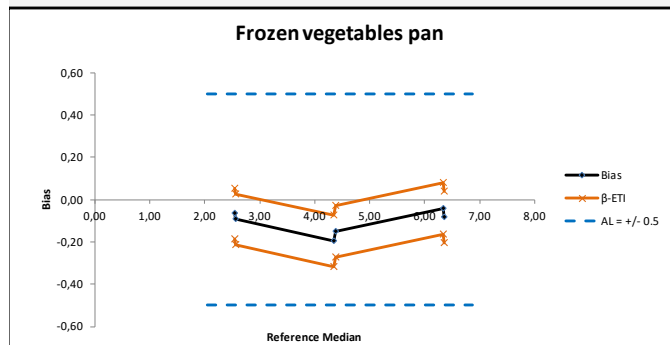
	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,097	0,100	YES	$\pm 0.500$



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1665745-1665749	2.23	-0,054	-0,115	0,006	YES	YES
1665750-1665754	2.20	0,026	-0,034	0,087	YES	YES
1665755-1665759	4.00	-0,046	-0,106	0,015	YES	YES
1665760-1665764	4.00	-0,046	-0,106	0,015	YES	YES
1665765-1665769	6.04	-0,138	-0,199	-0,078	YES	YES
1665770-1665774	6.15	-0,243	-0,304	-0,182	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,066	0,042	YES	$\pm 0.500$

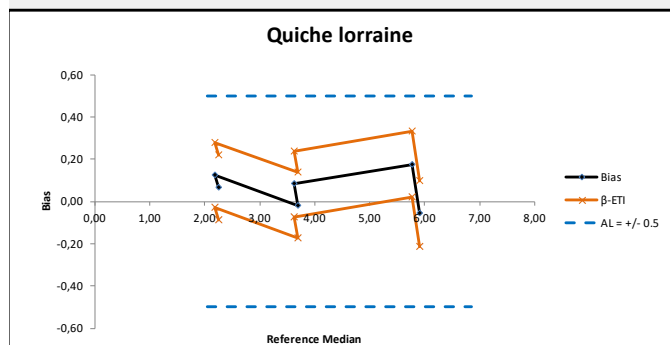
(Food) Category	Vegetables
(Food) Type	Frozen vegetables pan



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1663454-1663758	2.54	-0,067	-0,188	0,054	YES	YES
1663759-1663763	2.56	-0,094	-0,215	0,027	YES	YES
1663764-1663768	4.34	-0,196	-0,318	-0,075	YES	YES
1663769-1663773	4.38	-0,150	-0,271	-0,028	YES	YES
1663774-1663778	6.34	-0,041	-0,163	0,080	YES	YES
1663779-1663783	6.36	-0,083	-0,204	0,038	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,091	0,084	YES	$\pm 0.500$

(Food) Category	Ready to eat and ready to
(Food) Type	Quiche lorraine

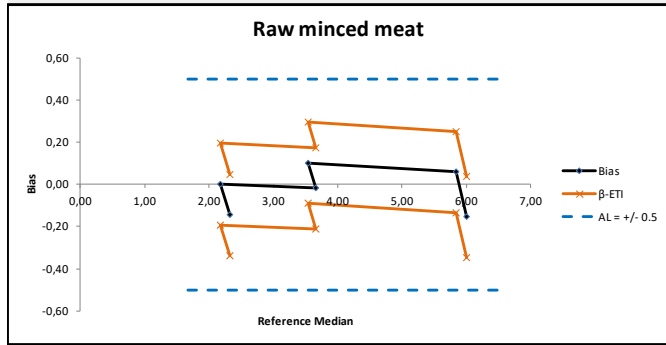


Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to $AL \pm 0.5$ Acceptable	$\beta$ -ETI compared to final AL Acceptable
1644578-1644582	2.26	0,067	-0,088	0,221	YES	YES
1644583-1644587	2.18	0,125	-0,030	0,279	YES	YES
1644588-1644592	3.69	-0,018	-0,173	0,136	YES	YES
1644593-1644597	3.63	0,083	-0,072	0,237	YES	YES
1644598-1644602	5.78	0,176	0,022	0,331	YES	YES
1644603-1644607	5.90	-0,058	-0,212	0,096	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq 0.125$	Final AL
SD Repeatability	0,087	0,107	YES	$\pm 0.500$

Figure 8: Accuracy profiles per category, using the pour plate inoculation

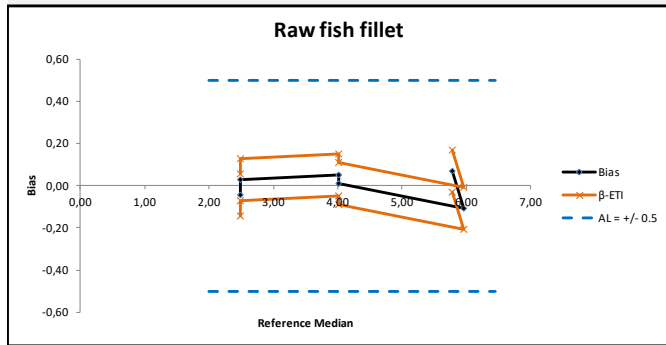
(Food) Category	Meats products
(Food) Type	Raw minced meat



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1665805-1665809	2.32	-0,146	-0,340	0,047	YES	YES
1665810-1665814	2.18	0,000	-0,193	0,193	YES	YES
1665819-1665819	3.66	-0,019	-0,213	0,174	YES	YES
1665820-1665824	3.53	0,102	-0,091	0,295	YES	YES
1665825-1665829	5.85	0,058	-0,135	0,251	YES	YES
1665830-1665834	6.00	-0,155	-0,348	0,039	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,085	0,134	YES	$\pm$ 0,500

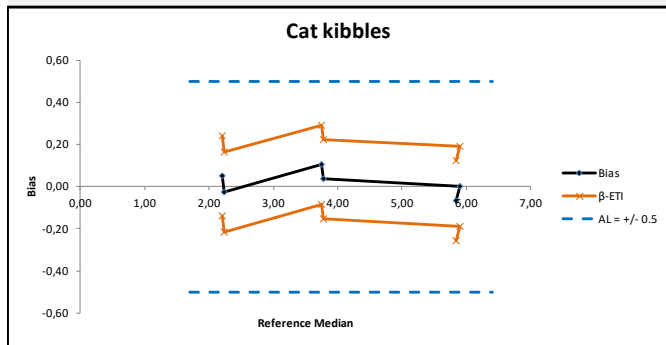
(Food) Category	Seafood products
(Food) Type	Raw fish fillet



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1690868-1690872	2.49	-0,044	-0,144	0,055	YES	YES
1690873-1690877	2.49	0,027	-0,072	0,127	YES	YES
1690878-1690882	4.02	0,051	-0,048	0,151	YES	YES
1690883-1690887	4.01	0,008	-0,091	0,108	YES	YES
1690888-1690892	5.95	-0,109	-0,209	-0,010	YES	YES
1690893-1690897	5.78	0,067	-0,033	0,167	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,076	0,069	YES	$\pm$ 0,500

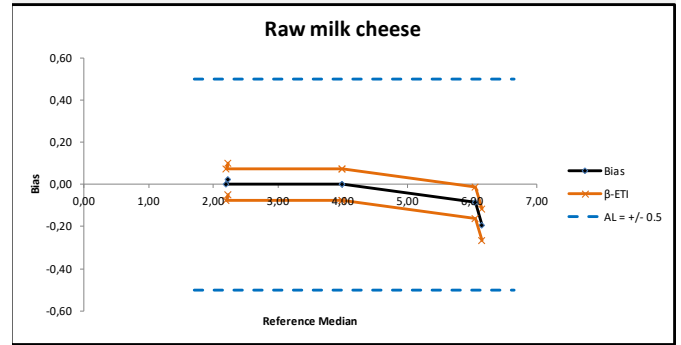
(Food) Category	Animal feed
(Food) Type	Cat kibbles



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1663784-1663788	2.20	0,051	-0,138	0,240	YES	YES
1663789-1663793	2.23	-0,026	-0,215	0,163	YES	YES
1663794-1663798	3.75	0,103	-0,086	0,292	YES	YES
1663799-1663803	3.78	0,035	-0,154	0,224	YES	YES
1663804-1663808	5.90	0,000	-0,189	0,189	YES	YES
1663809-1665744	5.85	-0,067	-0,256	0,122	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,106	0,131	YES	$\pm$ 0,500

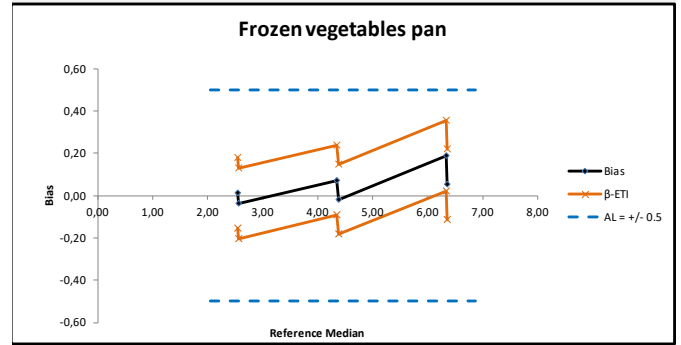
(Food) Category	Dairy products
(Food) Type	Raw milk cheese



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1665745-1665749	2.23	0,025	-0,050	0,100	YES	YES
1665750-1665754	2.20	0,000	-0,075	0,075	YES	YES
1665755-1665759	4.00	0,000	-0,075	0,075	YES	YES
1665760-1665764	4.00	0,000	-0,075	0,075	YES	YES
1665765-1665769	6.04	-0,087	-0,162	-0,012	YES	YES
1665770-1665774	6.15	-0,192	-0,267	-0,117	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,066	0,052	YES	$\pm$ 0,500

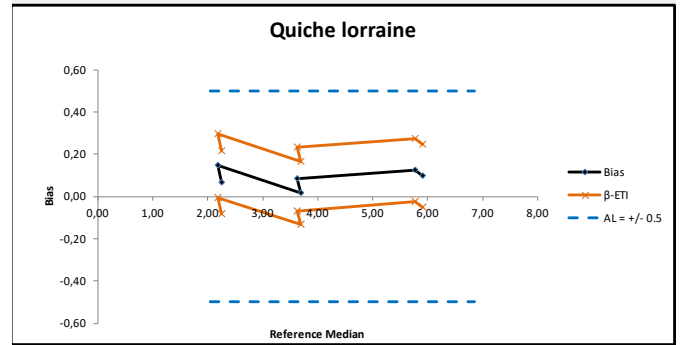
(Food) Category	Vegetables
(Food) Type	Frozen vegetables pan



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1663454-1663758	2.54	0,012	-0,154	0,178	YES	YES
1663759-1663763	2.56	-0,038	-0,204	0,128	YES	YES
1663764-1663768	4.34	0,073	-0,093	0,239	YES	YES
1663769-1663773	4.38	-0,018	-0,184	0,148	YES	YES
1663774-1663778	6.34	0,189	0,023	0,355	YES	YES
1663779-1663783	6.36	0,053	-0,113	0,219	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,091	0,115	YES	$\pm$ 0,500

(Food) Category	Ready to eat and ready to
(Food) Type	Quiche lorraine



Sample Name	Reference Central value	Bias	Lower $\beta$ -ETI	Upper $\beta$ -ETI	$\beta$ -ETI compared to AL $\pm$ 0.5 Acceptable	$\beta$ -ETI compared to final AL Acceptable
1644578-1644582	2.26	0,067	-0,083	0,217	YES	YES
1644583-1644587	2.18	0,146	-0,004	0,296	YES	YES
1644588-1644592	3.69	0,017	-0,133	0,168	YES	YES
1644593-1644597	3.63	0,083	-0,068	0,233	YES	YES
1644598-1644602	5.78	0,125	-0,025	0,275	YES	YES
1644603-1644607	5.90	0,097	-0,053	0,247	YES	YES

	Reference method	Alternative method	SD repeatability of reference method $\leq$ 0.125	Final AL
SD Repeatability	0,087	0,104	YES	$\pm$ 0,500

The tolerance intervals fall into the acceptability limits for all categories with the two modalities of inoculation for the REBECCA+EB method.

### 3.2.3. Conclusion

The alternative method is accepted as being equivalent to the reference method.

## 3.3. Specificity / selectivity

The aim of this study is to check that all the strains of Enterobacteriaceae are detected, and that no cross-reaction exists with strains from other groups.

The inclusivity and the exclusivity of the method are defined by analysis, respectively of 50 positive strains and 30 negative strains.

### 3.3.1. Protocols

#### 3.3.1.1. Initial validation study

The strains were tested in duplicate according to two types of inoculation of REBECCA agar media: spreading or inclusion method.

Thirty (30) target strains and twenty (20) non-target strains were tested.

The raw data are provided in Appendix G.

#### 3.3.1.2. Second renewal study

Twenty (20) additional target strains and ten (10) non-target strains were tested.

Tests for target microorganisms were performed once with the alternative method (by spreading and pour plate), the reference method and a non-selective agar at an inoculation level sufficient to obtain a countable number of colonies on the plate. A pure culture of the strains was calibrated and diluted in 90 ml of buffered peptone water then enumerated to obtain a countable number of colonies on Petri dishes.

Tests for non-target microorganisms were performed once with the alternative method (by spreading and pour plate) and the reference method. Pure cultures were grown on a non-selective broth for 24 hours and diluted at an appropriate level before testing. A pure culture of the strains in Brain Heart Infusion Broth was performed at the optimal growth temperature of the strains then enumerated to obtain a countable number of colonies on Petri dishes.

### 3.3.2. Results

#### 3.3.2.1. Initial validation study

- **Target strains**

The thirty target strains gave typical colonies on REBECCA+EB agar medium: pink to red colonies for Enterobacteriaceae strains and blue to blue violet with or without a blue halo for *E. coli* strains.

- **Non-target strains**

The twenty non-target strains did not grow on the REBECCA medium.

### 3.3.2.2. Second renewal study

The twenty target strains were typical onto the REBECCA+EB agar medium, showing pink to red colonies.

Nine (9) non-target strains did not grow on the REBECCA+EB agar medium. One strain of *Acinetobacter baumannii* gave very small typical pink colonies on REBECCA+EB pour plates and typical pink colonies on REBECCA+EB surface plates.

### 3.3.3. Conclusion

The inclusivity and the exclusivity of the alternative method are satisfactory.

## 3.4. Practicability

Practicability is studied as a function of the four criteria defined by the Technical Board in comparing the reference method EN ISO 21528-2:2017 with the REBECCA+EB method.

The criteria defined are informed below:

<b>Packaging Volume of reagents</b>	<ul style="list-style-type: none"><li>· AEB520020: pack of 20 plates of Ø 90 mm,</li><li>· AEB620027: 6 vials of 200 ml base medium</li><li>· AEB184135: EB supplement qsp 1.2 l</li><li>· AEB184135/10: EB supplement qsp 1.2 l x 10</li><li>· AEB150022: dehydrated REBECCA agar base, flask of 500 g</li></ul>
<b>Storage conditions and kit</b>	<p>The plates of pre-poured complete medium and REBECCA base flasks must be kept between +2°C and +8°C.</p> <p>The expiry date is shown on each plate and each vial.</p>
<b>Use after opening of the kit</b>	<p>The pre-poured complete medium plates must be stored at 2-8°C in their package until the expiry date.</p> <p>The plates poured from flasks can be kept up to 15 days at 2-8°C.</p> <p>After rehydration, the REBECCA enrichment supplement can be stored 24 hours between 2 and 8°C, or up to 7 days if frozen (only one freezing/thawing cycle).</p> <p>AEB184135 – AEB184135/10 – AEB520020 must be stored away from light.</p>
<b>Reagents</b>	<p>Supplements have to be kept protected from light.</p> <p>For the medium in flask, liquefy the medium in a water bath at 100°C and cool to 44-47°C.</p> <p>Per flask of 200 ml of REBECCA medium, add aseptically 1 ml of EB supplement. Homogenize slightly the agar base and the supplement.</p>

<b>Time to result</b>		
<b>Step</b>	<b>Time required (Day)</b>	
	REBECCA+EB method	EN ISO 21528-2 standard
Realization of first dilution and decimal dilutions	D0	D0
Media inoculation	D0	D0
Plates reading, interpretation and calculation	D1	D1
<b>Obtaining negative or positive results</b> (if no characteristic colony)	<b>D1</b>	<b>D1</b>
<b>Confirmation tests</b>	/	<b>D2 and D3</b>
<b>Obtaining negative results</b> (after negative confirmations if necessary)	<b>D1</b>	<b>D3</b>
<b>Obtaining positive results:</b> Confirmation by reference method test (including purification)	/	<b>D3</b>
<b>Alternative method</b>	<b>D1</b>	/
<b>Common step with reference method</b>	Preparation of initial suspension and decimal dilutions.	

### 3.5. General conclusion for the methods comparison study

The relative trueness study shows a good correlation between the alternative method and the reference method.

The accuracy profile study illustrates that the performances of the alternative method are comparable to those of the reference method.

The alternative method is specific and selective.

Time-to-result is equivalent to that of the reference method (one day) for negative results and faster if Enterobacteriaceae grow on agar media because the alternative method does not require confirmations.

## 4. Interlaboratory study

The aim of the interlaboratory study, as described in ISO 16140/A1:2011 standard, was to determine the variability of the results obtained in different laboratories using identical samples and to compare these results with those obtained during the methods comparison study.

### 4.1. Study organization

Twelve (12) collaborators took part in the interlaboratory study. Pasteurized milk was inoculated with an *Escherichia coli* strain coded I69, isolated from a camembert cheese and a *Citrobacter freundii* coded R40, strain ATCC 8090.

Eight samples were prepared per collaborator, representing 4 levels of contamination with 2 samples per level. Collaborators and Expert Laboratory carried out the analyses with the reference method and the alternative method.

### 4.2. Control of experimental parameters

#### 4.2.1. Contamination levels obtained after artificial contamination

The four contamination levels are detailed in the following table.

Table 6: Contamination levels of inoculated samples

Level	Sample	Targeted level (CFU/ml)	<i>E. coli</i> Real level (CFU/ml)	<i>C. freundii</i> Real level (CFU/ml)
Level 0 ( $L_0$ )	2 and 8	0	0	<1
Level 1 ( $L_1$ )	4 and 7	10-100	24	62
Level 2 ( $L_2$ )	5 and 6	100 - 1000	390	840
Level 3 ( $L_3$ )	1 and 3	1000 - 10 000	4900	8300

#### 4.2.2. Strain stability during shipping

In order to evaluate the *Escherichia coli* and the *Citrobacter freundii* strains variability during shipping, bacterial counts of inoculated milk at different levels were checked at different times, during storage at 4°C. Enumeration results (CFU/ml) are reported in table 8.

Table 7: Stability of the *Escherichia coli* and of the *Citrobacter freundii* strains at 4°C

Day	<i>Escherichia coli</i>						<i>Citrobacter freundii</i>					
	Level 1		Level 2		Level 3		Level 1		Level 2		Level 3	
	R1	R2	R1	R2	R1	R2	100	50	830	740	8200	5100
D0	65	30	380	410	4200	3100	35	80	830	850	6700	8400
D1	65	50	450	530	4000	4000	70	40	650	570	7600	6400
D2	30	60	450	430	4700	4500	100	50	830	740	8200	5100

The results showed a stability of the strains in the shipping conditions.

#### 4.2.3. Shipping conditions

Temperatures registered by the temperature probe during shipping were between 0.9°C and 2.6°C.

The temperatures at reception are recorded in the following table.

Table 8: temperatures at reception

Collaborator	Temperature (°C)	State of the samples	Probe temperature (°C)	
			Mean	Standard deviation
A	2,4	Correct	1,8	0,9
B	4,0	Correct	1,8	0,7
C	5,4	Correct	0,9	1,5
D	5,9	Correct	1,6	0,8
E	4,3	Correct	2,3	0,8
F	3,5	Correct	2,6	1,1
G	4,5	Correct	2,1	1,2
H	3,1	Correct	2,4	1,4
I	5,4	Correct	1,1	1,4
J	6,6	Correct	1,5	1,2
K	4,9	Correct	2,2	0,8
L	2,1	Correct	/	/

All collaborators received the packages and the reagents in one day, except:

- Collaborator E which received a part of the reagents one day after the samples,
- Collaborators K and L which received their packages in two days.

The data from collaborators E, K and L were excluded from the final analysis of the results.

#### 4.2.4. Conclusion

The results of 9 collaborators were exploited.

### 4.3. Results

#### 4.3.1. Expert laboratory

Results obtained for the Expert Laboratory are presented in the table 9.

Table 9: Results of the Expert Laboratory (in CFU/ml)

Level	Reference method		Alternative method	
	Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
$L_0$	<10	<10	<10	<10
$L_1$	35	55	20	40
$L_2$	500	490	500	440
$L_3$	5700	5000	4200	3800

Results according to the EN ISO 21528-2 standard and according to the alternative method were in agreement.



### 4.3.2. Results obtained by the collaborators

Detailed results of the 9 laboratories which realized the analysis are presented in Appendix H. The results are summarized in the table 10 for the low level  $L_1$ , intermediate level  $L_2$  and high level  $L_3$ .

Table 10: Results in CFU/ml of the collaborators per level of contamination (RM: reference method, AM: alternative method, D: duplicate)

Collaborator	$L_1$				$L_2$				$L_3$			
	RM		AM		RM		AM		RM		AM	
	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2	D1	D2
A	45	45	30	80	500	500	530	460	5600	4200	4100	5800
B	90	60	10	20	590	500	420	350	5000	4400	4500	4400
C	30	70	40	60	580	420	360	310	5400	5500	3500	4100
D	30	35	60	40	550	590	570	500	6100	6600	4500	5500
F	60	25	50	60	590	530	570	410	5500	5600	5100	4400
G	65	50	50	60	570	550	490	480	5500	5800	6100	4500
H	35	40	100	40	620	530	460	490	5600	5500	5900	5100
I	35	75	50	40	460	440	370	430	4500	5000	5200	4800
J	25	55	10	40	360	440	370	400	5200	4800	4000	3500

It's important to note that a part of the results of the level  $L_1$  do not fulfill the requirements of the standard EN ISO 7218/A1:2013 for the low counts of colonies (§ 10.3.2.4.1). Because of the low levels of *E. coli* inoculated, some results are expressed despite of their concentration inferior to 40 CFU/g (less than 4 colonies on Petri dishes for the enumeration of the initial suspension) while they should be noted: "Microorganisms are present but less than 40 per ml".

Seven results of the reference method and four results of the alternative method are concerned.

After the presentation of the project of renewal study of the method in July 2019, the Technical Board agreed with the proposition of the Expert Laboratory to still interpret all the data of the level  $L_1$ .

### 4.3.3. Conclusion

Results of 9 laboratories were finally statistically exploited.

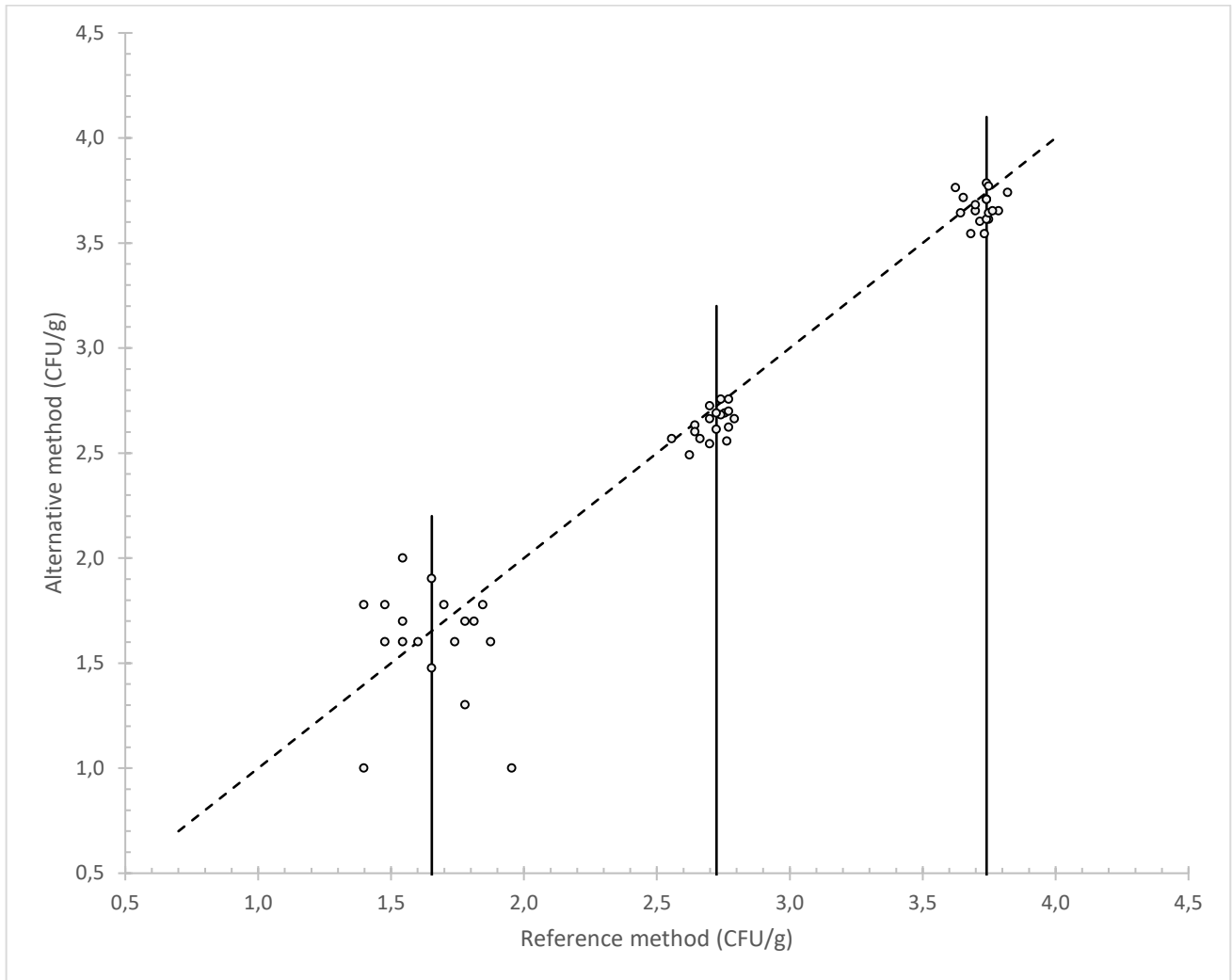
The results obtained by the collaborators were in agreement with those of the Expert Laboratory.

## 4.4. Statistical interpretations and calculations

### 4.4.1. Visual linearity checking

After the log<sub>10</sub> transformation of all test results, data are plotted with the results of the reference method on the x-axis and the results of the alternative method on the y-axis (figure 9).

Figure 9: Scatter plot of reference-method versus alternative-method results for the interlaboratory study (dotted line: first bisecting line, vertical lines: medians of the measurements obtained with the reference method)



Data are well balanced around the median values of the reference method for each level, but a very slight negative bias is observed for the alternative method at levels  $L_2$  and  $L_3$ .

#### 4.4.2. Calculation of the accuracy profile and interpretation

An accuracy profile is drawn according to the calculations provided in the Excel spreadsheet named [AP calculation tool ILS \(clause 6-2-3 Calculations summary and interpretation of data\) ver 14-03-2016.xlsx](#), available at: <http://standards.iso.org/iso/16140/-2/ed-1/en>.

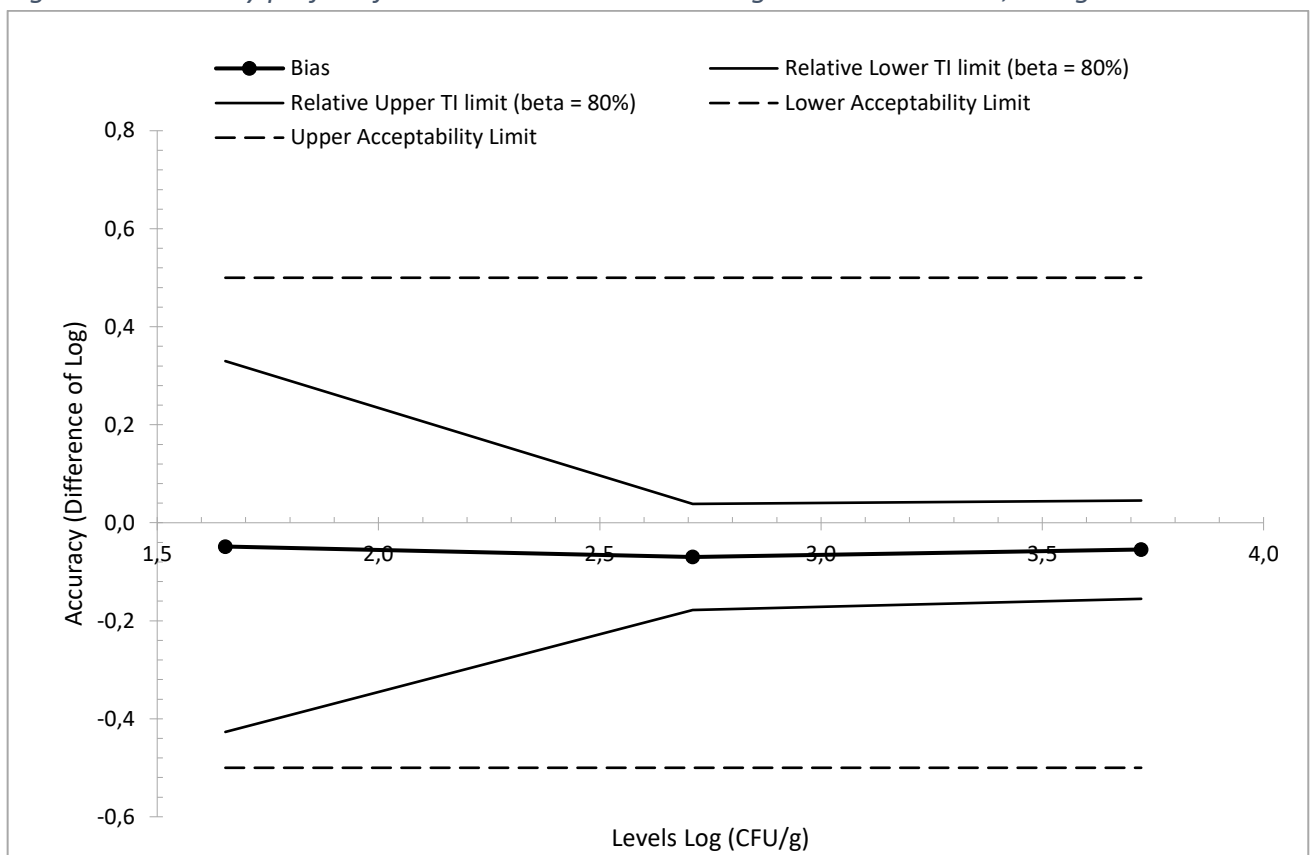
The results of the calculations are provided in table 11.

The graphical representation of the accuracy profile lies in figure 10.

Table 11: Summary of the accuracy profile calculations (AL: acceptability limit, TI: tolerance interval, SD: standard deviation)

Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
<b>Tolerance probability (beta)</b>	80%	80%	80%			
<b>Acceptability limit in log (lambda)</b>	0.50	0.50	0.50			
<b>Target value</b>	<b>1,654</b>	<b>2,710</b>	<b>3,723</b>			
Number of participants (K)	9	9	9	9	9	9
Average for alternative method	1,606	2,640	3,669	1,654	2,710	3,723
Repeatability standard deviation (sr)	0,221	0,050	0,060	0,176	0,048	0,036
Between-labs standard deviation (sL)	0,159	0,058	0,040	0,000	0,043	0,035
Reproducibility standard deviation (sR)	0,272	0,077	0,072	0,176	0,064	0,050
Corrected number of dof	14,646	12,048	14,987	16,941	13,534	13,168
Coverage factor	1,391	1,414	1,388			
Interpolated Student t	1,342	1,356	1,341			
Tolerance interval standard deviation	0,2819	0,0799	0,0749			
Lower TI limit	1,227	2,532	3,568			
Upper TI limit	1,984	2,748	3,769			
<b>Bias</b>	<b>-0,048</b>	<b>-0,070</b>	<b>-0,055</b>			
<b>Relative Lower TI limit (beta = 80%)</b>	<b>-0,427</b>	<b>-0,178</b>	<b>-0,155</b>			
<b>Relative Upper TI limit (beta = 80%)</b>	<b>0,330</b>	<b>0,038</b>	<b>0,046</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 of reference	0.112					

Figure 18: Accuracy profile of the alternative method using  $\beta = 80\%$  and  $\lambda = 0,50 \log_{10}$  unit



The AL is met for all the contamination levels: the tolerance interval limits of the alternative method are within the acceptability limits of 0.5 log CFU/g

#### 4.5. General conclusion for the interlaboratory study

The tolerance intervals of all levels of contamination fall within the default acceptability limits ( $\pm 0.5$  log CFU/ml).

Despite the very slight bias observed for the all levels of contamination (from -0.070 to -0.048 log CFU/ml), the alternative method is regarded as being equivalent to the reference method.

## 5. General conclusion

The data and the interpretation of the methods comparison study and of the interlaboratory study fulfilled the requirements of the EN ISO 16140-2:2016 standard. The REBECCA+EB method is considered as equivalent to the reference method described in the EN ISO 21528-2:2017 standard.

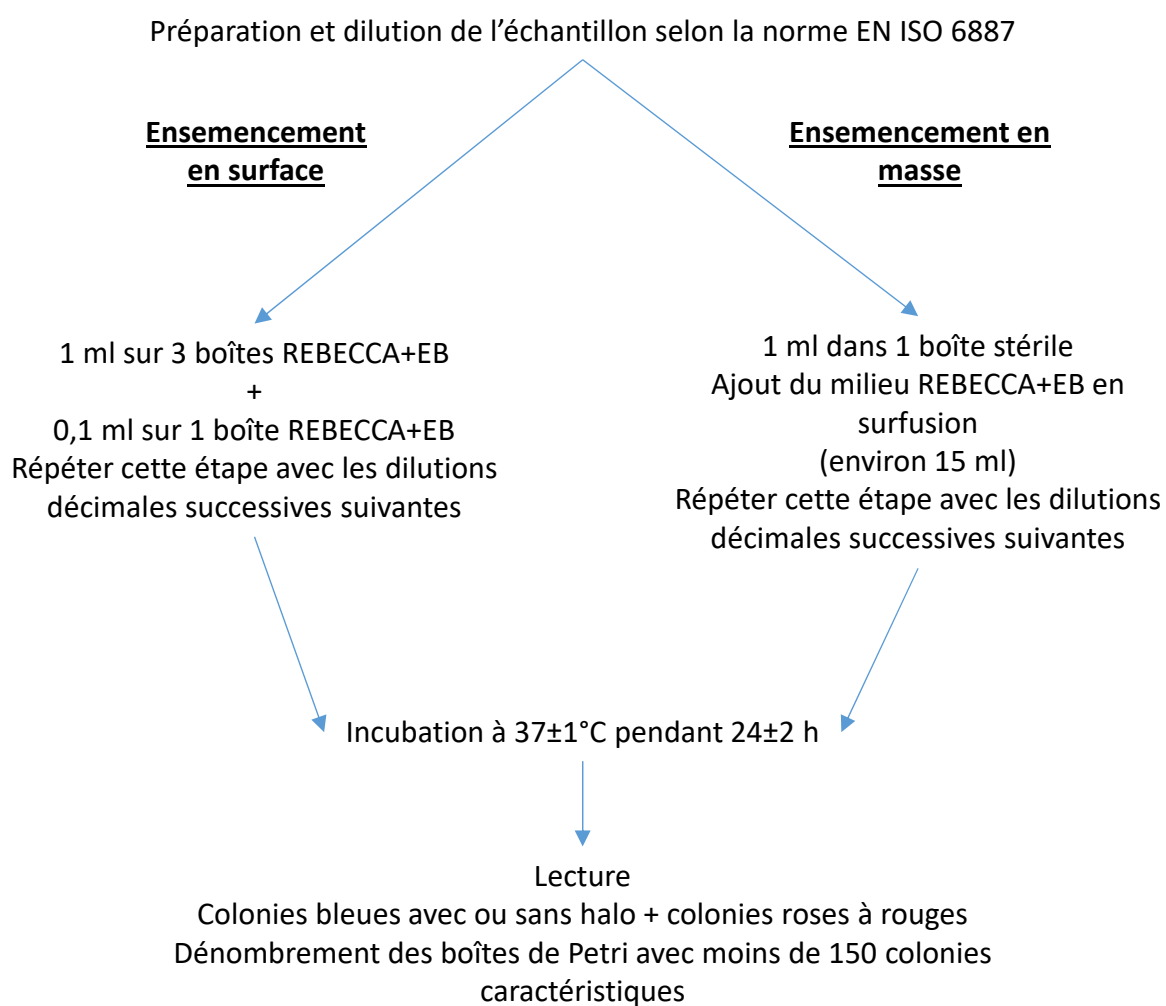
Le Lion d'Angers, December 31, 2019  
François Le Nestour  
Head of the Microbiology Department

A handwritten signature in black ink, consisting of a stylized 'F' and 'N' enclosed within a large, sweeping loop.

## **APPENDICES**

## APPENDIX A

### PROTOCOLE DE LA METHODE REBECCA+EB



## **APPENDIX B**

### **PROTOCOLE DE LA METHODE DECRITE DANS LA NORME EN ISO 21528-2**

Préparation et dilution de l'échantillon selon la norme EN ISO 6887



1 ml dans 1 boîte stérile  
Ajout du milieu VRBG en surfusion (environ 15 ml) et ajout d'une  
double couche de 5 à 10 ml après solidification  
Répéter cette étape avec les dilutions décimales successives  
suivantes



Incubation à  $37\pm 1^{\circ}\text{C}$  pendant  $24\pm 2$  h



Lecture  
Colonies roses à rouges ou violettes, avec ou sans halo  
Dénombrement des boîtes avec moins de 150 colonies  
caractéristiques



Confirmation  
Retenir 5 colonies caractéristiques par boîte (ou 5 colonies  
blanchâtres si aucune colonie caractéristique n'est présente)  
Les repiquer sur gélose nutritive et incuber  
les boîtes à  $37\pm 1^{\circ}\text{C}$  pendant  $24\pm 2$ h  
A partir d'une colonie isolée, réaliser un test oxydase et un test de  
fermentation du glucose sur gélose OF glucosée (piqûre centrale et  
incubation à  $37^{\circ}\text{C}$  pendant  $24\pm 2$  h avec 1 cm d'huile minérale)  
Les colonies oxydase-négatives et glucose-positives sont considérées  
comme des Enterobacteriaceae



**APPENDIX C - Artificial contaminations**


Study	Strain	Code	Origin	Stress type	Treatment and intensity	Samples	Number of uses
Initial validation study	<i>Hafnia alvei</i>	I3	Tabbouleh	Spiking	20 min à 57°C (0.7)	RD 1269/ 1274/ 1278/ 1279/ 1280	5
	<i>Citrobacter freundii</i>	R35	CIP 53.62	Spiking	20 min à 57°C (0.7)	RD 1276/ 1277/ 1281/ 1282/ 1283	5
	<i>Klebsiella oxytoca</i>	I17	Soy salad	Spiking	8 jours à -20°C (0.9)	RD 1270/ 1275	2
Third renewal study	<i>Leclercia adecarboxylata</i>	FAT267	Shell garniture St Jacques	Seeding	48 to 72 h at 5°C	1698384 -1698386 -1698391	3
	<i>Pantoea agglomerans</i>	CCA775	Organic thyme	Seeding	48 to 72 h at 5°C	1698387 - 1698388 -1698389	3
	<i>Serratia liquefaciens</i>	AER835	Raw beef	Seeding	48 to 72 h at 5°C	1698392 - 1698393 - 1698394 - 1698396	4
	<i>Klebsiella oxytoca</i>	ACZ526	Raw chicken wings	Seeding	48 to 72 h at 5°C	1692056 - 1692057 - 1692058	3
	<i>Enterobacter cloacae</i>	VBT249	Whey	Seeding	48 to 72 h at 5°C	1698397	1
	<i>Serratia liquefaciens</i>	WBH449	Salmon filet	Seeding	48 to 72 h at 5°C	1698398 - 1698399 - 1698400 - 1698401	4
	<i>Escherichia coli</i>	EZN508	Chopped steak	Seeding	48 to 72 h at 5°C	1692051 - 1692052 - 1692065	3
	<i>Escherichia coli</i>	TDW583	Raw milk cheese	Seeding	48 to 72 h at 5°C	1692054	1
	<i>Klebsiella oxytoca</i>	CGR888	Tiramisu	Seeding	48 to 72 h at 5°C	1692059 - 1692061 - 1692068	3
	<i>Hafnia alvei</i>	BMH115	Fish balls	Seeding	48 to 72 h at 5°C	1729337 - 1729338	2
	<i>Escherichia coli</i>	UVS777	Minced beef	Seeding	48 to 72 h at 5°C	1729339	1
	<i>Escherichia coli</i>	TZP821	Hummus and beets	Spiking	15 min at 56°C/cold water (0.9)	1729342 - 1729343	2
	<i>Escherichia coli</i>	UDD835	Spinach, potatoes and bechamel sauce	Seeding	48 to 72 h at 5°C	1729344	1


## APPENDIX D

### Relative trueness study

#### Raw results

##### Key:


 replicate analyzed during the initial validation study,  
not considered as part of the renewal study ac. EN ISO 16140-2:2016

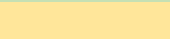
 not realized: only one replicate analyzed during the renewal study  
ac. EN ISO 16140-2:2016

Results are expressed in CFU/g or ml

nc: not countable

 Estimated number

 Microorganisms are present but less than 40 per g or ml

 Results not countable or inferior to the limit of quantification

**Meats products**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)										REBECCA + EB- Surface spreading					REBECCA + EB- Pour plate				
						R1				R2				R1	R2	Dil.	R1	R2	R1	R2	Dil.	R1	R2	R1	R2
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.	Result	Result		CFU	CFU	Result	Result		CFU	CFU	Result	Result
Initial validation study	HA 4187	Saucisse aux herbes crue	NC	c	-2	97	100%	119	100%	102	100%	85	100%	11000	9220	-2	85	68	8100	6600	-2	62	77	6200	7700
					-3	6	100%	9	100%	8	100%	10				-3	4	5			-3	6	8		
					-1	0		0		0		0				-1	0	0			-1	0	0		
					-2	0		0		0		0		<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
					-2	0		0		0		0				-2	0	0			-2	0	0		
					-2	37	100%	38	100%	30	100%	35		3700	3270	-2	35	29	3300	2900	-2	36	30	3500	3000
					-3	4	100%	3	100%	5	100%	4				-3	1	3			-3	2	3		
					-1	54	100%	58	100%	65	100%	75		580	780	-2	4	5	400	500	-1	57	60	550	570
					-2	6	100%	9	100%	8	100%	5				-3	0	0			-2	4	3		
					-1	>150		>150		>150		>150		15000	1,5E+04	-2	113	150	11000	10600	-1	>150	>150	9900	12000
					-2	151	100%	154	100%	148	100%	144				-3	7	11			-2	99	124		
					-1	0		0		0		0				-1	0	0			-1	0	0		
				-2	0		0		0		0		<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10	
				-1	0		0		0		0				-1	0	0			-1	0	0			
				-2	0		0		0		0		<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10	
				-1	123	100%	152	100%	104	100%	64		140000	9,6E+04	-3	98	83	98000	8,2E+04	-3	85	86	84000	8,5E+04	
				-4	23	100%	12	100%	25	100%	18				-4	10	7			-4	7	8			
				-1	0		0		0		0				-1	0	0			-1	0	0			
				-2	0		0		0		0		<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10	
				-3	>150		>150		>150		>150		560000	3,6E+05	-3	>150	>150	210000	2,1E+05	-3	>150	>150	450000	3,5E+05	
				-4	58	100%	53	100%	37	100%	25				-4	21	24			-4	45	35			
				-2	45	100%	45	100%	55	100%	52		4500	5100	-2	56	51	5400	5000	-2	45	49	4600	4800	
				-3	4	100%	6	100%	4	100%	4				-3	3	4			-3	6	4			
				-2	22	100%	23	100%	20	100%	17		2200	1800	-2	21	16	2200	1500	-2	19	15	2000	1500	
				-3	2	100%	2	100%	1	100%	1				-3	3	1			-3	3	2			
Renewal study	1714579	Saucisses fumées	No	c	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
					-1	115	100%						1100		-1	nc				-1	nc				
					-2	11	100%								-2	27				-2	22				
					-1	nc	100%						1900		-1	nc				-1	nc				
					-2	19	100%								-2	26				-2	13				
					-1	nc	100%						2100		-1	nc				-1	nc				
					-2	21	100%								-2	35				-2	36				
					-2	nc	100%						85000		-2	nc				-2	nc				
					-3	85	100%								-3	34				-3	94				
					-1	114	100%						1100		-1	nc				-1	nc				
					-2	10	100%								-2	18				-2	17				
					-1	0								<10		-1	0		<10		-1	0		<10	
					-2	0									-2	0				-2	0				
					-1	0								<10		-1	0		<10		-1	0		<10	
					-2	0									-2	0				-2	0				
					-1	0								<10		-1	0		<10		-1	0		<10	
					-2	0									-2	0				-2	0				
					-1	6	100%						60		-1	8			80		-1	7		70	
					-2	0									-2	0				-2	0				
					-1	2	100%						20		-1	4			40		-1	2		20	
					-2	0									-2	0				-2	0				
				-1	4	100%						40		-1	2			20		-1	1		10		
				-2	0									-2	0				-2	0					
				-1	nc	100%						1600		-1	114			1100		-1	nc		1600		
				-2	16	100%								-2	11				-2	16					
				-1	13	100%						120		-1	17			160		-1	11		100		
				-2	0									-2	1				-2	0					
				-1	25	100%						250		-1	35			350		-1	44		430		
				-2	3	100%								-2	3				-2	3					
				-1	nc	100%						4800		-1	nc			5200		-1	nc		6100		
				-2	48	100%								-2	52				-2	61					
				-1	14	100%						140		-1	13			120		-1	16		170		
				-2	1	100%								-2	0				-2	3					
				-1	2	100%						20		-1	2			20		-1	0		<10		
				-2	0									-2	0				-2	0					
				-1	12	100%						120		-1	13			130		-1	14		150		
				-2	1	100%								-2	1				-2	2					

**Dairy and egg products**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)								REBECCA +EB - Surface spreading				REBECCA + EB - Pour plate							
						R1				R2				R1	R2	Dil.	R1	R2	R1	R2	Dil.	R1	R2	R1	R2
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.	Result	Result		CFU	CFU	Result	Result		CFU	CFU	Result	Result
Initial validation study	RD 1600	Roquefort	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	RD 1601	Camembert	No	b	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	M 46205	Crème fraîche fluide	No	a	-5	>150	100%	>150	100%	>150	100%	>150	100%	22000000	3E+07	-5	>150	>150	25000000	3,2E+07	-5	>150	>150	29000000	3E+07
	RD 1602	Gruyère	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	VR 4896	Glace antillaise	No	a	-1	83	100%	104	100%	70	100%	85	95	930	790	-2	11	18	1100	1700	-1	113	110	1100	1100
	RD 1603	Fromage de chèvre	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	RD 1604	Lait	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	RD 1605	Crème épaisse	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	0	0	<10	<10			
	R 36162	Camembert	No	b	-3	32	100%	30	100%	28	100%	36	4	31000	3,1E+04	-3	37	29	36000	3,0E+04	-3	35	39	35000	3,9E+04
	RD 1278	Fromage au lait cru	Yes	b	-1	28	100%	20	100%	11	100%	15	2	240	145	-1	19	21	170	180	-1	24	36	250	360
	RD 1279	Comté	Yes	b	-1	39	100%	32	100%	42	100%	44	4	370	445	-2	4	4	400	480	-1	42	40	440	440
	RD 1280	Fromage blanc	Yes	a	-2	19	100%	14	100%	14	100%	20	1	1600	1600	-2	15	15	1500	1450	-2	24	28	2500	3100
	RD 1281	Fromage au lait de vache	Yes	b	-2	24	100%	36	100%	25	100%	26	2	3100	2750	-2	24	15	2500	1450	-2	48	25	4800	3400
	RD 1282	Lait entier	Yes	b	-3	10	100%	9	100%	20	100%	15	1	9500	1,6E+04	-3	15	24	15000	2,0E+04	-3	29	27	29000	3,0E+04
	RD 1283	Glace au caramel	Yes	a	-4	13	100%	18	100%	15	100%	11	1	140000	1,2E+05	-4	4	9	40000	9,1E+04	-4	18	23	180000	2,3E+05
	RD 1283	Glace au caramel	Yes	a	-5	0	0	0	0	0	0	0	0	140000	1,2E+05	-5	1	1	40000	9,1E+04	-5	2	2	180000	2,3E+05
	Renewal study	1714574	Lait de vache thermisé	No	a	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
		1714580	Tiramisu	No	c	-2	18	100%	1	100%	1	100%	1	1	1700	1700	-2	23	23	2500	2500	-2	11	11	1100
1714585		Coule d'oeufs	No	c	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
1691974		Lait	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
1691975		Pâte à gâteau	No	c	-2	nc	100%	19	100%	19	100%	19	1	19000	19000	-2	nc	52	52000	52000	-2	nc	57	57000	57000
1691979		Gâteau 3 chocolats	No	c	-1	27	100%	1	100%	1	100%	1	1	250	250	-1	37	37	350	350	-1	41	41	390	390
1691976		Tomme de Savoie	No	a	-2	nc	100%	69	100%	69	100%	69	1	69000	69000	-2	nc	57	57000	57000	-2	nc	26	26000	26000
1691977		Camembert	No	a	-1	65	100%	4	100%	4	100%	4	1	630	630	-1	68	68	690	690	-1	65	65	680	680
1691978		Yaourt chèvre	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
1691983		Jaune d'œuf liquide	No	c	-6	80	100%	10	100%	10	100%	10	1	82000000	82000000	-6	77	77	79000000	79000000	-6	70	70	65000000	65000000
1691984		Tiramisu aux fraises	No	c	-1	1	100%	0	100%	0	100%	0	0	10	10	-1	1	1	10	10	-1	4	4	40	40
1692000		Fromage blanc pasteurisé	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
1692007		Mousse pistache	No	c	-4	94	100%	11	100%	11	100%	11	1	950000	950000	-4	74	74	750000	750000	-4	nc	nc	1700000	1700000
1692013		Tomme angevine	No	a	-2	23	100%	1	100%	1	100%	1	1	2200	2200	-2	12	12	1300	1300	-2	20	20	1800	1800
1692017		Mousse chocolat chantilly	No	c	-2	nc	100%	53	100%	53	100%	53	1	53000	53000	-2	nc	72	72000	72000	-2	nc	94	94000	94000
1692054		Crème fraîche	Yes	a	-1	5	100%	0	100%	0	100%	0	0	50	50	-1	1	1	10	10	-1	3	3	30	30
1692054		Crème fraîche	Yes	a	-2	0	100%	0	100%	0	100%	0	0	50	50	-2	0	0	10	10	-2	0	0	30	30

**Seafood products**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)								Dil.	REBECCA + EB- Surface spreading				Dil.	REBECCA + EB - Pour plate					
						R1				R2					R1	R2	R1	R2		R1	R2	R1	R2		
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.		Result	Result	CFU	CFU		Result	Result	CFU	CFU	Result	Result
Initial validation study	RG 3029	Tarama	No	c	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10	
	VR 5037	Bouchées aux crevettes	No	c	-2	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10	
					-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Q 2271	Raviolis aux crevettes	No	c	-3	>150	>150	>150	>150	>150	>150	>150	340000	4,0E+05	-3	>150	>150	690000	7,8E+05	-3	>150	>150	480000	3,6E+05	
					-4	32	100%	35	100%	39	100%	40	100%	40	100%	-4	69	78	690000	7,8E+05	-4	48	36	480000	3,6E+05
	RG 3033	Presse de sole	No	c	-1	102	100%	95	100%	86	100%	112	100%	910	950	-2	15	11	1500	1100	-1	102	148	980	1400
					-2	0	0	4	100%	6	100%	5	100%	5	100%	-3	1	1	1500	1100	-2	6	3	980	1400
	VI 311	Saumon mariné	No	b	-5	14	100%	13	100%	8	100%	12	100%	1200000	1,0E+06	-5	15	9	1500000	9,1E+05	-5	14	10	1500000	1,1E+06
					-6	0	0	0	0	1	100%	1	100%	1	100%	-6	1	1	1500000	9,1E+05	-6	2	2	1500000	1,1E+06
	C 85	Tartare de bar	No	a	-3	43	100%	56	100%	48	100%	53	100%	56000	5,3E+04	-3	56	51	57300	5,2E+04	-3	41	38	38000	3,7E+04
					-4	8	100%	17	100%	5	100%	11	100%	11	100%	-4	7	6	57300	5,2E+04	-4	1	3	38000	3,7E+04
	C118	Moules	No	c	-2	>150	>150	>150	>150	>150	>150	>150	21000	1,8E+04	-2	>150	140	19000	1,5E+04	-1	>150	>150	18000	1,6E+04	
					-3	20	100%	22	100%	18	100%	18	100%	18	100%	-3	19	21	19000	1,5E+04	-2	183	164	18000	1,6E+04
	VI 397	Crevettes cuites	No	c	-2	>150	>150	>150	>150	>150	>150	>150	55000	4,5E+04	-2	>150	164	24000	1,6E+04	-2	>150	>150	62000	6,5E+04	
-3					48	100%	61	100%	43	100%	47	100%	47	100%	-3	24	13	24000	1,6E+04	-3	62	65	62000	6,5E+04	
HA 4520	Crevettes impériales	No	c	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10		
CI 141	Parmentière de poisson	No	c	-4	>150	>150	>150	218	100%	260	100%	2100000	2,4E+06	-4	>150	>150	2300000	2,1E+06	-4	>150	384	2600000	3,1E+06		
				-5	24	100%	18	100%	21	100%	27	100%	27	100%	-5	23	21	2300000	2,1E+06	-5	26	36	2600000	3,1E+06	
M 46488	Tarama	No	c	-1	9	100%	9	100%	9	100%	7	100%	90	80	-1	9	12	90	120	-1	12	11	120	110	
				-2	1	100%	1	100%	0	100%	1	100%	1	100%	-2	0	0	90	120	-2	1	1	120	110	
Renewal study	1714572	Saumon fumé	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0		<10		-2	0		<10		
	1714573	Filet de julienne	No	a	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0		<10		-2	0		<10		
	1691991	Filet de merlan	No	a	-1	16	100%						160		-1	12		120		-1	12		110		
					-2	2	100%								-2	1		120		-2	0		110		
	1691993	Filet de saumon	No	a	-2	57	100%						5500		-2	80		8000		-2	93		9600		
					-3	3	100%								-3	8		8000		-3	13		9600		
	1691994	Dos de cabillaud	No	a	-2	25	100%						2500		-2	18		1800		-2	16		1700		
					-3	3	100%								-3	2		1800		-3	3		1700		
	1691996	Maquereau fumé	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0		<10		-2	0		<10		
	1691997	Espadon	No	a	-2	nc	100%						43000		-2	nc		37000		-2	nc		14000		
					-3	43	100%								-3	37		37000		-3	14		14000		
	1691998	Pavé de saumon	No	a	-1	76	100%						750		-1	91		910		-1	80		810		
					-2	7	100%								-2	9		910		-2	9		810		
	1698391	Maquereau fumé poivron	Yes	b	-1	41	100%						420		-1	53		540		-1	58		610		
					-2	5	100%								-2	6		540		-2	9		610		
	1698398	Harengs fumés	Yes	b	-1	53	100%						580		-1	35		380		-1	44		450		
-2					11	100%								-2	7		380		-2	6		450			
1698400	Saumon fumé	Yes	b	-1	54	100%						550		-1	32		310		-1	54		560			
				-2	6	100%								-2	2		310		-2	8		560			
1698401	Anchois marinés	Yes	b	-1	47	100%						480		-1	39		380		-1	42		410			
				-2	6	100%								-2	3		380		-2	3		410			

**Relative trueness study - Vegetables**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)								Dil.	REBECCA + EB - Surface spreading				Dil.	REBECCA + EB - Pour plate					
						R1				R2					R1	R2	R1	R2		R1	R2	R1	R2		
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.		Result	Result	CFU	CFU		Result	Result	CFU	CFU	Result	Result
Initial validation study	Q 2237	Déchets de pâte recyclés	No	c	-3	111	100%	97	100%	96	100%	92	100%	100000	9,3E+04	-3	80	88	76000	8,9E+04	-3	84	75	85000	7,4E+04
					-4	11	100%	9	100%	10	100%	7	100%			-4	4	10			-4	9	6		
	ST 3672	Pommes de terre vapeur persillées	No	c	-1	0		0		0		0		<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0		0		0		0				-2	0	0			-2	0	0		
	VR 4459	Riz créole	No	c	-1	0		0		0		0		<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0		0		0		0				-2	0	0			-2	0	0		
	S 9941	Salade végétarienne	No	c	-6	29	100%	35	100%	25	100%	35	100%	30000000	2,9E+07	-6	21	28	20000000	2,5E+07	-6	14	50	15000000	4,8E+07
					-7	2	100%	1	100%	2	100%	2	100%			-7	1	0			-7	2	3		
	VI 313	Tajine de légumes	No	c	-1	>150		>150		>150		>150		15000	1,3E+04	-2	150	128	14000	1,2E+04	-1	>150	>150	12000	1,3E+04
					-2	149	100%	152	100%	140	100%	119	100%			-3	6	8			-2	124	132		
	VI 279	Concombres en sauce	No	c	-4	>150		>150		>150		>150		3300000	3,0E+06	-4	>150	>150	3100000	3,8E+06	-4	>150	>150	3800000	4,9E+06
					-5	38	100%	28	100%	23	100%	36	100%			-5	31	38			-5	38	49		
VI 413	Thé en feuilles sucré	No	c	-1	0		0		0		0		<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10	
				-2	0		0		0		0				-2	0	0			-2	0	0			
HA 4680	Pain aux raisins	No	c	-1	23	100%	14	100%	26	100%	22	100%	170	230	-2	1	1	100	160	-1	19	16	190	160	
				-2	0		1	100%	0		2	100%			-3	0	0			-2	2	2			
DJ 3361	Salade riz tomates	No	c	-1	8	100%	4	100%	11	100%	6	100%	60	90	-2	1	1	100	160	-1	8	6	80	70	
				-2	0		0		4	100%	1	100%			-3	0	0			-2	1	2			
DJ 3510	Salade tomate maïs	No	c	-1	7	100%	6	100%	11	100%	3	100%	65	70	-1	6	8	60	80	-1	11	7	100	70	
				-2	2	100%	0		1	100%	0				-2	0	0			-2	0	0			
Renewal study	1714582	Basilic frais	No	a	-1	124	100%						1200		-1	nc		3900		-1	72		730		
					-2	11	100%								-2	39				-2	8				
	1714587	Poellée de légumes	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1691985	Menthe	No	a	-6	30	100%						29000000		-6	22		22000000		-6	23		23000000		
					-7	2	100%								-7	2				-7	2				
	1691986	Aneth	No	a	-6	nc	100%						440000000		-6	nc		330000000		-6	nc		410000000		
					-7	44	100%								-7	33				-7	41				
	1692001	Poellée champêtre	No	b	-4	65	100%						660000		-4	39		450000		-4	89		860000		
					-5	8	100%								-5	10				-5	6				
	1692022	Ciboulette	No	a	-4	35	100%						360000		-4	25		270000		-4	40		360000		
					-5	5	100%								-5	5				-5	0				
	1692030	Haricots mungo	No	a	-6	47	100%						47000000		-6	20		19000000		-6	28		28000000		
					-7	5	100%								-7	1				-7	3				
1692031	Mélange de laitue	No	b	-2	70	100%						7700		-2	34		4100		-2	60		6100			
				-3	15	100%								-3	11				-3	7					
1692026	Carottes râpées non assaisonnées	No	b	-5	87	100%						8900000		-5	18		1700000		-5	70		7500000			
				-6	11	100%								-6	1				-6	12					
1692003	Poellée de légumes	No	b	-1	29	100%						300		-1	42		420		-1	43		420			
				-2	4	100%								-2	4				-2	3					
1729344	Mélange mâche betterave	Yes	b	-2	41	100%						4100		-2	56		5600		-2	37		3600			
				-3	4	100%								-3	6				-3	3					

**Feeds**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)								REBECCA + EB - Surface spreading				REBECCA + EB - Pour plate							
						R1				R2				R1	R2	Dil.	R1	R2	R1	R2					
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.	Result	Result		CFU	CFU	Result	Result	CFU	CFU	Result	Result	
Initial validation study	RD 1272	Viande pour animaux	No	c	-3	110	100%	120	100%	115	100%	130	100%	110000	1,2E+05	-3	91	94	97000	9,7E+04	-3	92	90	95000	8,8E+04
					-4	9	100%	12	100%	6	100%	15	100%			-4	16	13			-4	13	7		
	RD 1273	Viande pour animaux	No	c	-3	>150		>150		>150		>150		360000	2,2E+05	-3	>150	>150	280000	2,8E+05	-3	>150	>150	200000	2,3E+05
					-4	35	100%	36	100%	25	100%	19	100%			-4	28	28			-4	20	23		
	P 2743	Farine animale	No	b	-1	26	100%	23	100%	44	100%	36	100%	250	410	-1	28	37	250	370	-1	32	27	320	260
					-2	4	100%	2	100%	5	100%	5	100%			-2	0	0			-2	3	2		
	P 1450	Farine animale	No	b	-1	0		0		0		0		<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0		0		0		0				-2	0	0			-2	0	0		
	P 1451	Farine animale	No	b	-1	0		0		0		0		<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0		0		0		0				-2	0	0			-2	0	0		
	P 2742	Farine animale	No	b	-1	1	100%	3	100%	4	100%	4	100%	20	40	-1	2	3	30	30	-1	3	4	30	40
					-2	0		0		0		0				-2	0	0			-2	0	0		
	RD 1269	Terrine pour chat à l'agneau	Yes	a	-1	14	100%	22	100%	20	100%	12	100%	180	160	-2	1	2	100	200	-1	14	21	130	230
					-2	2	100%	1	100%	5	100%	1	100%			-3	0	0			-2	0	4		
RD 1274	Eminés pour chat bœuf volaille	Yes	a	-2	15	100%	19	100%	13	100%	11	100%	1600	1200	-2	16	19	1600	2100	-2	14	23	1400	2200	
				-3	1	100%	1	100%	5	100%	1	100%			-3	2	4			-3	1	1			
RD 1276	Eminés pour chat canard agneau	Yes	a	-3	9	100%	17	100%	11	100%	3	100%	13000	7000	-3	27	23	25000	2,4E+04	-3	43	35	43000	3,5E+04	
				-4	1	100%	1	100%	0	100%	0	100%			-4	1	3			-4	4	4			
RD 1277	Eminés pour chat lapin foie	Yes	a	-4	10	100%	18	100%	10	100%	4	100%	140000	7,0E+04	-4	20	42	210000	4,4E+05	-4	28	38	310000	3,6E+05	
				-5	2	100%	1	100%	2	100%	1	100%			-5	3	6			-5	6	2			
RD 1275	Eminés pour chat poulet dinde	Yes	a	-2	23	100%	22	100%	29	100%	11	100%	2200	2000	-2	29	24	2800	2400	-2	36	45	3500	4400	
				-3	1	100%	3	100%	0	100%	0	100%			-3	2	2			-3	2	3			
RD 1270	Terrine pour chat truite cabillaud	Yes	a	-1	29	100%	35	100%	34	100%	28	100%	340	310	-1	29	34	330	345	-1	35	28	350	270	
				-2	7	100%	3	100%	4	100%	2	100%			-2	7	4			-2	3	2			
Renewal study	1714583	Granulé d'avoine	No	b	-1	8	100%						80		-1	9		90		-1	9		90		
					-2	0									-2	0				-2	0				
	1714584	Tourteau de colza	No	b	-2	>150							70000		-2	>150		74000		-2	>150		61000		
					-3	70	100%								-3	74				-3	61				
	1692071	Viande crue pour animaux	No	c	-3	>150							1100000		-3	>150		810000		-3	>150		1100000		
					-4	106	100%								-4	81				-4	110				
	1726637	Granulé pour porc	No	c	-1	5	100%						50		-1	9		90		-1	7		70		
					-2	0									-2	0				-2	0				
	1726638	Granulé pour porcelet	No	c	-1	12	100%						120		-1	9		90		-1	10		100		
					-2	1	100%								-2	0				-2	1				
1726639	Soja	No	c	-1	57	100%						560		-1	54		550		-1	61		620			
				-2	5	100%								-2	6				-2	7					
1729342	Aliment poules pondeuses	Yes	b	-2	120	100%						12000		-2	101		10000		-2	108		11000			
				-3	13	100%								-3	9				-3	11					
1729343	Aliment lapins	Yes	b	-1	20	100%						190		-1	14		140		-1	25		250			
				-2	1	100%								-2	1				-2	3					

**Ready-to-eat and ready-to-reheat products**

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)												REBECCA + EB - Surface spreading				REBECCA + EB - Pour plate			
						R1				R2				R1	R2	Dil.	R1	R2	R1	R2	Dil.	R1	R2	R1	R2
						CFU/plate 1	Conf.	CFU/plate 2	Conf.	CFU/plate 1	Conf.	CFU/plate 2	Conf.	Result	Result		CFU	CFU	Result	Result		CFU	CFU	Result	Result
Initial validation study	S 9938	Salade au fromage	No	a	-4	43	100%	40	100%	23	100%	39	100%	400000	3.1E+05	-4	48	37	4600000	3.4E+05	-4	41	39	410000	3.9E+05
	S 9939	Salade poulet asiatique	NC	a	-6	34	100%	60	100%	36	100%	33	100%	46000000	33181818,18	-6	30	40	31000000	39000000	-6	20	26	20000000	3.5E+07
	S 9940	Salade au jambon	NC	a	-5	>150	100%	>150	100%	>150	100%	>150	100%	66000000	74500000	-5	>150	>150	72000000	9.0E+07	-5	>150	>150	50000000	65000000
	DJ 3253	Brioche au porc et crevettes	NC	a	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
	S 9942	Salade océane	No	a	-6	>150	100%	>150	100%	>150	100%	>150	100%	300000000	2.3E+08	-6	>150	>150	200000000	3.1E+08	-6	>150	>150	100000000	2.0E+08
	MV 2234	Taboulé de la mer	No	a	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	S 10132	Salade d'écrevisse	No	a	-2	>150	100%	>150	100%	>150	100%	>150	100%	36000	2.6E+04	-2	>150	>150	57000	4.0E+04	-2	>150	>150	40000	2.2E+04
	MV 2163	Salade tomate mozzarella	No	a	-1	40	100%	34	100%	43	100%	33	100%	370	480	-2	3	2	300	270	-1	28	27	280	260
	S 9937	Salade composée	No	a	-6	18	100%	19	100%	17	100%	7	100%	20000000	1.2E+07	-6	13	45	12000000	4.2E+07	-6	17	28	19000000	2.5E+07
	VR 4497	Salade de pommes de terre	No	a	-3	40	100%	60	100%	59	100%	48	100%	51000	5.2E+04	-3	58	54	55000	5.2E+04	-3	48	57	46000	5.6E+04
Renewal study	1714575	Pizza jambon	No	b	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1714576	Bouchée ris de veau	No	b	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1714578	Potée cuite	No	b	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1714581	Quiche lorraine	No	b	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1698389	Flan brocolis	Yes	b	-1	nc	100%	148	100%	0	100%	0	100%	15000	15000	-1	nc	130	13000	13000	-1	nc	150	15000	15000
	1714577	Magret fumé tranché	No	c	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1698384	Coquille de poisson	Yes	b	-1	7	100%	1	100%	0	100%	0	100%	70	70	-1	4	4	40	40	-1	9	9	90	90
	1692052	Tomate farcie	Yes	b	-1	nc	100%	24	100%	0	100%	0	100%	2400	2400	-1	nc	26	2600	2600	-1	nc	34	3400	3400
	1692056	Tartiflette	Yes	b	-1	6	100%	0	100%	0	100%	0	100%	60	60	-1	3	3	30	30	-1	5	5	50	50
	1698387	Tourte aux épinards	Yes	b	-1	104	100%	10	100%	0	100%	0	100%	1000	1000	-1	65	7	650	650	-1	120	11	1200	1200
	1692059	Quiche aux légumes	Yes	b	-1	2	100%	0	100%	0	100%	0	100%	20	20	-1	3	3	30	30	-1	2	2	20	20
	1692061	Soufflé au chorizo	Yes	b	-1	1	100%	0	100%	0	100%	0	100%	10	10	-1	2	2	20	20	-1	5	5	50	50
	1692068	Bouchée à la reine	Yes	b	-1	3	100%	0	100%	0	100%	0	100%	30	30	-1	4	4	40	40	-1	1	1	10	10
	1698396	Magret de canard fumé	Yes	c	-1	37	100%	4	100%	0	100%	0	100%	370	370	-1	28	3	280	280	-1	44	2	420	420
	1698399	Rollmops	Yes	c	-1	60	100%	6	100%	0	100%	0	100%	600	600	-1	40	4	400	400	-1	60	6	600	600
	1698386	Maquereau fumé	Yes	c	-1	2	100%	0	100%	0	100%	0	100%	20	20	-1	0	0	<10	<10	-1	2	0	20	20
	1698392	Pizza vénitienne	Yes	b	-1	17	100%	1	100%	0	100%	0	100%	160	160	-1	15	1	150	150	-1	20	1	190	190
	1698394	Paëlla	Yes	b	-2	44	100%	4	100%	0	100%	0	100%	4400	4400	-2	33	3	3300	3300	-2	49	5	4900	4900
	1726634	Saumon fumé	No	c	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
	1726635	Hareng fumé	No	c	-1	0	100%	0	100%	0	100%	0	100%	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10
1726636	Saumon mariné	No	c	-2	60	100%	7	100%	0	100%	0	100%	6100	6100	-2	53	8	5500	5500	-2	64	6	6400	6400	
1729337	Saumon fumé	Yes	c	-1	9	100%	0	100%	0	100%	0	100%	90	90	-1	8	8	80	80	-1	9	9	90	90	
1729338	Hareng fumé	Yes	c	-2	77	100%	5	100%	0	100%	0	100%	7500	7500	-2	87	9	8700	8700	-2	66	8	6700	6700	



## **APPENDIX E**

**Relative trueness study**

**Statistical calculations**

## Results summary and statistical calculations

### Surface spreading for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Meat products	a	RG 3243	2,76	2,60	2,68	-0,16
		HA 4490	4,18	4,04	4,11	-0,13
		V 7798	5,75	5,32	5,54	-0,43
		VR 4768	3,65	3,73	3,69	0,08
		VI 585	3,34	3,34	3,34	0,00
		1691980	3,04	3,43	3,24	0,39
		1691981	3,28	3,41	3,35	0,14
		1691982	3,32	3,54	3,43	0,22
		1692011	4,93	4,53	4,73	-0,40
	b	1698388	3,20	3,04	3,12	-0,16
		1714586	2,08	2,20	2,14	0,12
		1698388	2,40	2,54	2,47	0,15
		1698393	3,68	3,72	3,70	0,03
		1729339	2,08	2,11	2,10	0,03
	c	HA 4187	4,04	3,91	3,97	-0,13
		MV 1916	3,57	3,52	3,54	-0,05
		VR 4755	5,15	4,99	5,07	-0,15
		1692018	3,04	3,26	3,15	0,21
		1692070	1,78	1,90	1,84	0,12
		1692058	2,15	2,08	2,11	-0,07
	<b>Average difference of the category</b>					
<b>Standard deviation of differences</b>						<b>0,20</b>
Dairy & egg products	a	M 46205	7,34	7,40	7,37	0,06
		VR 4896	2,97	3,04	3,00	0,07
		RD 1280	3,20	3,18	3,19	-0,03
		RD 1283	5,15	4,60	4,87	-0,54
		1691976	4,84	4,76	4,80	-0,08
		1691977	2,80	2,84	2,82	0,04
		1692013	3,34	3,11	3,23	-0,23
	b	R 36162	4,49	4,56	4,52	0,06
		RD 1278	2,38	2,23	2,31	-0,15
		RD 1279	2,57	2,60	2,59	0,03
		RD 1281	3,49	3,40	3,44	-0,09
		RD 1282	3,98	4,18	4,08	0,20
	c	1714580	3,23	3,40	3,31	0,17
		1691975	4,28	4,72	4,50	0,44
		1691979	2,40	2,54	2,47	0,15
		1691983	7,91	7,90	7,91	-0,02
1692007		5,98	5,88	5,93	-0,10	
1692017	4,72	4,86	4,79	0,13		
<b>Average difference</b>						<b>0,01</b>
<b>Standard deviation of differences</b>						<b>0,20</b>

## Results summary and statistical calculations

### Surface spreading for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Seafood products	a	C 85	4,75	4,76	4,75	0,01
		1691991	2,20	2,08	2,14	-0,12
		1691993	3,74	3,90	3,82	0,16
		1691994	3,40	3,26	3,33	-0,14
		1691997	4,63	4,57	4,60	-0,07
		1691998	2,88	2,96	2,92	0,08
	b	VI 311	6,08	6,18	6,13	0,10
		1698391	2,62	2,73	2,68	0,11
		1698398	2,76	2,58	2,67	-0,18
		1698400	2,74	2,49	2,62	-0,25
		1698401	2,68	2,58	2,63	-0,10
	c	Q 2271	5,53	5,84	5,69	0,31
		RG 3033	2,96	3,18	3,07	0,22
		C118	4,32	4,28	4,30	-0,04
		VI 397	4,74	4,38	4,56	-0,36
		CI 141	6,32	6,36	6,34	0,04
		M 46488	1,95	1,95	1,95	0,00
<b>Average difference</b>						<b>-0,01</b>
<b>Standard deviation of differences</b>						<b>0,17</b>
Vegetal products	a	1714582	3,08	3,59	3,34	0,51
		1691985	7,46	7,34	7,40	-0,12
		1691986	8,64	8,52	8,58	-0,12
		1692022	5,56	5,43	5,49	-0,12
		1692030	7,67	7,28	7,48	-0,39
	b	1692001	5,82	5,65	5,74	-0,17
		1692031	3,89	3,61	3,75	-0,27
		1692026	6,95	6,23	6,59	-0,72
		1692003	2,48	2,62	2,55	0,15
		1729344	3,61	3,75	3,68	0,14
	c	Q 2237	5,00	4,88	4,94	-0,12
		S 9941	7,48	7,30	7,39	-0,18
		VI 313	4,18	4,15	4,16	-0,03
		VI 279	6,52	6,49	6,50	-0,03
		DJ 3510	1,81	1,78	1,80	-0,03
<b>Average difference</b>						<b>-0,10</b>
<b>Standard deviation of differences</b>						<b>0,27</b>

## Results summary and statistical calculations

### Surface spreading for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference	
Feed products	a	RD 1274	3,20	3,20	3,20	0,00	
		RD 1276	4,11	4,40	4,26	0,28	
		RD 1277	5,15	5,32	5,23	0,18	
		RD 1275	3,34	3,45	3,39	0,10	
		RD 1270	2,53	2,52	2,52	-0,01	
	b	P 2743	2,40	2,40	2,40	0,00	
		1714583	1,90	1,95	1,93	0,05	
		1714584	4,85	4,87	4,86	0,02	
		1729342	4,08	4,00	4,04	-0,08	
		1729343	2,28	2,15	2,21	-0,13	
	c	RD 1272	5,04	4,99	5,01	-0,05	
		RD 1273	5,56	5,45	5,50	-0,11	
		1692071	6,04	5,91	5,97	-0,13	
		1726637	1,70	1,95	1,83	0,26	
		1726638	2,08	1,95	2,02	-0,12	
		1726639	2,75	2,74	2,74	-0,01	
	<b>Average difference</b>						<b>0,02</b>
	<b>Standard deviation of differences</b>						<b>0,13</b>
Ready-to-eat & ready-to-reheat products	a	S 9938	5,60	5,66	5,63	0,06	
		S 9939	7,66	7,49	7,58	-0,17	
		S 9940	7,82	7,86	7,84	0,04	
		S 9942	8,48	8,30	8,39	-0,18	
		S 10132	4,56	4,76	4,66	0,20	
		S 9937	7,30	7,08	7,19	-0,22	
		VR 4497	4,71	4,74	4,72	0,03	
	b	1698389	4,18	4,11	4,15	-0,06	
		1698384	1,85	1,60	1,72	-0,24	
		1692052	3,38	3,41	3,40	0,03	
		1698387	3,00	2,81	2,91	-0,19	
		1698392	2,20	2,18	2,19	-0,03	
	c	1698394	3,64	3,52	3,58	-0,12	
		1698396	2,57	2,45	2,51	-0,12	
		1698399	2,78	2,60	2,69	-0,18	
		1726636	3,79	3,74	3,76	-0,04	
	1729337	1,95	1,90	1,93	-0,05		
	1729338	3,88	3,94	3,91	0,06		
<b>Average difference</b>						<b>-0,07</b>	
<b>Standard deviation of differences</b>						<b>0,12</b>	

## Results summary and statistical calculations

### Surface spreading for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Average difference all categories						-0,03
Standard deviation of differences						0,19

n = 104

$\beta$  = 95%

$T(0.025;97)=$  1,98

Lower confidence limit	Upper confidence limit
-0,40	0,35

### Data not used for the calculations

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
MP	1692051	b	1,30	1,60	1,45	0,30
	1692057	b	1,60	1,30	1,45	-0,30
	1692065	c	1,30	1,30	1,30	0,00
DP	1692054	a	1,70	1,00	1,35	-0,70
	1691984	c	1,00	1,00	1,00	0,00
VP	HA 4680	c	2,23	2,00	2,12	-0,23
	DJ 3361	c	1,78	2,00	1,89	0,22
FP	RD 1269	a	2,26	2,00	2,13	-0,26
	P 2742	b	1,30	1,48	1,39	0,18
RTE	MV 2163	a	2,57	2,48	2,52	-0,09
	1692056	b	1,78	1,48	1,63	-0,30
	1692059	b	1,30	1,48	1,39	0,18
	1692061	b	1,00	1,30	1,15	0,30
	1692068	b	1,48	1,60	1,54	0,12
	1698386	c	1,30	0,00	0,65	-1,30

## Results summary and statistical calculations

### Pour plates for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Meat products	a	RG 3243	2,76	2,74	2,75	-0,02
		HA 4490	4,18	4,00	4,09	-0,18
		V 7798	5,75	5,65	5,70	-0,09
		VR 4768	3,65	3,66	3,66	0,01
		VI 585	3,34	3,30	3,32	-0,04
		1691980	3,04	3,34	3,19	0,30
		1691981	3,28	3,11	3,20	-0,16
		1691982	3,32	3,56	3,44	0,23
	1692011	4,93	4,97	4,95	0,04	
	b	1698388	3,20	3,20	3,20	0,00
		1714586	2,08	2,00	2,04	-0,08
		1698388	2,40	2,63	2,52	0,24
		1698393	3,68	3,79	3,73	0,10
		1729339	2,08	2,18	2,13	0,10
	c	HA 4187	4,04	3,79	3,92	-0,25
		MV 1916	3,57	3,54	3,56	-0,02
		VR 4755	5,15	4,92	5,04	-0,22
		1692018	3,04	3,23	3,14	0,19
		1692070	1,78	1,85	1,81	0,07
		1692058	2,15	2,23	2,19	0,08
<b>Average difference of the category</b>						<b>0,01</b>
<b>Standard deviation of differences</b>						<b>0,15</b>
Dairy & egg products	a	M 46205	7,34	7,46	7,40	0,12
		VR 4896	2,97	3,04	3,00	0,07
		RD 1280	3,20	3,40	3,30	0,19
		RD 1283	5,15	5,26	5,20	0,11
		1691976	4,84	4,41	4,63	-0,42
		1691977	2,80	2,83	2,82	0,03
		1692013	3,34	3,26	3,30	-0,09
	b	R 36162	4,49	4,54	4,52	0,05
		RD 1278	2,38	2,40	2,39	0,02
		RD 1279	2,57	2,64	2,61	0,08
		RD 1281	3,49	3,68	3,59	0,19
		RD 1282	3,98	4,46	4,22	0,48
	c	1714580	3,23	3,04	3,14	-0,19
		1691975	4,28	4,76	4,52	0,48
		1691979	2,40	2,59	2,49	0,19
		1691983	7,91	7,81	7,86	-0,10
1692007		5,98	6,23	6,10	0,25	
1692017	4,72	4,97	4,85	0,25		
<b>Average difference</b>						<b>0,10</b>
<b>Standard deviation of differences</b>						<b>0,22</b>

## Results summary and statistical calculations

### Pour plates for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Seafood products	a	C 85	4,75	4,58	4,66	-0,17
		1691991	2,20	2,04	2,12	-0,16
		1691993	3,74	3,98	3,86	0,24
		1691994	3,40	3,23	3,31	-0,17
		1691997	4,63	4,15	4,39	-0,49
		1691998	2,88	2,91	2,89	0,03
	b	VI 311	6,08	6,18	6,13	0,10
		1698391	2,62	2,79	2,70	0,16
		1698398	2,76	2,65	2,71	-0,11
		1698400	2,74	2,75	2,74	0,01
		1698401	2,68	2,61	2,65	-0,07
	c	Q 2271	5,53	5,68	5,61	0,15
		RG 3033	2,96	2,99	2,98	0,03
		C118	4,32	4,26	4,29	-0,07
		VI 397	4,74	4,79	4,77	0,05
		CI 141	6,32	6,41	6,37	0,09
		M 46488	1,95	2,08	2,02	0,12
<b>Average difference</b>						<b>-0,01</b>
<b>Standard deviation of differences</b>						<b>0,17</b>
Vegetal products	a	1714582	3,08	2,86	2,97	-0,22
		1691985	7,46	7,36	7,41	-0,10
		1691986	8,64	8,61	8,63	-0,03
		1692022	5,56	5,56	5,56	0,00
		1692030	7,67	7,45	7,56	-0,22
	b	1692001	5,82	5,93	5,88	0,11
		1692031	3,89	3,79	3,84	-0,10
		1692026	6,95	6,88	6,91	-0,07
		1692003	2,48	2,62	2,55	0,15
		1729344	3,61	3,56	3,58	-0,06
	c	Q 2237	5,00	4,93	4,96	-0,07
		S 9941	7,48	7,18	7,33	-0,30
		VI 313	4,18	4,08	4,13	-0,10
		VI 279	6,52	6,58	6,55	0,06
		HA 4680	2,23	2,28	2,25	0,05
DJ 3361		1,78	1,90	1,84	0,12	
DJ 3510	1,81	2,00	1,91	0,19		
<b>Average difference</b>						<b>-0,03</b>
<b>Standard deviation of differences</b>						<b>0,14</b>

## Results summary and statistical calculations

### Pour plates for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Feed products	a	RD 1269	2,26	2,11	2,18	-0,14
		RD 1274	3,20	3,15	3,18	-0,06
		RD 1276	4,11	4,63	4,37	0,52
		RD 1277	5,15	5,49	5,32	0,35
		RD 1275	3,34	3,54	3,44	0,20
		RD 1270	2,53	2,54	2,54	0,01
	b	P 2743	2,40	2,51	2,45	0,11
		1714583	1,90	1,95	1,93	0,05
		1714584	4,85	4,79	4,82	-0,06
		1729342	4,08	4,04	4,06	-0,04
		1729343	2,28	2,40	2,34	0,12
	c	RD 1272	5,04	4,98	5,01	-0,06
		RD 1273	5,56	5,30	5,43	-0,26
		1692071	6,04	6,04	6,04	0,00
		1726637	1,70	1,85	1,77	0,15
		1726638	2,08	2,00	2,04	-0,08
		1726639	2,75	2,79	2,77	0,04
	<b>Average difference</b>					
<b>Standard deviation of differences</b>						<b>0,18</b>
Ready-to-eat & ready-to-reheat products	a	S 9938	5,60	5,61	5,61	0,01
		S 9939	7,66	7,30	7,48	-0,36
		S 9940	7,82	7,70	7,76	-0,12
		S 9942	8,48	8,00	8,24	-0,48
		S 10132	4,56	4,60	4,58	0,05
		MV 2163	2,57	2,45	2,51	-0,12
		S 9937	7,30	7,28	7,29	-0,02
		VR 4497	4,71	4,66	4,69	-0,04
	b	1698389	4,18	4,18	4,18	0,00
		1698384	1,85	1,95	1,90	0,11
		1692052	3,38	3,53	3,46	0,15
		1692056	1,78	1,70	1,74	-0,08
		1698387	3,00	3,08	3,04	0,08
		1698392	2,20	2,28	2,24	0,07
		1698394	3,64	3,69	3,67	0,05
	c	1698396	2,57	2,62	2,60	0,06
		1698399	2,78	2,78	2,78	0,00
		1726636	3,79	3,81	3,80	0,02
		1729337	1,95	1,95	1,95	0,00
		1729338	3,88	3,83	3,85	-0,05
<b>Average difference</b>						<b>-0,03</b>
<b>Standard deviation of differences</b>						<b>0,15</b>



## Results summary and statistical calculations

### Pour plates for REBECCA+EB

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
Average difference all categories						<b>0,01</b>
Standard deviation of differences						<b>0,17</b>

n = 109

$\beta$  = 95%

$T(0.025;97)=$  1,98

Lower confidence limit	Upper confidence limit
-0,33	0,36

### Data not used for the calculations

Category	Type	Sample code	Reference method	Alternative method	Mean	Difference
MP	1692051	b	1,30	1,30	1,30	0,00
	1692057	b	1,60	1,00	1,30	-0,60
	1692065	c	1,30	0,00	0,65	-1,30
DP	1692054	a	1,70	1,48	1,59	-0,22
	1691984	c	1,00	1,60	1,30	0,60
FP	P 2742	b	1,30	1,48	1,39	0,18
RTE	1692059	b	1,30	1,30	1,30	0,00
	1692061	b	1,00	1,70	1,35	0,70
	1692068	b	1,48	1,00	1,24	-0,48
	1698386	c	1,30	1,30	1,30	0,00

## APPENDIX F

### Accuracy profile study

#### Raw results

**Key:**

 Estimated number

**ACCURACY PROFILE - Meat products**

Matrix: Ground beef

Strain: *Citrobacter youngae*, RAX819A

Enumeration of the microorganisms at 30°C - batch 1: 840 000 CFU/g

Enumeration of the microorganisms at 30°C - batch 2: 870 000 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	20	-2	2	100%	200	-1	13	-2	2	140	-1	16	-2	5	190
		R2	-1	22	-2	1	100%	210	-1	10	-2	1	100	-1	19	-2	0	170
		R3	-1	29	-2	2	100%	280	-1	21	-2	2	210	-1	17	-2	3	180
		R4	-1	18	-2	2	100%	180	-1	12	-2	4	150	-1	23	-2	2	230
		R5	-1	27	-2	1	100%	260	-1	21	-2	2	210	-1	16	-2	0	150
	Batch 2	R1	-1	17	-2	1	100%	160	-1	14	-2	2	150	-1	11	-2	2	120
		R2	-1	16	-2	2	100%	160	-1	14	-2	2	150	-1	14	-2	1	140
		R3	-1	14	-2	2	100%	150	-1	10	-2	4	130	-1	14	-2	0	130
		R4	-1	11	-2	2	100%	120	-1	18	-2	3	190	-1	10	-2	0	100
		R5	-1	11	-2	1	100%	110	-1	14	-2	0	130	-1	16	-2	1	160
Level 2	Batch 1	R1	-2	55	-3	4	100%	5400	-2	43	-3	2	4100	-2	32	-3	5	3400
		R2	-2	51	-3	5	100%	5100	-2	45	-3	8	4800	-2	35	-3	3	3500
		R3	-2	49	-3	2	100%	4600	-2	43	-3	5	4400	-2	34	-3	4	3500
		R4	-2	40	-3	5	100%	4100	-2	46	-3	2	4400	-2	35	-3	3	3500
		R5	-2	47	-3	2	100%	4400	-2	40	-3	3	3900	-2	31	-3	3	3100
	Batch 2	R1	-2	36	-3	6	100%	3800	-2	44	-3	3	4300	-2	36	-3	2	3400
		R2	-2	41	-3	4	100%	4100	-2	48	-3	7	5000	-2	34	-3	4	3500
		R3	-2	35	-3	3	100%	3400	-2	38	-3	8	4200	-2	34	-3	4	3500
		R4	-2	31	-3	5	100%	3300	-2	50	-3	5	5000	-2	31	-3	9	3600
		R5	-2	37	-3	1	100%	3400	-2	35	-3	1	3300	-2	27	-3	2	2600
Level 3	Batch 1	R1	-5	11	-6	1	100%	1100000	-5	14	-6	0	1300000	-5	4	-6	1	400000
		R2	-5	7	-6	1	100%	700000	-5	12	-6	2	1300000	-5	5	-6	0	500000
		R3	-5	7	-6	0	100%	700000	-5	8	-6	0	800000	-5	6	-6	0	600000
		R4	-5	7	-6	0	100%	700000	-5	6	-6	0	600000	-5	5	-6	0	500000
		R5	-5	6	-6	0	100%	600000	-5	8	-6	1	800000	-5	5	-6	0	500000
	Batch 2	R1	-5	11	-6	2	100%	1200000	-5	12	-6	1	1200000	-5	4	-6	1	400000
		R2	-5	13	-6	1	100%	1300000	-5	13	-6	0	1200000	-5	6	-6	0	600000
		R3	-5	9	-6	1	100%	900000	-5	4	-6	0	400000	-5	7	-6	0	700000
		R4	-5	6	-6	3	100%	600000	-5	7	-6	0	700000	-5	8	-6	1	800000
		R5	-5	11	-6	0	100%	1000000	-5	4	-6	0	400000	-5	5	-6	0	500000

**ACCURACY PROFILE - Dairy products**

Matrix: Raw milk cheese

Strain: *Hafnia alvei*, BEY899

Enumeration of the microorganisms at 30°C - batch 1: 1700 CFU/g

Enumeration of the microorganisms at 30°C - batch 2: 2000 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	18	-2	2	100%	180	-1	20	-2	1	190	-1	17	-2	1	160
		R2	-1	14	-2	1	100%	140	-1	18	-2	1	170	-1	14	-2	1	140
		R3	-1	18	-2	1	100%	170	-1	17	-2	1	160	-1	16	-2	1	150
		R4	-1	19	-2	2	100%	190	-1	18	-2	2	180	-1	15	-2	1	140
		R5	-1	16	-2	1	100%	150	-1	20	-2	1	190	-1	17	-2	1	160
	Batch 2	R1	-1	18	-2	2	100%	180	-1	17	-2	1	160	-1	19	-2	2	190
		R2	-1	17	-2	1	100%	160	-1	16	-2	1	150	-1	16	-2	1	150
		R3	-1	16	-2	1	100%	150	-1	19	-2	1	180	-1	18	-2	1	170
		R4	-1	19	-2	2	100%	190	-1	20	-2	1	190	-1	17	-2	1	160
		R5	-1	17	-2	1	100%	160	-1	17	-2	1	160	-1	19	-2	1	180
Level 2	Batch 1	R1	-3	13	-4	1	100%	13000	-3	10	-4	0	10000	-3	8	-4	1	8000
		R2	-3	9	-4	1	100%	9000	-3	9	-4	1	9000	-3	10	-4	1	10000
		R3	-3	12	-4	1	100%	12000	-3	12	-4	1	12000	-3	11	-4	1	11000
		R4	-3	10	-4	1	100%	10000	-3	10	-4	1	10000	-3	9	-4	0	9000
		R5	-3	9	-4	1	100%	9000	-3	12	-4	1	12000	-3	8	-4	1	8000
	Batch 2	R1	-3	12	-4	1	100%	12000	-3	8	-4	0	8000	-3	9	-4	1	9000
		R2	-3	11	-4	1	100%	11000	-3	9	-4	1	9000	-3	10	-4	1	10000
		R3	-3	8	-4	1	100%	8200	-3	12	-4	1	12000	-3	10	-4	0	10000
		R4	-3	10	-4	1	100%	10000	-3	11	-4	0	10000	-3	8	-4	1	8000
		R5	-3	9	-4	1	100%	9000	-3	10	-4	1	10000	-3	9	-4	1	9000
Level 3	Batch 1	R1	-5	12	-6	1	100%	1200000	-5	9	-6	1	900000	-5	8	-6	0	800000
		R2	-5	10	-6	1	100%	1000000	-5	8	-6	1	800000	-5	9	-6	0	900000
		R3	-5	11	-6	1	100%	1100000	-5	10	-6	0	900000	-5	8	-6	1	800000
		R4	-5	12	-6	1	100%	1200000	-5	10	-6	1	1000000	-5	10	-6	0	900000
		R5	-5	9	-6	0	100%	900000	-5	11	-6	1	1100000	-5	8	-6	0	800000
	Batch 2	R1	-5	10	-6	1	100%	1000000	-5	11	-6	0	1100000	-5	9	-6	1	910000
		R2	-5	9	-6	0	100%	900000	-5	8	-6	0	800000	-5	8	-6	0	800000
		R3	-5	14	-6	1	100%	1400000	-5	9	-6	1	900000	-5	7	-6	0	700000
		R4	-5	15	-6	0	100%	1400000	-5	10	-6	0	900000	-5	8	-6	1	800000
		R5	-5	14	-6	1	100%	1400000	-5	11	-6	1	1100000	-5	9	-6	0	900000

**ACCURACY PROFILE - Seafood products**

Matrix: Raw fish fillet

Strain: *Klebsiella oxytoca*, CGR888

Enumeration of the microorganisms at 30°C - batch 1: 1200 CFU/g

Enumeration of the microorganisms at 30°C - batch 2: 1300 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	30	-2	7	100%	340	-1	26	-2	1	250	-1	24	-2	1	230
		R2	-1	27	-2	1	100%	260	-1	32	-2	2	310	-1	21	-2	3	220
		R3	-1	30	-2	4	100%	310	-1	33	-2	2	320	-1	34	-2	2	330
		R4	-1	33	-2	1	100%	310	-1	30	-2	1	280	-1	33	-2	4	340
		R5	-1	39	-2	1	100%	360	-1	27	-2	4	280	-1	22	-2	2	220
	Batch 2	R1	-1	25	-2	7	100%	290	-1	33	-2	3	330	-1	30	-2	1	280
		R2	-1	34	-2	3	100%	340	-1	34	-2	4	350	-1	24	-2	4	260
		R3	-1	36	-2	4	100%	360	-1	25	-2	2	250	-1	30	-2	1	280
		R4	-1	24	-2	4	100%	260	-1	40	-2	1	370	-1	33	-2	5	350
		R5	-1	31	-2	3	100%	310	-1	25	-2	5	270	-1	31	-2	1	290
Level 2	Batch 1	R1	-2	104	-3	14	100%	10700	-2	124	-3	11	12300	-2	108	-3	11	10800
		R2	-2	111	-3	3	100%	10400	-2	121	-3	8	11700	-2	117	-3	20	12400
		R3	-2	109	-3	13	100%	11100	-2	89	-3	14	9400	-2	123	-3	13	13600
		R4	-2	91	-3	12	100%	9400	-2	90	-3	11	9200	-2	93	-3	7	9000
		R5	-2	93	-3	11	100%	9500	-2	125	-3	11	12400	-2	84	-3	5	8100
	Batch 2	R1	-2	90	-3	8	100%	8900	-2	117	-3	15	12000	-2	122	-3	9	12000
		R2	-2	99	-3	14	100%	10300	-2	100	-3	13	10300	-2	103	-3	10	10300
		R3	-2	108	-3	15	100%	11200	-2	96	-3	10	9600	-2	82	-3	8	8200
		R4	-2	94	-3	8	100%	9400	-2	124	-3	14	12500	-2	99	-3	10	9900
		R5	-2	102	-3	11	100%	10300	-2	100	-3	15	10500	-2	107	-3	8	11500
Level 3	Batch 1	R1	-5	10	-6	0	100%	900000	-5	9	-6	2	900000	-5	5	-6	0	500000
		R2	-5	6	-6	0	100%	600000	-5	7	-6	0	700000	-5	7	-6	0	700000
		R3	-5	14	-6	0	100%	1300000	-5	6	-6	2	600000	-5	6	-6	0	600000
		R4	-5	11	-6	1	100%	1100000	-5	10	-6	1	1000000	-5	10	-6	0	1000000
		R5	-5	8	-6	2	100%	800000	-5	6	-6	3	600000	-5	8	-6	0	800000
	Batch 2	R1	-5	9	-6	0	100%	900000	-5	7	-6	0	700000	-5	9	-6	1	900000
		R2	-5	6	-6	0	100%	600000	-5	6	-6	1	600000	-5	8	-6	0	800000
		R3	-5	6	-6	0	100%	600000	-5	5	-6	1	500000	-5	6	-6	0	600000
		R4	-5	9	-6	2	100%	900000	-5	7	-6	0	700000	-5	5	-6	0	500000
		R5	-5	6	-6	0	100%	600000	-5	7	-6	0	700000	-5	7	-6	0	700000

**ACCURACY PROFILE - Vegetal products**

Matrix: Frozen vegetables pan

Strain: *Serratia liquefaciens*, AGL470

Enumeration of the microorganisms at 30°C - batch 1: 4200 CFU/g

Enumeration of the microorganisms at 30°C - batch 2: 7800 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	38	-2	3	100%	370	-1	38	-2	2	360	-1	31	-2	3	310
		R2	-1	33	-2	4	100%	340	-1	39	-2	3	380	-1	38	-2	4	380
		R3	-1	39	-2	4	100%	390	-1	37	-2	3	360	-1	29	-2	3	290
		R4	-1	30	-2	3	100%	300	-1	33	-2	3	330	-1	27	-2	3	270
		R5	-1	35	-2	4	100%	350	-1	38	-2	2	360	-1	30	-2	3	300
	Batch 2	R1	-1	38	-2	4	100%	380	-1	34	-2	2	330	-1	26	-2	3	260
		R2	-1	35	-2	2	100%	340	-1	32	-2	4	330	-1	30	-2	2	290
		R3	-1	32	-2	2	100%	310	-1	35	-2	4	350	-1	34	-2	2	330
		R4	-1	39	-2	2	100%	370	-1	32	-2	5	340	-1	27	-2	4	280
		R5	-1	36	-2	4	100%	360	-1	34	-2	2	330	-1	31	-2	3	310
Level 2	Batch 1	R1	-3	22	-4	1	100%	21000	-3	35	-4	2	34000	-3	18	-4	2	18000
		R2	-3	26	-4	0	100%	24000	-3	28	-4	4	29000	-3	18	-4	1	17000
		R3	-3	25	-4	2	100%	24000	-3	11	-4	1	11000	-3	15	-4	0	14000
		R4	-3	21	-4	2	100%	21000	-3	18	-4	3	19000	-3	13	-4	1	13000
		R5	-3	23	-4	1	100%	22000	-3	27	-4	2	26000	-3	14	-4	1	14000
	Batch 2	R1	-3	23	-4	3	100%	24000	-3	20	-4	2	20000	-3	20	-4	1	19000
		R2	-3	12	-4	1	100%	12000	-3	19	-4	2	19000	-3	19	-4	1	18000
		R3	-3	27	-4	1	100%	25000	-3	28	-4	4	29000	-3	12	-4	2	13000
		R4	-3	29	-4	2	100%	28000	-3	29	-4	2	28000	-3	17	-4	1	17000
		R5	-3	20	-4	2	100%	20000	-3	25	-4	0	23000	-3	13	-4	0	12000
Level 3	Batch 1	R1	-5	21	-6	2	100%	2100000	-5	41	-6	4	4100000	-5	17	-6	2	1700000
		R2	-5	23	-6	1	100%	2200000	-5	32	-6	5	3400000	-5	21	-6	1	2000000
		R3	-5	20	-6	2	100%	2000000	-5	27	-6	1	2500000	-5	20	-6	3	2100000
		R4	-5	29	-6	1	100%	2700000	-5	34	-6	2	3300000	-5	17	-6	1	1600000
		R5	-5	27	-6	0	100%	2400000	-5	38	-6	5	3900000	-5	22	-6	0	2000000
	Batch 2	R1	-5	19	-6	0	100%	1700000	-5	26	-6	3	2600000	-5	25	-6	1	2400000
		R2	-5	22	-6	3	100%	2300000	-5	11	-6	2	1200000	-5	10	-6	4	1300000
		R3	-5	26	-6	1	100%	2400000	-5	32	-6	3	3200000	-5	10	-6	1	1000000
		R4	-5	26	-6	8	100%	2900000	-5	27	-6	2	2600000	-5	18	-6	3	1900000
		R5	-5	11	-6	2	100%	1200000	-5	30	-6	1	2800000	-5	17	-6	5	2000000

**ACCURACY PROFILE - Feed products**

Matrix: Cat kibbles

Strain: *Enterobacter cloacae*, EBJ453

Enumeration of the microorganisms at 30°C - batch 1: 140 CFU/g

Enumeration of the microorganisms at 30°C - batch 2: 120 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	14	-2	0	100%	130	-1	24	-2	1	230	-1	11	-2	0	100
		R2	-1	16	-2	1	100%	160	-1	14	-2	0	130	-1	9	-2	0	90
		R3	-1	14	-2	4	100%	160	-1	16	-2	4	180	-1	10	-2	0	100
		R4	-1	17	-2	2	100%	170	-1	20	-2	3	210	-1	9	-2	0	90
		R5	-1	18	-2	0	100%	180	-1	17	-2	3	180	-1	10	-2	1	100
	Batch 2	R1	-1	18	-2	3	100%	190	-1	28	-2	0	260	-1	9	-2	0	90
		R2	-1	16	-2	0	100%	150	-1	17	-2	1	160	-1	9	-2	1	90
		R3	-1	10	-2	1	100%	100	-1	14	-2	1	140	-1	11	-2	3	110
		R4	-1	19	-2	2	100%	190	-1	18	-2	0	160	-1	9	-2	0	90
		R5	-1	17	-2	1	100%	170	-1	19	-2	1	180	-1	12	-2	1	120
Level 2	Batch 1	R1	-2	64	-3	2	100%	6000	-2	80	-3	10	8200	-2	41	-3	2	3900
		R2	-2	51	-3	3	100%	4900	-2	80	-3	4	7600	-2	43	-3	1	4000
		R3	-2	52	-3	7	100%	5400	-2	65	-3	13	7100	-2	37	-3	5	3800
		R4	-2	60	-3	7	100%	6100	-2	70	-3	4	6700	-2	33	-3	5	3500
		R5	-2	55	-3	7	100%	5600	-2	53	-3	5	5300	-2	30	-3	0	2700
	Batch 2	R1	-2	60	-3	9	100%	6300	-2	66	-3	5	6500	-2	40	-3	2	3800
		R2	-2	55	-3	9	100%	5800	-2	69	-3	12	7400	-2	33	-3	6	3600
		R3	-2	60	-3	6	100%	6000	-2	68	-3	6	6700	-2	18	-3	6	2200
		R4	-2	62	-3	8	100%	6400	-2	62	-3	6	6200	-2	42	-3	5	4300
		R5	-2	59	-3	6	100%	5900	-2	51	-3	6	5200	-2	38	-3	1	3600
Level 3	Batch 1	R1	-5	8	-6	4	100%	800000	-5	15	-6	2	1500000	-5	4	-6	0	400000
		R2	-5	9	-6	0	100%	900000	-5	8	-6	0	800000	-5	7	-6	0	700000
		R3	-5	8	-6	1	100%	800000	-5	12	-6	0	1100000	-5	8	-6	0	800000
		R4	-5	4	-6	1	100%	400000	-5	7	-6	0	700000	-5	6	-6	0	600000
		R5	-5	7	-6	2	100%	700000	-5	6	-6	1	600000	-5	4	-6	0	400000
	Batch 2	R1	-5	9	-6	1	100%	900000	-5	6	-6	2	600000	-5	6	-6	2	600000
		R2	-5	8	-6	0	100%	800000	-5	16	-6	1	1600000	-5	5	-6	1	500000
		R3	-5	7	-6	0	100%	700000	-5	4	-6	0	400000	-5	6	-6	0	600000
		R4	-5	6	-6	0	100%	600000	-5	8	-6	1	800000	-5	9	-6	0	900000
		R5	-5	4	-6	1	100%	400000	-5	6	-6	0	600000	-5	4	-6	0	400000

**ACCURACY PROFILE - Ready-to-eat and ready-to-reheat products**

Matrix: Quiche lorraine

Strain: *Escherichia coli*, UBS981

Enumeration of the microorganisms at 30°C - | 2500 CFU/g

Enumeration of the microorganisms at 30°C - | 2200 CFU/g

Level	Batch	R.	EN ISO 21528-2 (■)					REBECCA+EB - Pour plates					REBECCA+EB - Surface spreading					
			Dil. 1	CFU	Dil. 2	CFU	Conf.	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)	Dil. 1	CFU	Dil. 2	CFU	Result (CFU/g)
Level 1	Batch 1	R1	-1	18	-2	2	100%	180	-1	24	-2	2	240	-1	15	-2	2	150
		R2	-1	19	-2	2	100%	190	-1	20	-2	3	210	-1	19	-2	2	190
		R3	-1	20	-2	3	100%	210	-1	10	-2	2	110	-1	27	-2	3	270
		R4	-1	15	-2	1	100%	150	-1	25	-2	1	240	-1	30	-2	3	300
		R5	-1	16	-2	1	100%	160	-1	13	-2	3	150	-1	20	-2	3	210
	Batch 2	R1	-1	13	-2	1	100%	130	-1	23	-2	1	220	-1	15	-2	5	180
		R2	-1	19	-2	5	100%	220	-1	21	-2	6	250	-1	19	-2	3	200
		R3	-1	13	-2	1	100%	130	-1	16	-2	2	160	-1	25	-2	3	250
		R4	-1	15	-2	2	100%	150	-1	20	-2	3	210	-1	19	-2	2	190
		R5	-1	18	-2	2	100%	180	-1	20	-2	1	190	-1	21	-2	1	200
Level 2	Batch 1	R1	-2	53	-3	4	100%	5200	-2	49	-3	7	5100	-2	53	-3	11	5800
		R2	-2	48	-3	5	100%	4800	-2	48	-3	8	5100	-2	41	-3	9	4600
		R3	-2	66	-3	8	100%	6700	-2	51	-3	6	5200	-2	38	-3	7	4100
		R4	-2	46	-3	8	100%	4900	-2	50	-3	6	5100	-2	44	-3	8	4700
		R5	-2	41	-3	7	100%	4400	-2	47	-3	7	4900	-2	50	-3	7	5200
	Batch 2	R1	-2	41	-3	5	100%	4200	-2	60	-3	7	6100	-2	51	-3	10	5600
		R2	-2	44	-3	3	100%	4300	-2	38	-3	6	4000	-2	49	-3	5	4900
		R3	-2	41	-3	7	100%	4400	-2	50	-3	7	5200	-2	48	-3	9	5200
		R4	-2	45	-3	8	100%	4800	-2	53	-3	6	5400	-2	50	-3	6	5100
		R5	-2	31	-3	5	100%	3300	-2	48	-3	6	4900	-2	61	-3	12	6600
Level 3	Batch 1	R1	-5	9	-6	1	100%	900000	-5	10	-6	2	1100000	-5	5	-6	0	500000
		R2	-5	8	-6	0	100%	800000	-5	8	-6	1	800000	-5	12	-6	1	1200000
		R3	-5	6	-6	1	100%	600000	-5	5	-6	0	500000	-5	9	-6	1	900000
		R4	-5	6	-6	1	100%	600000	-5	8	-6	0	800000	-5	6	-6	2	600000
		R5	-5	5	-6	1	100%	500000	-5	9	-6	1	900000	-5	13	-6	1	1300000
	Batch 2	R1	-5	5	-6	1	100%	500000	-5	6	-6	1	600000	-5	6	-6	0	600000
		R2	-5	6	-6	2	100%	600000	-5	9	-6	0	900000	-5	6	-6	1	600000
		R3	-5	9	-6	1	100%	900000	-5	14	-6	0	1300000	-5	8	-6	2	800000
		R4	-5	8	-6	1	100%	800000	-5	10	-6	3	1200000	-5	7	-6	0	700000
		R5	-5	9	-6	0	100%	900000	-5	10	-6	1	1000000	-5	11	-6	1	1100000



## APPENDIX G

### Inclusivité - Résultats bruts

#	Code	Souche	Origine	REBECCA+EB		EN ISO 21528-2	
				Replicate 1	Replicate 2	Replicate 1	Replicate 2
1	R35	<i>Citrobacter freundii</i>	CIP 53.62	21	38	43	30
2	R40	<i>Citrobacter freundii</i>	ATCC 8090	27	38	69	63
3	R2	<i>Citrobacter koserii</i>	CIP 72.11	67	70	64	48
4	I25	<i>Enterobacter aerogenes</i>	Industrie laitière	53	61	58	64
5	R8	<i>Enterobacter aerogenes</i>	CIP 60.86 T	41	47	49	38
6	R67	<i>Enterobacter cloacae</i>	CIP 60 85	26	42	51	50
7	I37	<i>Enterobacter sakazakii</i>	Poudre de lait	15	12	31	30
8	R123	<i>Enterobacter sakazakii</i>	CIP 103183	86	79	126	125
9	I2	<i>Escherichia coli</i>	Carottes râpées	101	119	63	59
10	I23	<i>Escherichia coli</i>	Industrie laitière	33	43	31	37
11	R3	<i>Escherichia coli</i>	CIP 54.127	35	41	43	35
12	R74	<i>Escherichia coli</i>	ATCC 8739	56	48	52	49
13	R82	<i>Escherichia hermanii</i>	CIP 103176	36	35	51	45
14	I3	<i>Hafnia alvei</i>	Taboulé	29	33	58	53
15	R14	<i>Hafnia alvei</i>	CNRZ 713	31	37	7	17
16	I17	<i>Klebsiella oxytoca</i>	Salade soja	38	46	53	53
17	I6	<i>Klebsiella pneumoniae</i>	Pâtisserie	60	78	58	56
18	R60	<i>Klebsiella pneumoniae</i>	CIP 82.91	16	17	49	34
19	R121	<i>Pantoea agglomerans</i>	CIP A181	69	75	67	73
20	R122	<i>Pantoea agglomerans</i>	CIP 57.51	34	36	20	56
21	R95	<i>Proteus mirabilis</i>	CIP 103181	53	54	54	58
22	R117	<i>Serratia ficaria</i>	CIP 79.23	20	29	10	9
23	R118	<i>Serratia fonticola</i>	CIP 103580	139	153	118	110
24	R81	<i>Shigella flexneri</i>	CIP 82.48T	11	12	28	32
25	R80	<i>Shigella sonnei</i>	ATCC 9290	16	15	24	38
26	P39	<i>Salmonella</i> Agona	Industrie laitière	84	79	77	73
27	S7	<i>Salmonella</i> Hadar	Merguez	36	40	52	35
28	S65	<i>Salmonella</i> Javiana	Champignons séchés	65	50	76	92
29	P35	<i>Salmonella</i> Typhimurium	Gorge de porc	31	33	47	30
30	R120	<i>Escherichia coli</i> O:157.H7	CIP 105917	14	12	11	19

#	Code	Souche	Origine	REBECCA+EB		EN ISO 21528-2	TSA
				Masse	Surface		
31	SWS017	<i>Ewingella americana</i>	DSMZ 4581	9	8	8	9
32	VRY654	<i>Providencia burhodogranariea</i>	DSMZ 19968	63	63	66	81
33	UJF619	<i>Erwinia persicina</i>	DSMZ 19328	19	15	17	18
34	BJK3652	<i>Serratia marcescens</i>	Aliment	55	50	53	56
35	RKL458	<i>Yersinia enterocolitica</i>	Chou crème chantilly	24	20	25	30
36	WDP406	<i>Enterobacter hormachaei</i>	DSMZ 101093	28	31	32	64
37	GAR051	<i>Escherichia coli</i>	Andouillette cuite	56	61	69	87
38	GBL293	<i>Escherichia coli</i>	Biscuit chocolat non cuit	37	40	42	85
39	DBP642	<i>Enterobacter cloacae</i>	Crème glacée vanille	16	14	14	18
40	AER835	<i>Serratia liquefaciens</i>	Côte de bœuf crue	63	76	9	66
41	AAY895	<i>Enterobacter asburiae</i>	Poudre lait infantile	33	40	38	41
42	DEC391	<i>Klebsiella pneumonia</i>	Mousse bavaroise fraise	26	14	19	55
43	VBT249	<i>Enterobacter cloacae</i>	Lactosérum	43	34	40	50
44	NAB548	<i>Proteus</i> sp.	Environnement ovoproduit	56	44	54	45
45	QAJ806	<i>Escherichia vulneris</i>	Extrait de mûrier blanc	18	17	<1	62
46	RAX819	<i>Citrobacter youngae</i>	Viande hachée bœuf	31	13	36	56
47	ARP296	<i>Citrobacter brakii</i>	Araignée de porc marinée crue	9	11	8	46
48	FAT267	<i>Leclercia adecarboxylata</i>	Garniture de Saint-Jacques	7	9	4	23
49	EZN508	<i>Escherichia coli</i>	Steak haché	18	19	19	38
50	XAL298	<i>Serratia odorifera</i>	Saumon mariné	15	17	<1	40

## APPENDIX G

### Exclusivité - Résultats bruts

#	Code	Souche	Origine	REBECCA+ EB	EN ISO 21528-2
1	I5	<i>Acinetobacter baumannii</i>	Sandwich fromage	<1	<1
2	I24	<i>Alcaligenes xylosoxydans</i>	Industrie laitière	<1	<1
3	R200	<i>Enterococcus faecalis</i>	ATCC 33186	<1	<1
4	I29	<i>Enterococcus faecium</i>	Industrie laitière	<1	<1
5	I16	<i>Pseudomonas aeruginosa</i>	Omelette gruyère	<1	<1
6	R65	<i>Pseudomonas aeruginosa</i>	ATCC 19429	<1	<1
7	R58	<i>Pseudomonas aeruginosa</i>	CIP 100.720	<1	<1
8	R53	<i>Bacillus cereus</i>	CIP 54.9	<1	<1
9	I21	<i>Bacillus circulans</i>	Industrie laitière	<1	<1
10	I35	<i>Brevibacterium casei</i>	Produit laitier	<1	<1
11	I31	<i>Hansenula anomala</i>	Industrie laitière	<1	<1
12	R73	<i>Staphylococcus aureus</i>	ATCC 6538	<1	<1
13	R83	<i>Staphylococcus aureus</i>	CIP 53.154	<1	<1
14	I34	<i>Staphylococcus epidermidis</i>	Produit laitier	<1	<1
15	P51	<i>Pseudomonas aeruginosa</i>	Viande hachée	<1	<1
16	P52	<i>Pseudomonas aeruginosa</i>	Filet de bœuf	<1	<1
17	I30	<i>Micrococcus luteus</i>	Industrie laitière	<1	<1
18	I21	<i>Bacillus circulans</i>	Industrie laitière	<1	<1
19	I22	<i>Bacillus subtilis</i>	Crème dessert	<1	<1
20	I36	<i>Aeromonas aerophila</i>	Saumon fumé	<1	<1

#	Code	Souche	Origine	TSA	EN ISO 21528-2	R+EB masse	R+EB surface
21	SAE286	<i>Acinetobacter baumannii</i>	Poudre de lait infantile	630	<10	<10*	10
22	EFG554	<i>Corynebacterium callunae</i>	Pizza calzone	850	<10	<10	<10
23	FBM018	<i>Corynebacterium flavescens</i>	Morbier AOP	550	<10	<10	<10
24	ABB472	<i>Aeromonas sp</i>	Saumon mariné	610	<10	<10	<10
25	AAZ671	<i>Pseudomonas fragi</i>	Crèmeux fruits rouges	510	<10	<10	<10
26	BDK055	<i>Pseudomonas fluorescens</i>	Produits carnés	600	<10	<10	<10
27	DAR118	<i>Rhodococcus erythropolis</i>	Cubes de concombre avec peau	610	<10	<10	<10
28	ANW492	<i>Lactobacillus paracasei</i>	Produit laitier (CECALAIT)	500	<10	<10	<10
29	BHL731	<i>Stenotrophomonas maltophilia</i>	Croûte de St-Nectaire (CECALAIT)	1000	<10	<10	<10
30	YFJ492	<i>Carnobacterium divergens</i>	Steak de saumon	560	<10	<10	<10

\*: très petites colonies roses

## APPENDIX H

### Interlaboratory study

#### Raw results

#### TOTAL VIABLE COUNT OF THE PASTEURIZED MILK

##### Results per collaborator in CFU/ml

<b>Collaborator</b>	<b>Result</b>
A	<10
B	<10
C	<10
D	1900
F	2500
G	140
H	380
I	6500
J	430
Expert	1100



## Résultats bruts - Entérobactéries

### Niveau 1

#### NOMBRE DE COLONIES CARACTERISTIQUES COMPTEES

Contamination initiale: 62 Entérobactéries par mL

Laboratoires	Méthode de référence (NF ISO 21528-2)													
	Echantillon 4							Echantillon 7						
	-1		-2		-3		Résultats (UFC/mL)	-1		-2		-3		Résultats (UFC/mL)
	Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2		Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2	
A	5 <sup>a</sup>	4	<1	<1	<1	<1	4,5E+01	7	2	2	<1	<1	<1	4,5E+01
B	10	8	1	<1	<1	<1	9,0E+01	7	5	1	<1	<1	1	6,0E+01
C	4	2	<1	1	<1	<1	3,0E+01	6	8	<1	2	<1	<1	7,0E+01
D	4	2	<1	<1	<1	<1	3,0E+01	6	1	<1	2	<1	<1	3,5E+01
F	6	6	2	1	<1	<1	6,0E+01	2	3	1	2	<1	<1	2,5E+01
G	8	5	1	1	<1	<1	6,5E+01	3	7	<1	<1	<1	<1	5,0E+01
H	3	4	1	<1	<1	<1	3,5E+01	4	4	1	<1	<1	<1	4,0E+01
I	5	2	1	<1	<1	<1	3,5E+01	11	4	<1	<1	<1	<1	7,5E+01
J	4	1	1	<1	<1	<1	2,5E+01	6	5	<1	<1	<1	<1	5,5E+01
Laboratoire expert	4	3	<1	<1	<1	<1	3,5E+01	6	5	<1	<1	<1	<1	5,5E+01

Laboratoires	Méthode alternative (REBECCA + supplément EB - colonies bleu-violet + colonies rose à rouge)													
	Echantillon 4				Echantillon 7									
	-1		-2		-3		Résultats (UFC/mL)	-1		-2		-3		Résultats (UFC/mL)
	Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1		
A	3 <sup>a</sup>		1		<1		3,0E+01	8		1		<1		8,0E+01
B							1,0E+01	2						2,0E+01
C	4		1		<1		4,0E+01	6		<1		<1		6,0E+01
D	6		<1		<1		6,0E+01	4		1		<1		4,0E+01
F	5		<1		<1		5,0E+01	6		<1		<1		6,0E+01
G	5		<1		<1		5,0E+01	6		<1		<1		6,0E+01
H	10		1		<1		1,0E+02	4		<1		<1		4,0E+01
I	5		<1		<1		5,0E+01	4		<1		<1		4,0E+01
J	1		<1		<1		1,0E+01	4		<1		<1		4,0E+01
Laboratoire expert	2		1		<1		2,0E+01	4		<1		<1		4,0E+01

a=nombre estimés

## Résultats bruts - Entérobactéries

### Niveau 2

#### NOMBRE DE COLONIES CARACTERISTIQUES COMPTEES

Contamination initiale: 840 Entérobactéries par mL

Laboratoires	Méthode de référence (NF ISO 21528-2)													
	Echantillon 5							Echantillon 6						
	-1		-2		-3		Résultats (UFC/mL)	-1		-2		-3		Résultats (UFC/mL)
	Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2		Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2	
A	46	49	8	6	1	1	5,0E+02	53	43	8	5	<1	<1	5,0E+02
B	48	62	9	10	1	<1	5,9E+02	46	48	7	9	<1	1	5,0E+02
C	61	56	7	3	<1	<1	5,8E+02	44	39	3	6	<1	<1	4,2E+02
D	50	59	7	5	2	<1	5,5E+02	51	64	7	7	2	<1	5,9E+02
F	60	58	10	2	1	<1	5,9E+02	55	49	10	3	1	<1	5,3E+02
G	50	61	9	6	<1	<1	5,7E+02	56	56	3	6	1	<1	5,5E+02
H	59	66	5	6	<1	<1	6,2E+02	54	52	8	3	1	<1	5,3E+02
I	42	52	5	3	<1	<1	4,6E+02	46	43	4	4	1	<1	4,4E+02
J	38	35	3	3	1	<1	3,6E+02	47	40	5	4	1	<1	4,4E+02
Laboratoire expert	51	51	4	4	2	2	5,0E+02	41	54	8	4	1	<1	4,9E+02

Laboratoires	Méthode alternative (REBECCA + supplément EB - colonies bleu-violet + colonies rose à rouge)							
	Echantillon 5				Echantillon 6			
	-1	-2	-3	Résultats (UFC/mL)	-1	-2	-3	Résultats (UFC/mL)
	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1	
A	50	8	1	5,3E+02	44	6	<1	4,6E+02
B	44	2	<1	4,2E+02	35	3	<1	3,5E+02
C	32	8	1	3,6E+02	33	8	<1	3,1E+02
D	63	<1	1	5,7E+02	55	<1	<1	5,0E+02
F	58	5	<1	5,7E+02	41	4	<1	4,1E+02
G	50	4	<1	4,9E+02	47	6	<1	4,8E+02
H	42	8	<1	4,6E+02	48	6	1	4,9E+02
I	37	4	<1	3,7E+02	36	11	1	4,3E+02
J	38	3	<1	3,7E+02	41	3	<1	4,0E+02
Laboratoire expert	51	4	<1	5,0E+02	45	3	<1	4,4E+02

## Résultats bruts - Entérobactéries

### Niveau 3

#### NOMBRE DE COLONIES CARACTERISTIQUES COMPTEES

Contamination initiale: 8300 Entérobactéries par mL

Laboratoires	Méthode de référence (NF ISO 16649-2)													
	Echantillon 1							Echantillon 3						
	-1		-2		-3		Résultats (UFC/mL)	-1		-2		-3		Résultats (UFC/mL)
	Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2		Boîte 1	Boîte 2	Boîte 1	Boîte 2	Boîte 1	Boîte 2	
A	>150	>150	59	54	6	4	5,6E+03	>150	>150	54	26	7	5	4,2E+03
B	>150	>150	48	48	6	7	5,0E+03	>150	>150	53	39	2	3	4,4E+03
C	>150	>150	77	32	1	9	5,4E+03	>150	>150	53	54	5	8	5,5E+03
D	>150	>150	48	72	8	7	6,1E+03	>150	>150	65	71	6	4	6,6E+03
F	>150	>150	58	53	9	2	5,5E+03	>150	>150	60	49	12	3	5,6E+03
G	>150	>150	57	56	4	4	5,5E+03	>150	>150	58	51	7	12	5,8E+03
H	>150	>150	58	51	7	7	5,6E+03	>150	>150	48	50	16	6	5,5E+03
I	>150	>150	51	37	4	7	4,5E+03	>150	>150	41	51	10	8	5,0E+03
J	>150	>150	52	52	7	4	5,2E+03	>150	>150	43	55	4	4	4,8E+03
Laboratoire expert	>150	>150	60	57	5	3	5,7E+03	>150	>150	53	46	6	6	5,0E+03

Laboratoires	Méthode alternative (REBECCA + supplément EB - colonies bleu-violet + colonies rose à rouge)													
	Echantillon 1				Echantillon 3									
	-1		-2		-3		Résultats (UFC/mL)	-1		-2		-3		Résultats (UFC/mL)
	Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1	Boîte 1	Boîte 1		
A	>150		43		2		4,1E+03	>150		56		8		5,8E+03
B	>150		42		8		4,5E+03	>150		47		1		4,4E+03
C	>150		38		1		3,5E+03	>150		53		3		5,1E+03
D	>150		46		4		4,5E+03	>150		55		6		5,5E+03
F	>150		51		5		5,1E+03	>150		42		6		4,4E+03
G	>150		62		5		6,1E+03	>150		44		5		4,5E+03
H	>150		61		4		5,9E+03	>150		53		3		5,1E+03
I	>150		51		6		5,2E+03	>150		50		3		4,8E+03
J	>150		42		2		4,0E+03	>150		36		3		3,5E+03
Laboratoire expert	>150		40		6		4,2E+03	>150		39		3		3,8E+03