

**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 (project version 7).

- 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.

## Table of contents

1. Introduction.....	6
2. Protocols of the methods .....	7
2.1. Alternative method .....	7
2.1.1. Principle of the method.....	7
2.1.2. Protocol of the method .....	7
2.1.3. Restrictions.....	7
2.2. Reference method .....	8
3. Methods comparison study .....	8
3.1. Relative trueness study .....	8
3.1.1. Number and nature of the samples.....	8
3.1.2. Artificial contaminations .....	9
3.1.3. Protocols used during the study.....	9
3.1.4. Results .....	10
3.1.5. Calculation and interpretation of relative trueness study .....	14
3.1.6. Conclusion .....	16
3.2. Accuracy profile study .....	17
3.2.1. Protocols .....	17
3.2.2. Results .....	17
3.2.3. Conclusion .....	20
3.3. Specificity / selectivity .....	20
3.3.1. Protocols .....	20
3.3.1.1. Initial validation study.....	20
3.3.1.2. Second renewal study.....	20
3.3.2. Results .....	20
3.3.2.1. Initial validation study.....	20
3.3.2.2. Second renewal study.....	21
3.3.3. Conclusion .....	21
3.4. Practicability .....	21
3.5. General conclusion for the methods comparison study .....	22
4. Interlaboratory study.....	23
4.1. Study organization.....	23
4.2. Control of experimental parameters .....	23
4.2.1. Contamination levels obtained after artificial contamination .....	23

4.2.2.	Strain stability during shipping .....	23
4.2.3.	Shipping conditions .....	23
4.2.4.	Conclusion .....	24
4.3.	Results .....	24
4.3.1.	Expert laboratory .....	24
4.3.2.	Results obtained by the collaborators .....	25
4.3.3.	Conclusion .....	25
4.4.	Statistical interpretations and calculations.....	25
4.4.1.	Visual linearity checking .....	25
4.4.2.	Calculation of the accuracy profile and interpretation.....	26
4.5.	General conclusion for the interlaboratory study.....	28
5.	General conclusion .....	28

## **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
Project of fourth renewal	December 2023	ISO 16140-2:2016 ISO 21528-2:2017	Microsept	/

This 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

The study was conducted on a variety of samples and strains representative of food products. This is not an exhaustive list of the various matrices included in the application scope. For any remark on the alternative method, you can contact AFNOR Certification by connecting to the Internet page <http://nf-validation.afnor.org/contact-2/>.

#### 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.

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±1°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

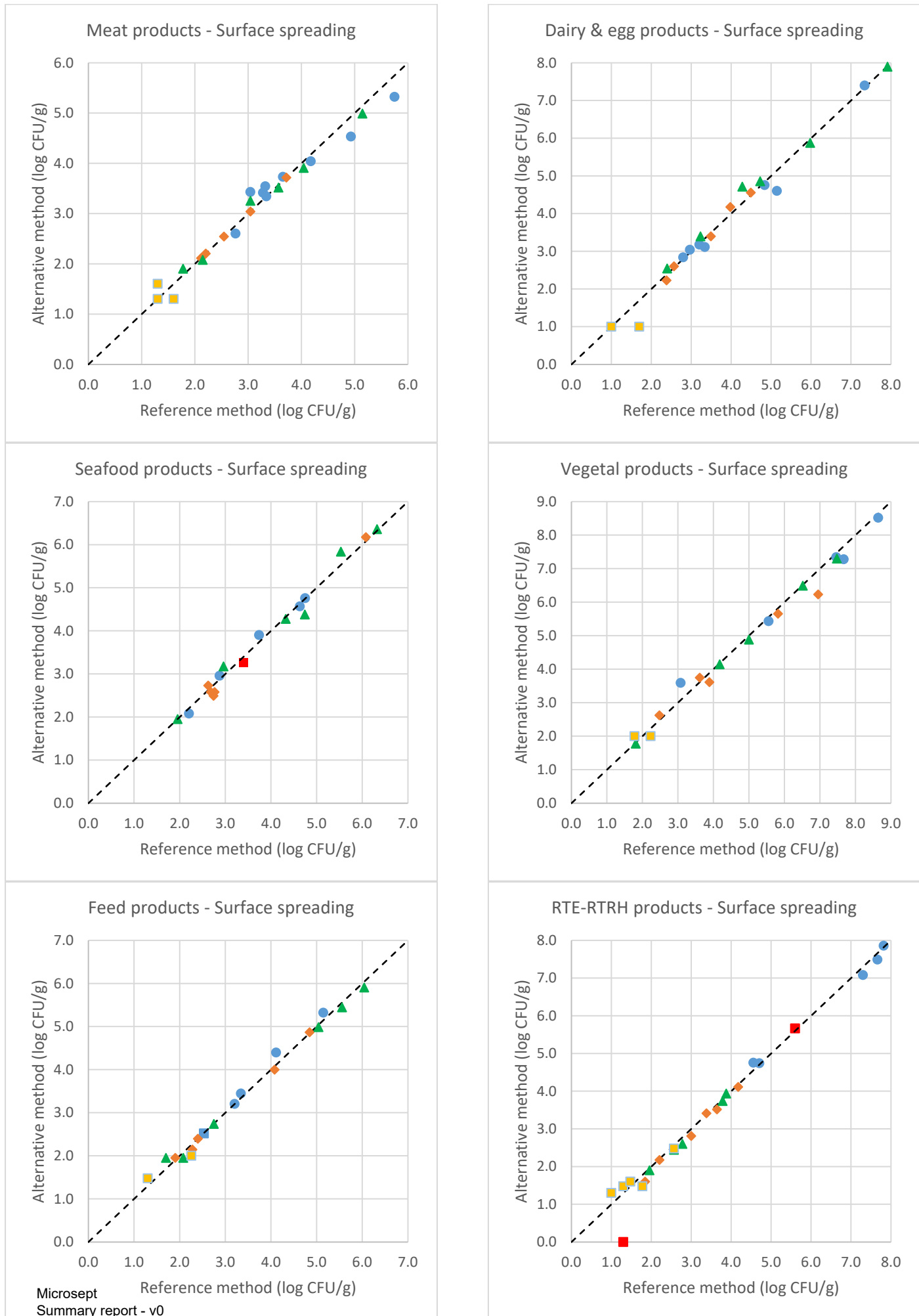


Figure 2: Two-dimensional plots per category, using the pour plate inoculation

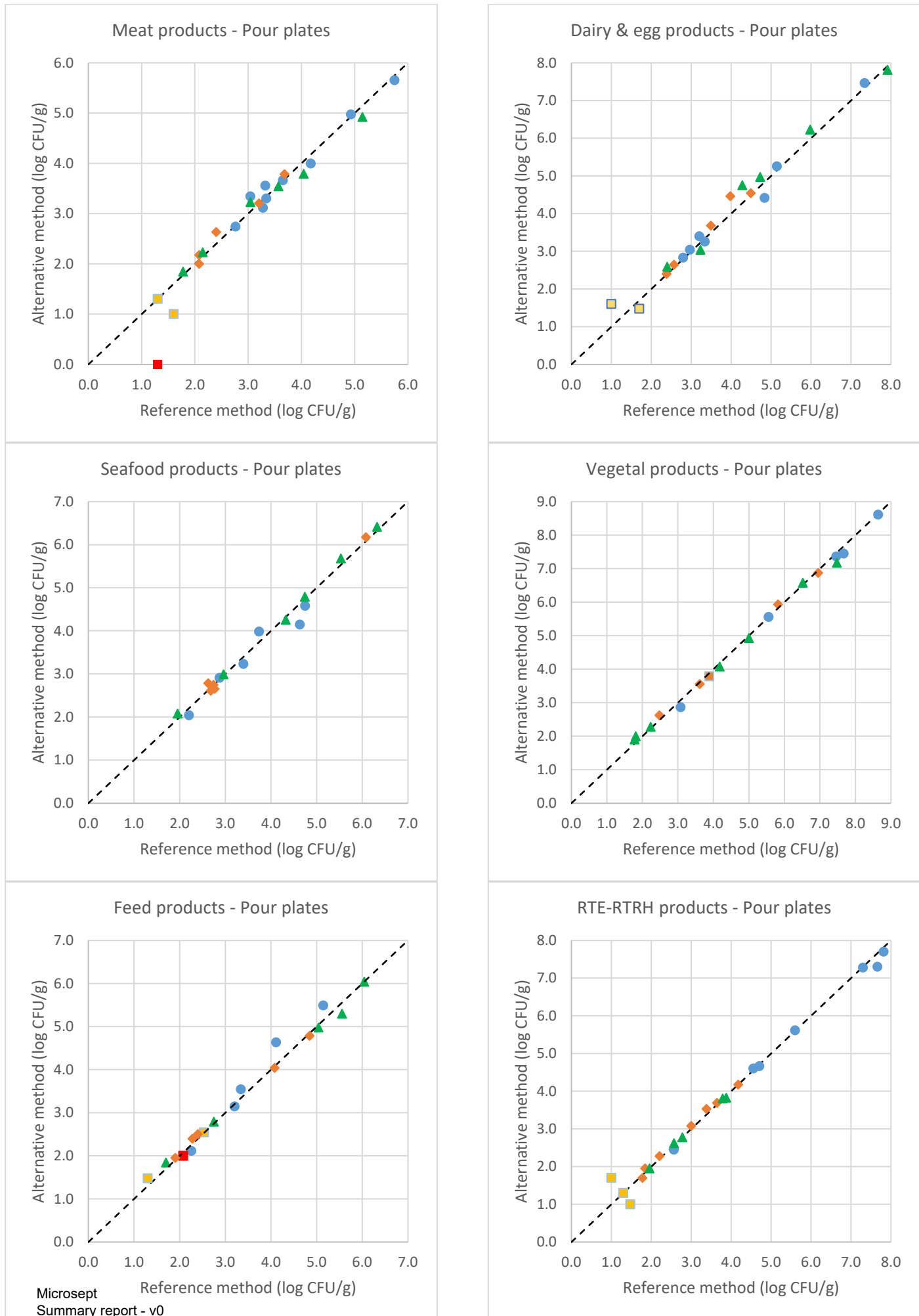


Figure 3: Two-dimensional plots for all categories using the surface spreading inoculation

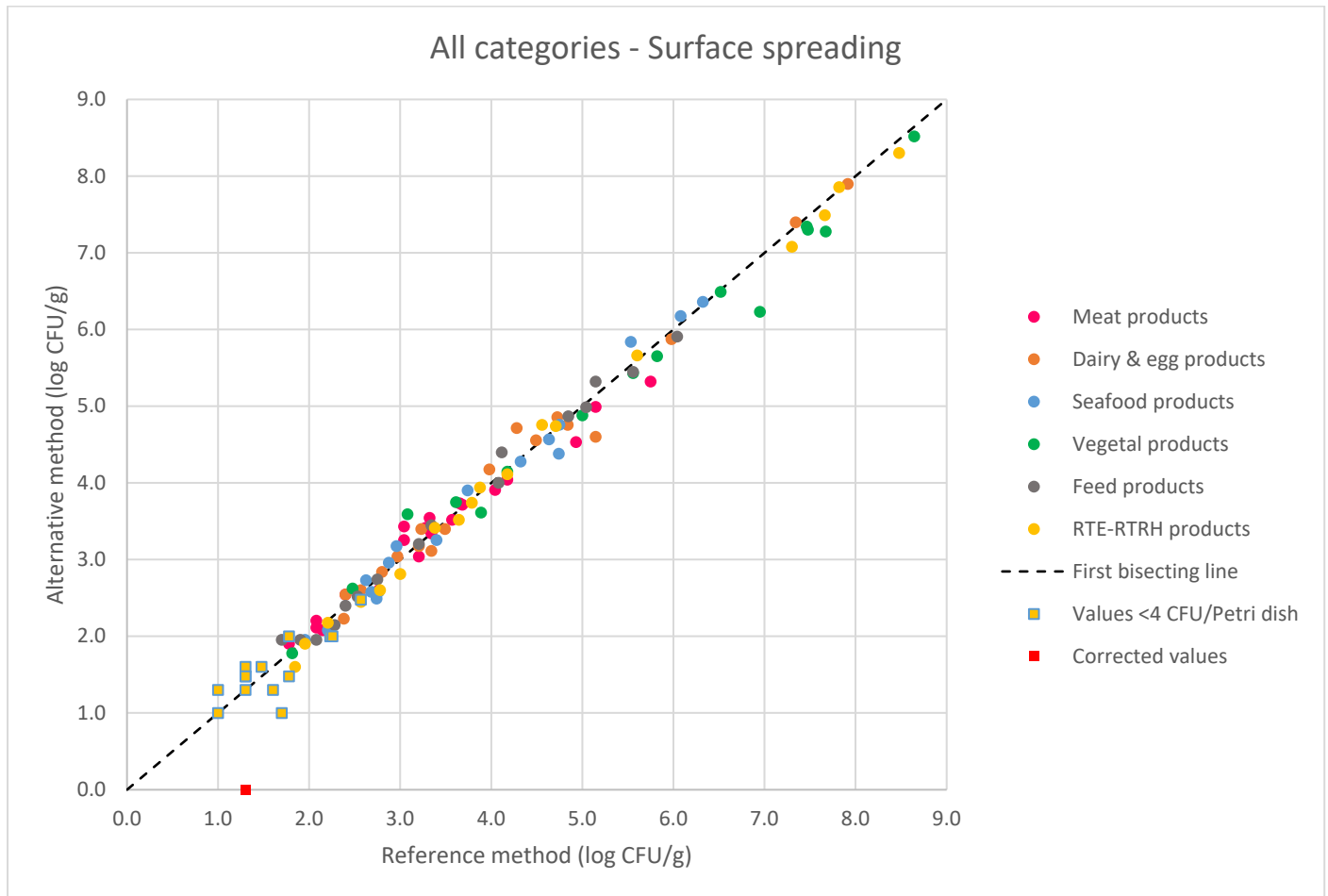
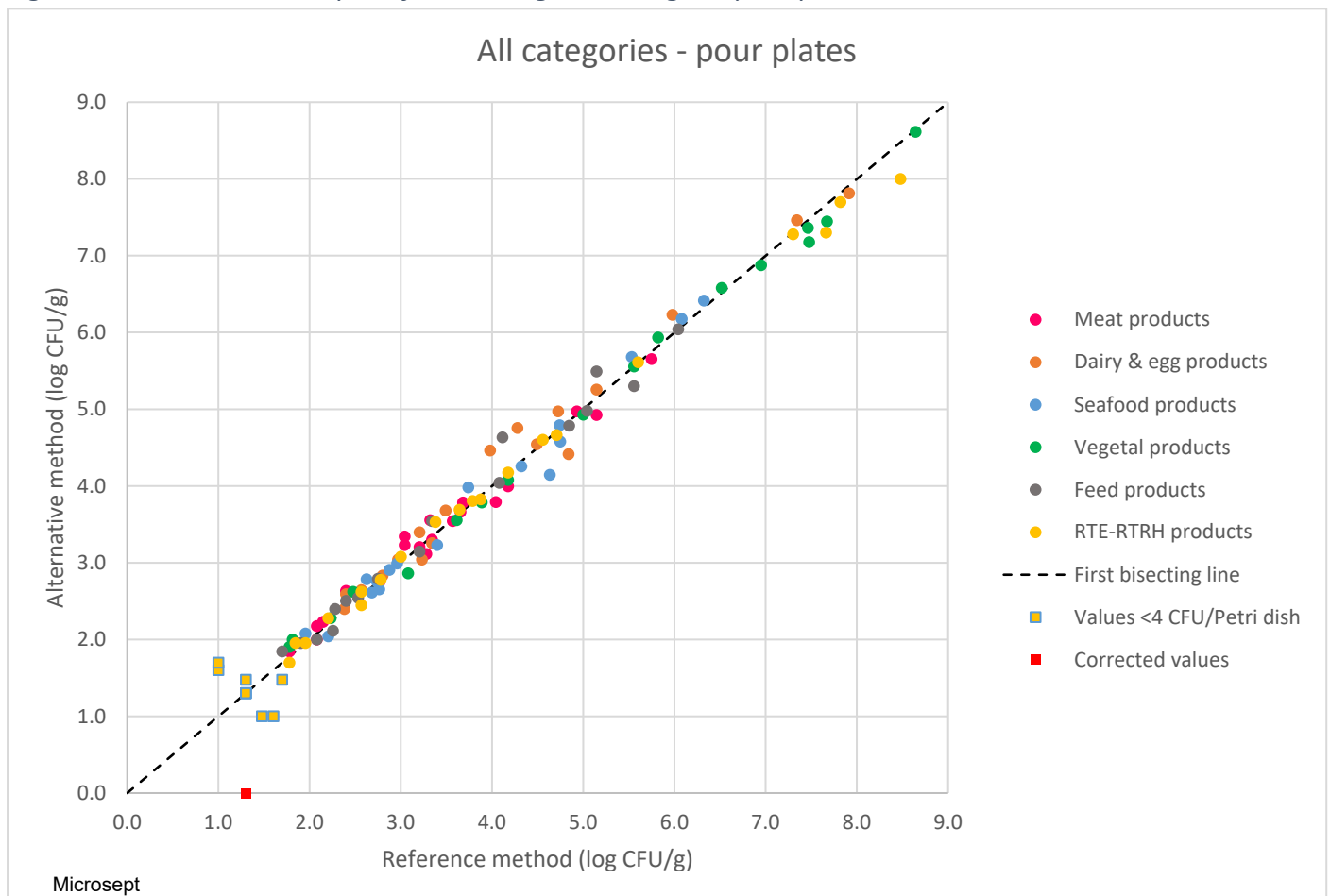


Figure 4: Two-dimensional plots for all categories using the pour plate inoculation



### 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

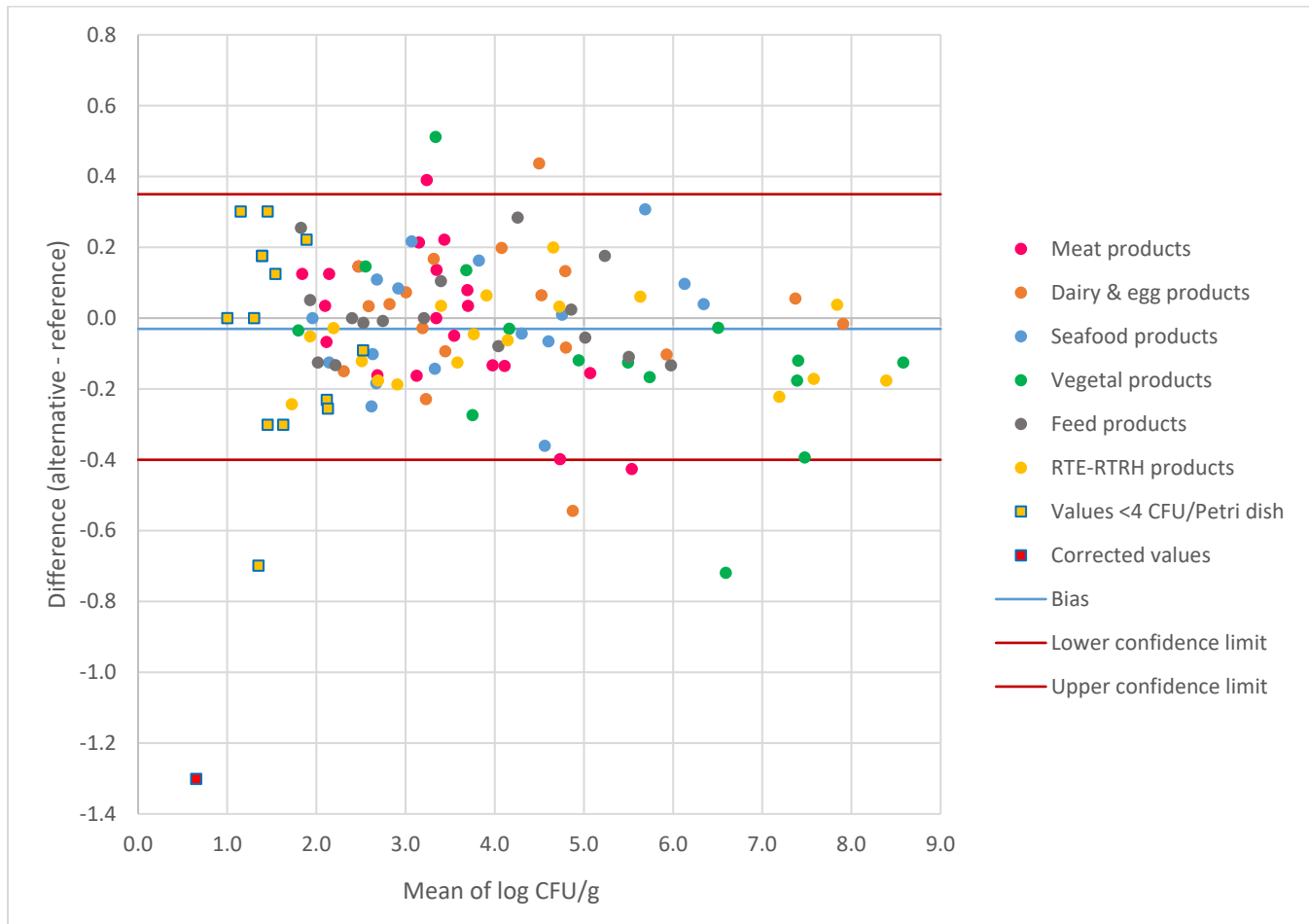
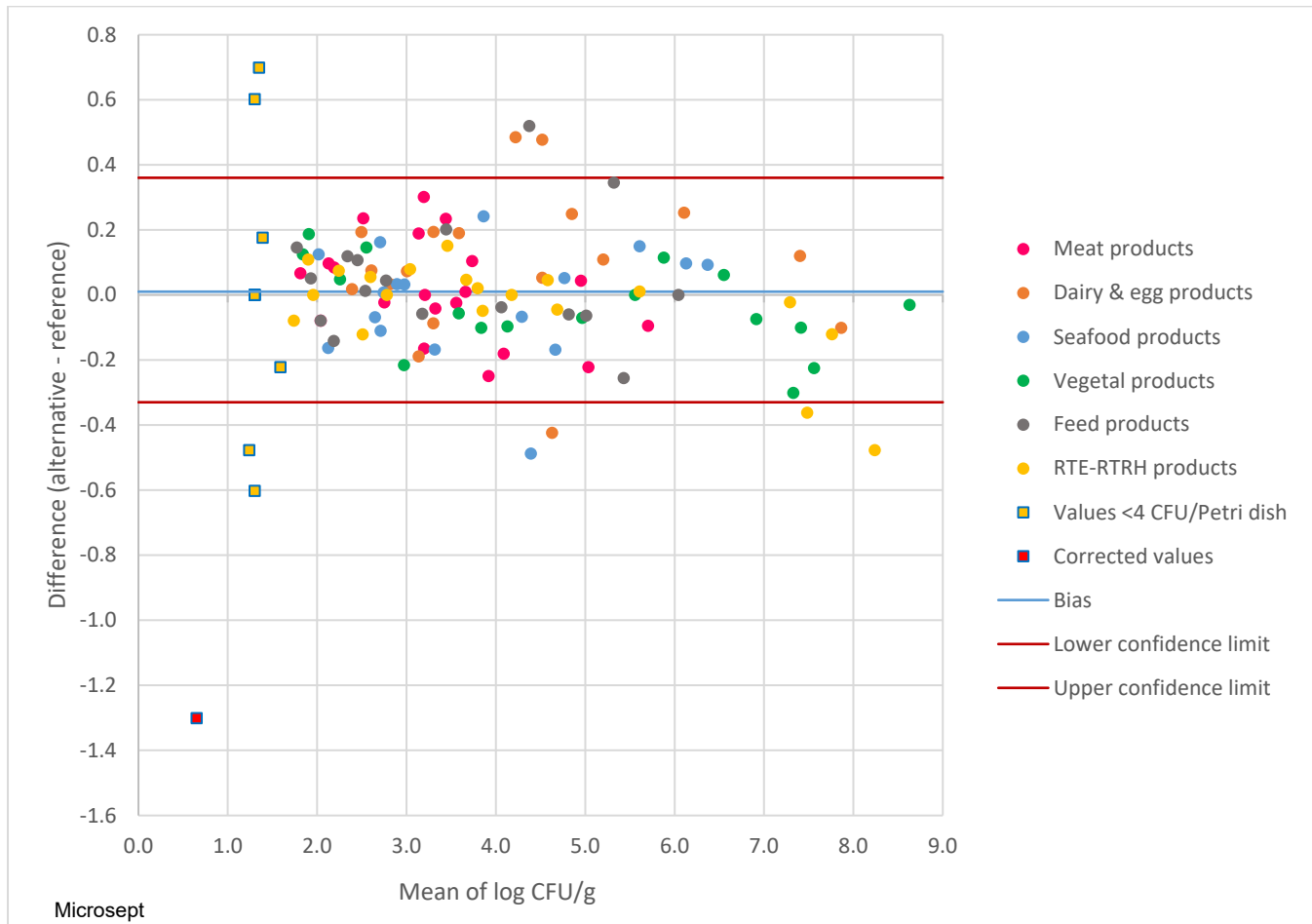


Figure 6: Bland-Altman difference plot for all categories with the pour plate technique



- **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.

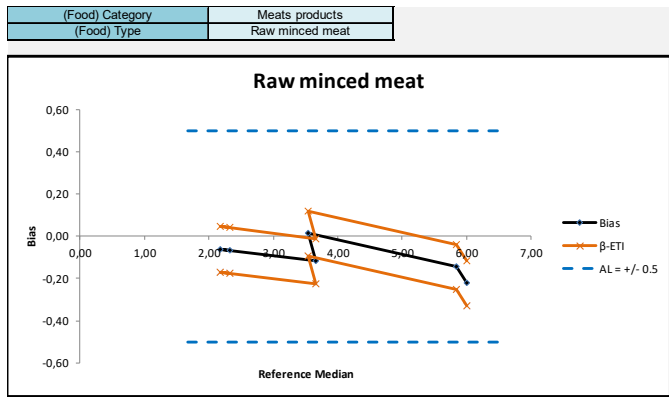
The statistical data and the accuracy profiles are shown:

- 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.

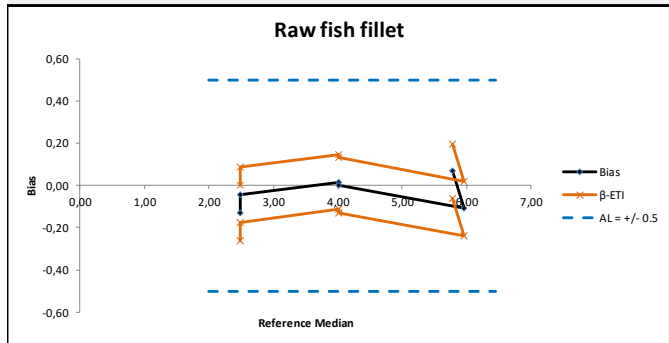
Figure 7: Accuracy profiles per category, using the surface spreading inoculation



Sample Name	Reference Central value	Bias	Lower beta-ETI	Upper beta-ETI	beta-ETI compared to AL=±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 <= 0.125	Final AL
SD Repeatability	0,085	0,074	YES	+/- 0,500

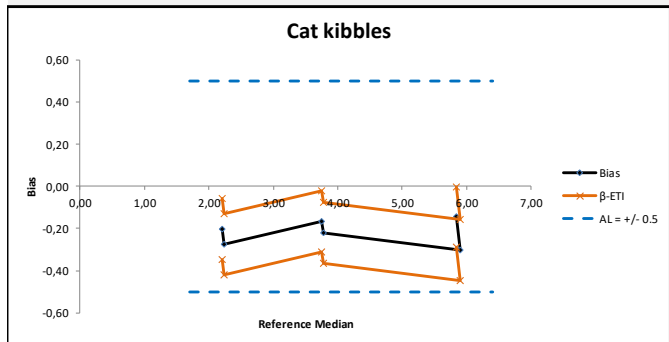
(Food) Category	Seafood products
(Food) Type	Raw fish fillet



Sample Name	Reference Central value	Bias	Lower beta-ETI	Upper beta-ETI	beta-ETI compared to AL=±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

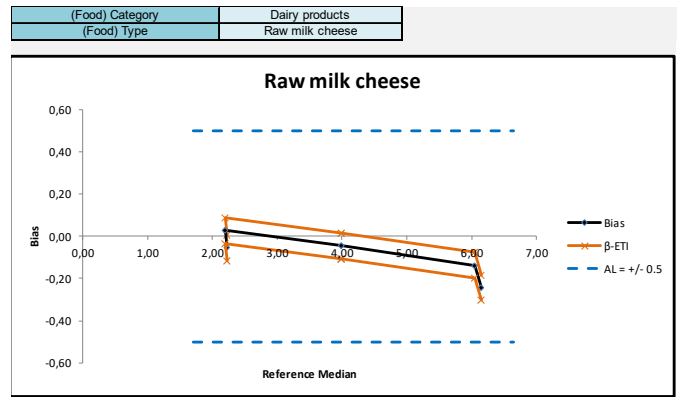
	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,076	0,090	YES	+/- 0,500

(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=±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
1663809-1663744	5,85	-0,146	-0,290	-0,002	YES	YES

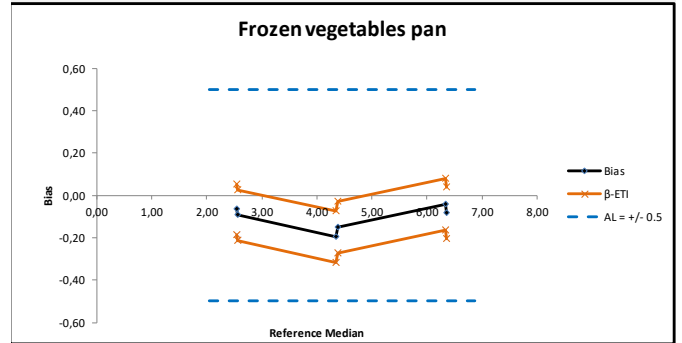
	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0,099	0,100	YES	+/- 0,500



Sample Name	Reference Central value	Bias	Lower beta-ETI	Upper beta-ETI	beta-ETI compared to AL=±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	6,00	-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 <= 0.125	Final AL
SD Repeatability	0,066	0,042	YES	+/- 0,500

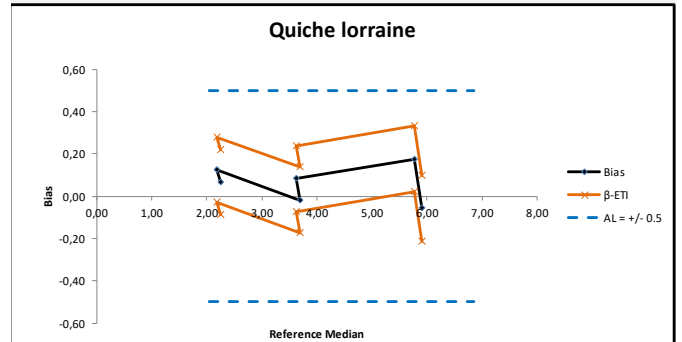
(Food) Category	Vegetables
(Food) Type	Frozen vegetables pan



Sample Name	Reference Central value	Bias	Lower beta-ETI	Upper beta-ETI	beta-ETI compared to AL=±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 <= 0.125	Final AL
SD Repeatability	0,091	0,084	YES	+/- 0,500

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

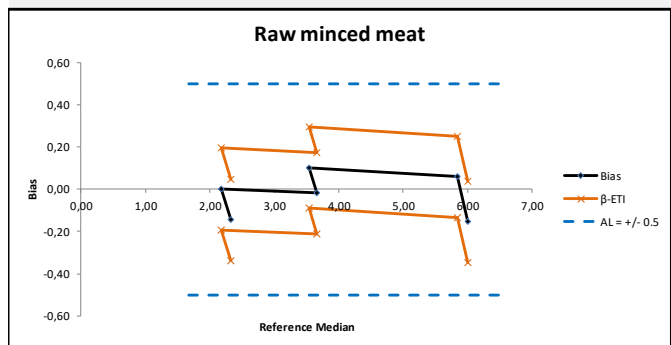


Sample Name	Reference Central value	Bias	Lower beta-ETI	Upper beta-ETI	beta-ETI compared to AL=±0.5 Acceptable	beta-ETI compared to final AL Acceptable
1644578-1644582	2,26	0,067	-0,088	0,221	YES	YES
1644583-1644587	2,18	0,125	-0,030	0,279	YES	YES
1644588-1644592	3,69	-0,018	-0,173	0,136	YES	YES
1644593-1644597	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 <= 0.125	Final AL
SD Repeatability	0,087	0,107	YES	+/- 0,500

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

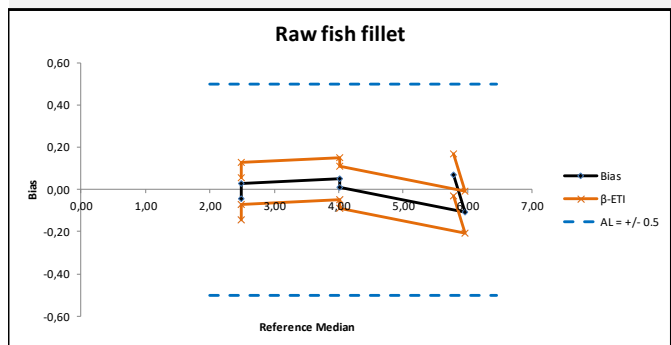
(Food) Category	Meats products
(Food) Type	Raw minced meat



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
1665815-1665819	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

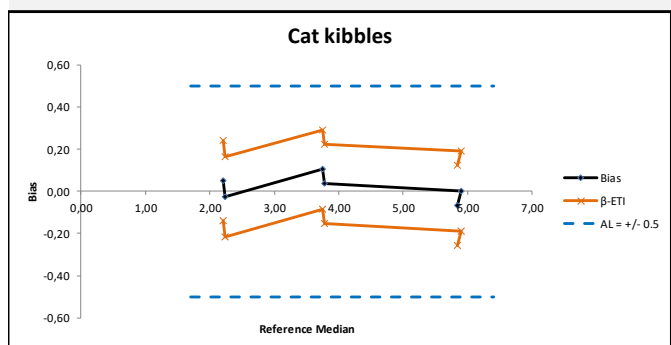
(Food) Category	Seafood products
(Food) Type	Raw fish fillet



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

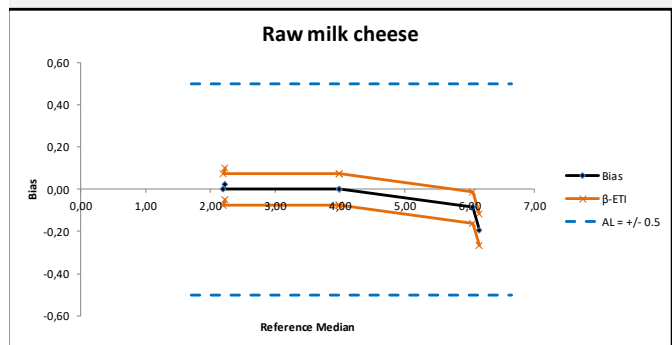
(Food) Category	Animal feed
(Food) Type	Cat kibbles



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
1663809-1663744	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	0,131	0,131	YES	$\pm$ 0,500

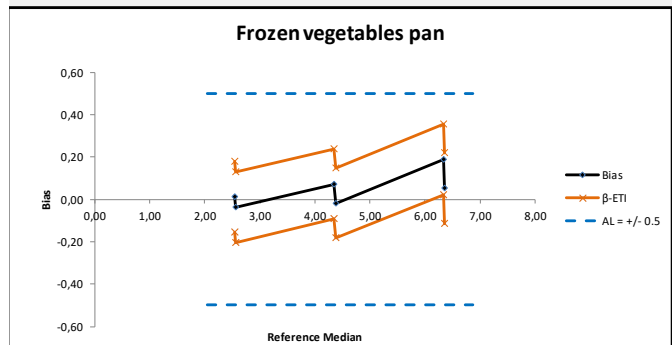
(Food) Category	Dairy products
(Food) Type	Raw milk cheese



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

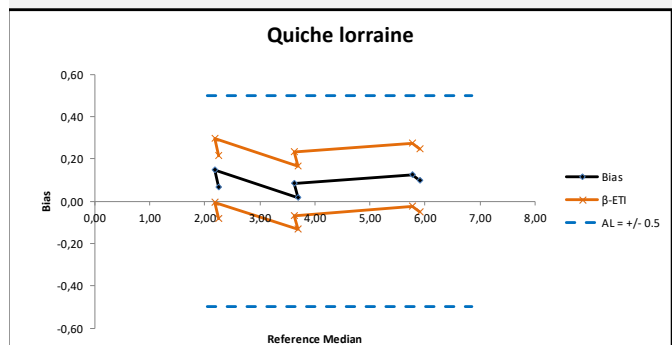
(Food) Category	Vegetables
(Food) Type	Frozen vegetables pan



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>	The plates of pre-poured complete medium and REBECCA base flasks must be kept between +2°C and +8°C. The expiry date is shown on each plate and each vial.
<b>Use after opening of the kit</b>	The pre-poured complete medium plates must be stored at 2-8°C in their package until the expiry date. The plates poured from flasks can be kept up to 15 days at 2-8°C. 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). AEB184135 – AEB184135/10 – AEB520020 must be stored away from light.
<b>Reagents</b>	Supplements have to be kept protected from light. For the medium in flask, liquefy the medium in a water bath at 100°C and cool to 44-47°C. Per flask of 200 ml of REBECCA medium, add aseptically 1 ml of EB supplement. Homogenize slightly the agar base and the supplement.

<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>/</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>/</b>	<b>D3</b>
<b>Alternative method</b>	<b>D1</b>	<b>/</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	5100
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	4100
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.

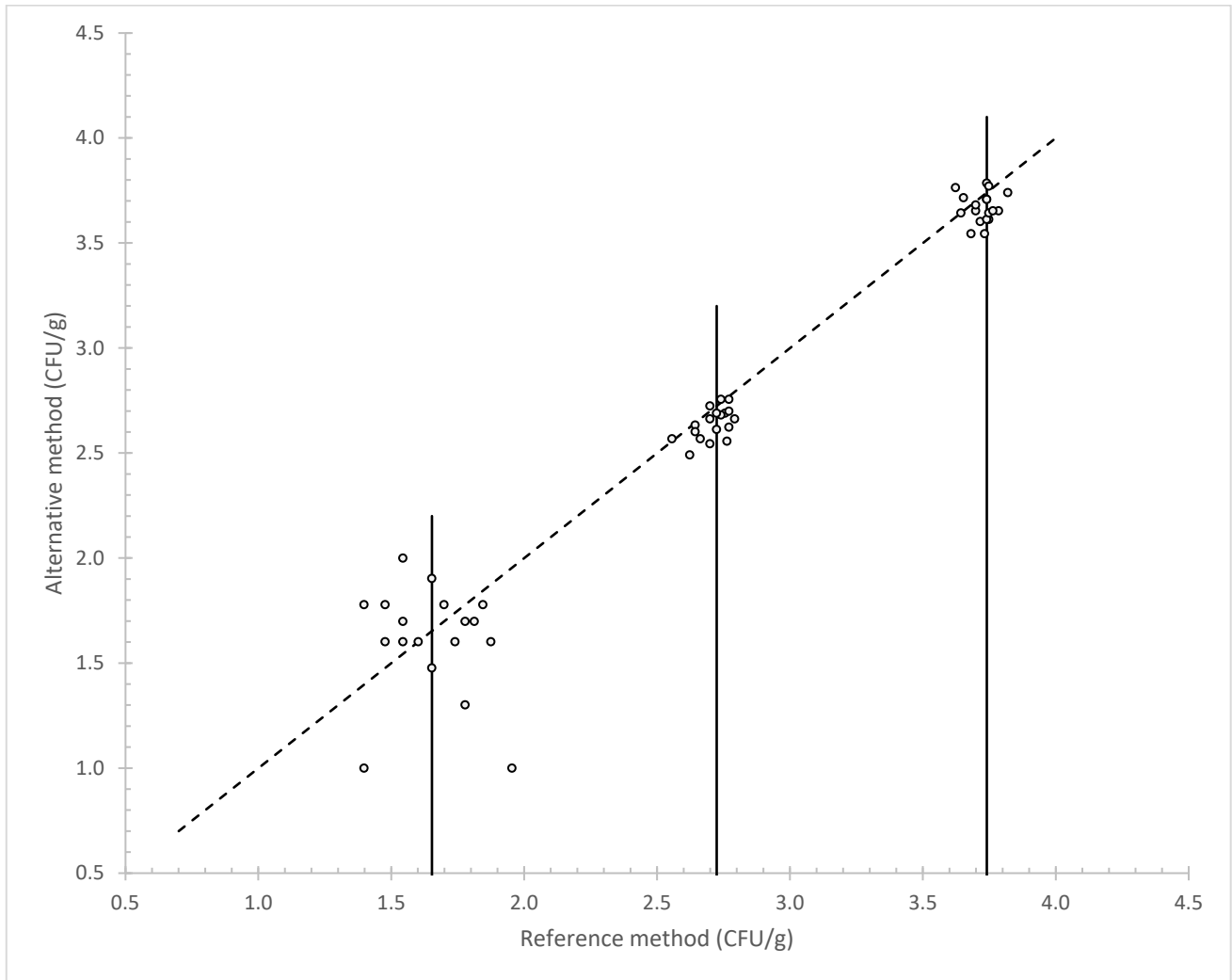
The results obtained by the collaborators were in agreement with those of the Expert Laboratory.

## 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>.

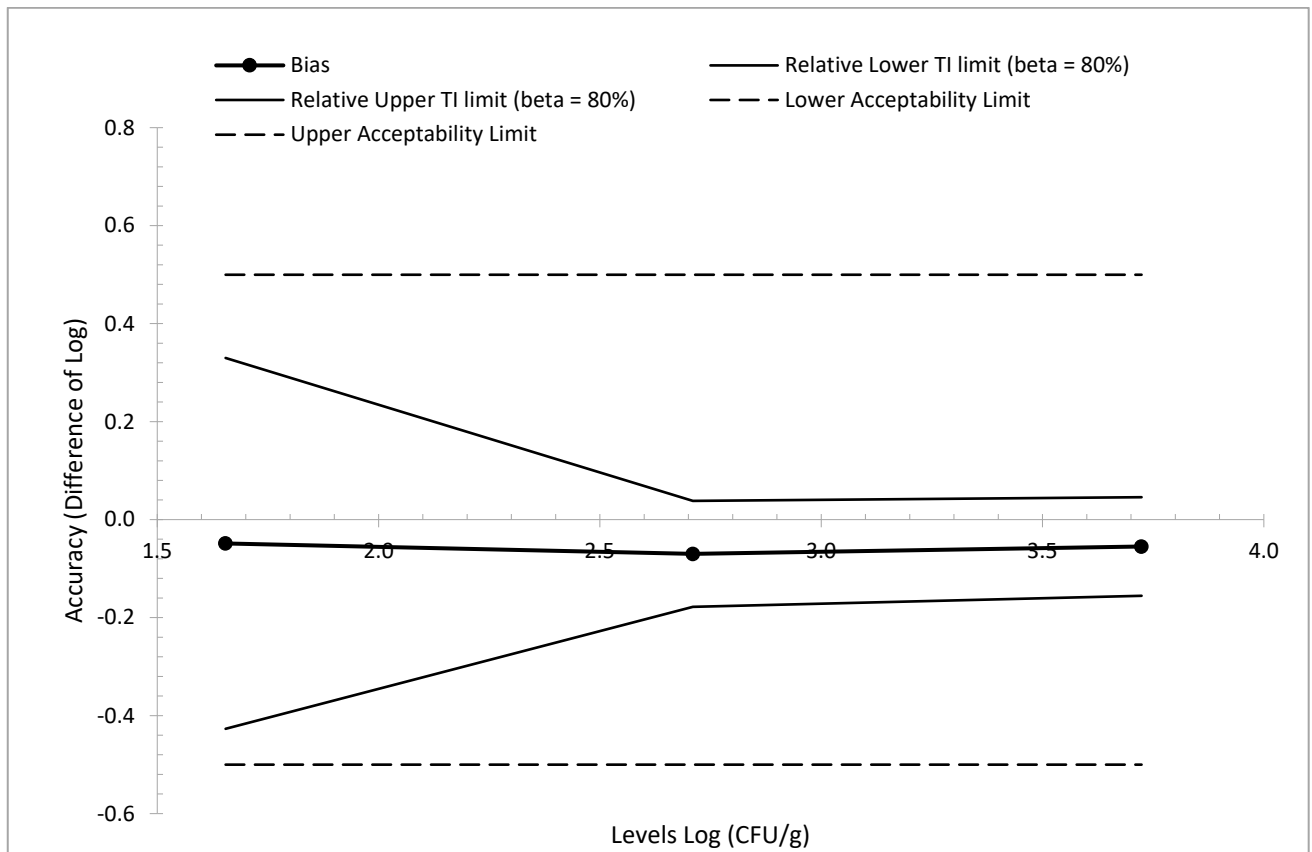
The results of the calculations are provided in table 11.

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)

Tolerance probability (beta)	80%			Reference method		
	Acceptability limit in log (lambda)					
	0.50	0.50	0.50			
Levels	Alternative method			Reference method		
	Low	Medium	High	Low	Medium	High
<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 10: 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 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 18<sup>th</sup>, 2023  
François Le Nestour  
Head of the Microbiology Department

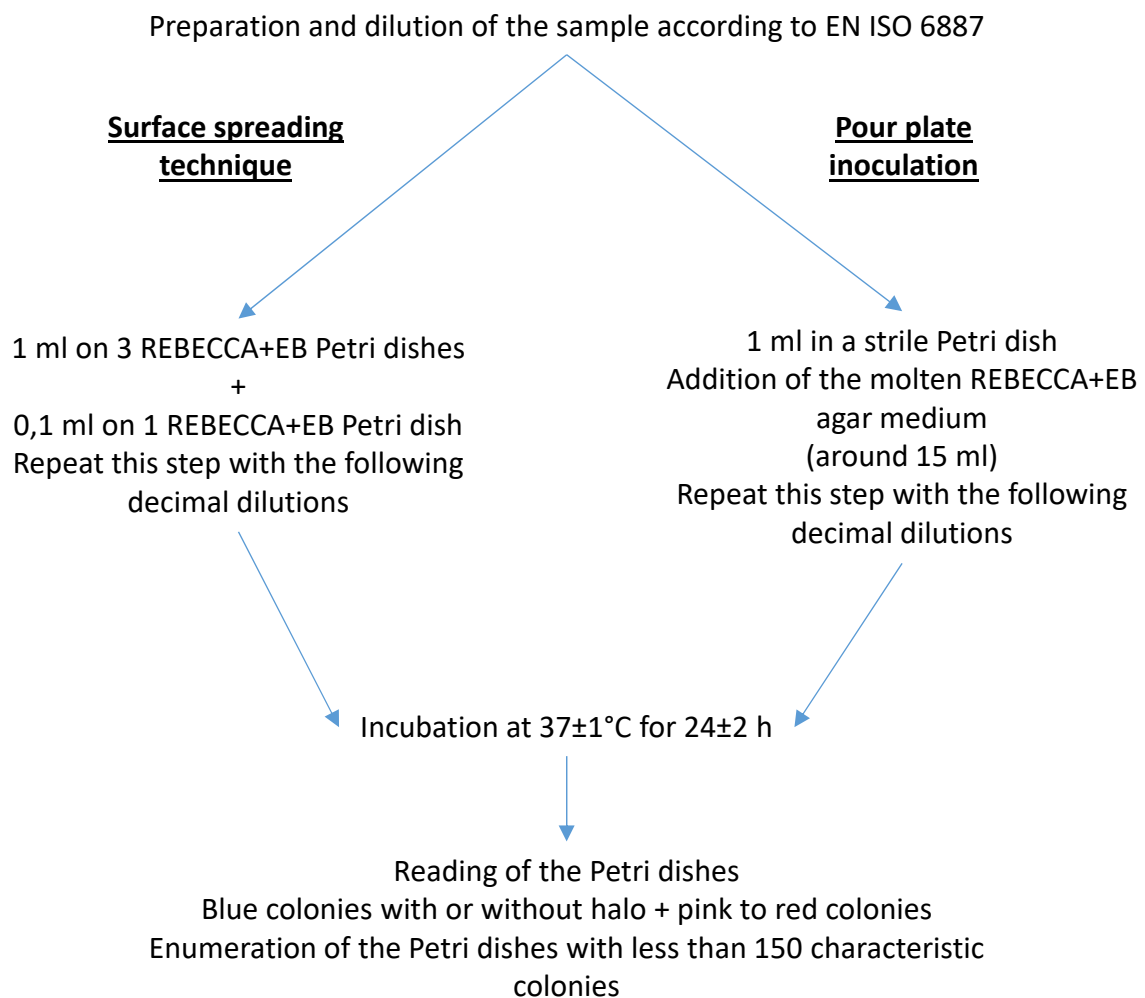
A handwritten signature in black ink, appearing to read 'F. Le Nestour', is written over a horizontal line.

# APPENDICES

# APPENDIX A

## REBECCA+EB METHOD

### TECHNICAL PROCEDURE



## **APPENDIX B**

### **EN ISO 21528-2** **TECHNICAL PROCEDURE**

Preparation and dilution of the sample according to EN ISO 6887



1 ml in a sterile Petri dish  
Addition of the molten VRBG agar medium (around 15 ml) and  
addition of a covering layer from 5 to 10 ml after solidification  
Repeat this step with the following decimal dilutions



Incubation at  $37\pm 1^{\circ}\text{C}$  for  $24\pm 2$  h



Reading  
Pink to red or purple colonies, with or without halo  
Enumeration of the Petri dishes with less than 150 characteristic  
colonies



Confirmation  
Choose 5 characteristic colonies per Petri dish (or 5 whitish colonies  
when no characteristic colonies are present)  
Streak the colonies on a non-selective agar medium and incubate the  
Petri dishes at  $37\pm 1^{\circ}\text{C}$  for  $24\pm 2$ h  
From an isolated colony, perform an oxidase reaction test and a  
fermentation test on glucose OF medium tube (stabbing and  
incubation at  $37^{\circ}\text{C}$  for  $24\pm 2$  h with 1 cm of mineral oil)  
Colonies that are oxidase-negative and glucose-positive are  
confirmed as 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	Cooked seafood preparation	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	Ground beef	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	Ground 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	

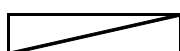


## APPENDIX D

### 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

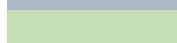


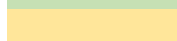
not realized: only one replicate analyzed during the renewal study  
ac. EN ISO 16140-2:2016

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					
						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	Raw sausage with herbs	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	100%			-3	4	5			-3	6	8		
	RG 3028	Raw duck breast	NC	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0			-2	0	0			-2	0	0		
	MV 1916	Merguez	NC	c	-2	37	100%	38	100%	30	100%	35	100%	3700	3270	-2	35	29	3300	2900	-2	36	30	3500	3000
					-3	4	100%	3	100%	3	100%	4	100%			-3	1	3			-3	2	3		
	RG 3243	Raw chicken breast	NC	a	-1	54	100%	58	100%	65	100%	75	100%	580	700	-2	4	5	400	500	-1	57	60	550	570
					-2	6	100%	9	100%	8	100%	5	100%			-3	0	0			-2	4	3		
	HA 4490	Rack of milk-fed lamb	NC	a	-1	>150		>150		>150		>150		15000	15000	-2	113	150	11000	14000	-1	>150	>150	9900	12000
					-2	151	100%	154	100%	148	100%	144	100%			-3	7	11			-2	99	124		
	VR 4754	Chicken breast	NC	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0			-2	0	0			-2	0	0		
	VI 717	Poultry livers	NC	b	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0			-2	0	0			-2	0	0		
VR 4755	Veal merguez	NC	c	-3	123	100%	152	100%	104	100%	64	100%	140000	96000	-3	98	83	98000	82000	-3	85	86	84000	85000	
				-4	23	100%	12	100%	25	100%	18	100%			-4	10	7			-4	7	8			
VI 693	Ground beef	NC	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10	
				-2	0	0	0	0	0	0	0	0			-2	0	0			-2	0	0			
V 7798	Pork cheeks	NC	a	-3	>150		>150		>150		>150		560000	360000	-3	>150	>150	210000	240000	-3	>150	>150	450000	350000	
				-4	58	100%	53	100%	37	100%	35	100%			-4	21	24			-4	45	35			
VR 4768	Groud beef	NC	a	-2	45	100%	45	100%	53	100%	52	100%	4500	5100	-2	56	51	5400	5000	-2	45	49	4600	4800	
				-3	4	100%	6	100%	4	100%	4	100%			-3	3	4			-3	6	4			
VI 585	Beef tartare	NC	a	-2	22	100%	23	100%	20	100%	17	100%	2200	1800	-2	21	16	2200	1500	-2	19	15	2000	1500	
				-3	2	100%	2	100%	1	100%	1	100%			-3	3	1			-3	3	2			
Renewal study	1714579	Smoked sausages	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10		
					-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0			
	1691980	Mechanically separated meat	No	a	-1	115	100%						1100		-1	nc		2700		-1	nc		2200		
					-2	11	100%								-2	27				-2	22				
	1691981	Turkey breast	No	a	-1	nc	100%						1900		-1	nc		2600		-1	nc		1300		
					-2	19	100%								-2	26				-2	13				
	1691982	Mechanically separated meat	No	a	-1	nc	100%						2100		-1	nc		3500		-1	nc		3600		
					-2	21	100%								-2	35				-2	36				
	1692011	Turket thigh	No	a	-2	nc	100%						85000		-2	nc		34000		-2	nc		94000		
					-3	85	100%								-3	34				-3	94				
	1692018	Raw sausage with herbs	No	c	-1	114	100%						1100		-1	nc		1800		-1	nc		1700		
					-2	10	100%								-2	18				-2	17				
1692019	Merguez	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10			
				-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0				
1692033	Slices of smoked duck	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10			
				-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0				
1692034	Unsmoked lardons	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10			
				-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0				
1692035	Salami	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10			
				-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0				
1692036	Bacon	No	c	-1	0	0	0	0	0	0	0	<10		-1	0	0	<10		-1	0	0	<10			
				-2	0	0	0	0	0	0	0			-2	0	0			-2	0	0				

Microsept

Summary report - v0

REBECCA+EB - Enterobacteriaceae

Study	Sample code	Sample name	AC	Type	Dil.	EN ISO 21528-2:2017 (■ for the renewal study)										REBECCA + EB- Surface spreading													
						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				
Renewal study	1692070	Slices of smoked pork belly	No	c	-1	6	100%								60		-1	8			80		-1	7			70		
					-2	0											-2	0					-2	0					
	1692051	Beef Parmentier	Yes	b	-1	2	100%								20		-1	4			40		-1	2			20		
					-2	0											-2	0					-2	0					
	1692057	Hamburger	Yes	b	-1	4	100%								40		-1	2			20		-1	1			10		
					-2	0											-2	0					-2	0					
	1698388	Vegetables and chorizo pie	Yes	b	-1	nc	100%								1600		-1	114			1100		-1	nc			1600		
					-2	16	100%										-2	11					-2	16					
	1714586	Beef Stroganoff	No	b	-1	13	100%								120		-1	17			160		-1	11			100		
					-2	0											-2	1					-2	0					
	1698388	Kidneys with Madeira sauce	Yes	b	-1	25	100%								250		-1	35			350		-1	44			430		
					-2	3	100%										-2	3					-2	3					
1698393	Jambalaya	Yes	b	-1	nc	100%								4800		-1	nc			5200		-1	nc			6100			
				-2	48	100%										-2	52					-2	61						
1692058	Bacon	Yes	c	-1	14	100%								140		-1	13			120		-1	16			170			
				-2	1	100%										-2	0					-2	3						
1692065	Slices of smoked duck	Yes	c	-1	2	100%								20		-1	2			20		-1	0			<10			
				-2	0											-2	0					-2	0						
1729339	Bolognese sauce	Yes	b	-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					
						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	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	RD 1601	Camembert	No	b	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	M 46205	Fluid fresh cream	No	a	-5	>150	>150	>150	>150	>150	>150	>150	>150	22000000	31500000	-5	>150	>150	25000000	32000000	-5	>150	>150	29000000	34000000
					-6	19	100%	25	100%	35	100%	28			-6	25	32			-6	29	34			
	RD 1602	Gruyère	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	VR 4896	Caribbean ice-cream	No	a	-1	83	100%	104	100%	70	100%	85		930	790	-2	11	18	1100	1700	-1	113	110	1100	1100
					-2	8	100%	10	100%	13	100%	6			-3	1	1			-2	11	9			
	RD 1603	Goat milk cheese	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	RD 1604	Milk	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	RD 1605	Thick cream	No	a	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
					-2	0	0	0	0	0	0	0	0	<10	<10	-2	0	0	<100	<100	-2	0	0	<10	<10
	R 36162	Camembert	No	b	-3	32	100%	30	100%	28	100%	36		31000	31000	-3	37	29	36000	30000	-3	35	39	35000	39000
					-4	3	100%	4	100%	1	100%	4			-4	3	4			-4	3	4			
RD 1278	Raw milk cheese	Yes	b	-1	28	100%	20	100%	11	100%	15		240	145	-1	19	21	170	190	-1	24	26	250	360	
				-2	2	100%	2	100%	4	100%	2			-2	0	0			-2	4	4				
RD 1279	Comté	Yes	b	-1	39	100%	32	100%	42	100%	44		370	445	-2	4	4	400	450	-1	42	40	440	430	
				-2	3	100%	7	100%	8	100%	4			-3	0	1			-2	6	7				
RD 1280	Cottage cheese	Yes	a	-2	19	100%	14	100%	14	100%	20		1600	1600	-2	15	15	1500	1450	-2	24	28	2500	3100	
				-3	2	100%	0	100%	0	100%	1			-3	2	1			-3	3	6				
RD 1281	Raw milk cheese	Yes	b	-2	24	100%	36	100%	25	100%	26		3100	2730	-2	24	15	2500	1450	-2	48	35	4800	3400	
				-3	6	100%	3	100%	2	100%	7			-3	4	1			-3	5	2				
RD 1282	Whole milk	Yes	b	-3	10	100%	9	100%	20	100%	15		9500	16000	-3	15	21	15000	20000	-3	29	27	29000	30000	
				-4	2	100%	0	100%	0	100%	1			-4	1	1			-4	3	6				
RD 1283	Caramel ice-cream	Yes	a	-4	13	100%	18	100%	15	100%	11		140000	120000	-4	4	9	40000	91000	-4	18	23	180000	230000	
				-5	0		0		0		0			-5	1	1			-5	2	2				
Renewal study	1714574	Thermised cow milk	No	a	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0							<10		-2	0		<10		-2	0		<10		
	1714580	Tiramisu	No	c	-2	18	100%						1700		-2	23		2500		-2	11		1100		
					-3	1	100%								-3	4				-3	1				
	1714585	Liquid eggs	No	c	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0							<10		-2	0		<10		-2	0		<10		
	1691974	Milk	No	a	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0							<10		-2	0		<10		-2	0		<10		
1691975	Cake dough	No	c	-2	nc	100%						19000		-2	nc		52000		-2	nc		57000			
				-3	19	100%								-3	52				-3	57					
1691979	3 chocolates cake	No	c	-1	27	100%						250		-1	37		350		-1	41		390			
				-2	1	100%								-2	2				-2	2					
1691976	Tomme de Savoie	No	a	-2	nc	100%						69000		-2	nc		57000		-2	nc		26000			
				-3	69	100%								-3	57				-3	26					
1691977	Camembert	No	a	-1	65	100%						630		-1	68		690		-1	65		680			
				-2	4	100%								-2	8				-2	10					

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Summary report - v0

REBECCA+EB - Enterobacteriaceae



**Seafood 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					
						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	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
	VR 5037	Shrimps bouchées	No	c	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
	Q 2271	Shrimps ravioli	No	c	-3	>150	>150	>150	>150	>150	>150	>150	>150	340000	400000	-3	>150	>150	690000	780000	-3	>150	>150	480000	360000
	RG 3033	Sole fillets	No	c	-1	102	100%	95	100%	86	100%	112	100%	910	950	-2	15	11	1500	1100	-1	102	148	980	1400
	VI 311	Marinated salmon	No	b	-5	14	100%	13	100%	8	100%	12	100%	1200000	1000000	-5	15	9	1500000	910000	-5	14	10	1500000	1100000
	C 85	Bass tartare	No	a	-3	43	100%	56	100%	48	100%	53	100%	56000	54000	-3	56	51	57000	52000	-3	41	38	38000	37000
	C118	Mussels	No	c	-2	>150	>150	>150	>150	>150	>150	>150	>150	21000	18000	-2	>150	140	19000	15000	-1	>150	>150	18000	16000
	VI 397	Cooked shrimps	No	c	-2	>150	>150	>150	>150	>150	>150	>150	>150	55000	45000	-2	>150	164	24000	16000	-2	>150	>150	62000	65000
	HA 4520	Imperial shrimps	No	c	-1	0	0	0	0	0	0	0	0	<10	<10	-1	0	0	<100	<100	-1	0	0	<10	<10
	CI 141	Fish Parmentier	No	c	-4	>150	>150	>150	>150	218	100%	260	100%	2100000	2400000	-4	>150	>150	2300000	2100000	-4	>150	304	2600000	3090000,1
	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
Renewal study	1714572	Smoked salmon	No	b	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10	
	1714573	Ling fillet	No	a	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10	
	1691991	Whitting fillet	No	a	-1	16	100%	2	100%	0	0	0	160	160	-1	12	0	120	0	-1	12	0	110	0	
	1691993	Salmon fillet	No	a	-2	57	100%	3	100%	0	0	0	5500	5500	-2	80	0	8000	0	-2	93	0	9600	0	
	1691994	Codfish loin	No	a	-2	25	100%	3	100%	0	0	0	2500	2500	-2	18	0	1800	0	-2	16	0	1700	0	
	1691996	Smoked mackerel	No	b	-1	0	0	0	0	0	0	0	<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10	
	1691997	Swordfish	No	a	-2	nc	100%	43	100%	0	0	0	43000	43000	-2	nc	0	37000	0	-2	nc	0	14000	0	
	1691998	Salmon fillet	No	a	-1	76	100%	7	100%	0	0	0	750	750	-1	91	0	910	0	-1	80	0	810	0	
	1698391	Smoked mackerel with bell peppers	Yes	b	-1	41	100%	5	100%	0	0	0	420	420	-1	53	0	540	0	-1	58	0	610	0	
	1698398	Smoked herrings	Yes	b	-1	53	100%	11	100%	0	0	0	580	580	-1	35	0	380	0	-1	44	0	450	0	
	1698400	Smoked salmon	Yes	b	-1	54	100%	6	100%	0	0	0	550	550	-1	32	0	310	0	-1	54	0	560	0	
	1698401	Marinated anchovies	Yes	b	-1	47	100%	6	100%	0	0	0	480	480	-1	39	0	380	0	-1	42	0	410	0	
						-2	6	100%								-2	3				-2	3			

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Summary report - v0

REBECCA+EB - Enterobacteriaceae

**Relative trueness study - Vegetables**

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	Conf.	CFU / plate	Conf.	CFU / plate	Conf.	CFU / plate	Conf.	Result	Result		CFU	CFU	Result	Result	CFU	CFU	Result	Result	
Initial validation study	Q 2237	Recycled doughs offcuts	No	c	-3	111	100%	97	100%	96	100%	92	100%	100000	93000	-3	80	88	76000	89000	-3	84	75	85000	74000
					-4	11	100%	9	100%	10	100%	7	100%			-4	4	10			-4	9	8		
	ST 3672	Steamed potatoes with parsil	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	Creole cooked rice	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	Vegetarian salad	No	c	-6	29	100%	35	100%	25	100%	35	100%	30000000	29000000	-6	21	28	20000000	25000000	-6	14	50	15000000	48000000
					-7	2	100%	1	100%	2	100%	2	100%			-7	1	0			-7	2	3		
	VI 313	Vegetables tajine	No	c	-1	>150		>150		>150		>150		15000	13000	-2	150	128	14000	12000	-1	>150	>150	12000	13000
					-2	149	100%	152	100%	140	100%	119	100%			-3	6	8			-2	124	132		
VI 279	Cucumbers with sauce	No	c	-4	>150		>150		>150		>150		3300000	3600000	-4	>150	>150	3100000	3800000	-4	>150	>150	3800000	4900000	
				-5	38	100%	28	100%	23	100%	36	100%			-5	31	38			-5	38	49			
VI 413	Leaf tea	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	Raisin bread	No	c	-1	23	100%	14	100%	26	100%	22	100%	170	230	-2	1	1	100	100	-1	19	16	190	160	
				-2	0		1	100%	0		2	100%			-3	0	0			-2	2	2			
DJ 3361	Rice and tomatoes salad	No	c	-1	8	100%	4	100%	11	100%	6	100%	60	90	-2	1	1	100	100	-1	8	6	80	70	
				-2	0		0		4	100%	1	100%			-3	0	0			-2	1	2			
DJ 3510	Tomatoes and corn salad	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	Fresh basil	No	a	-1	124	100%						1200		-1	nc		3900		-1	72		730		
					-2	11	100%								-2	39				-2	8				
	1714587	Vegetables pan	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1691985	Mint	No	a	-6	30	100%						29000000		-6	22		22000000		-6	23		23000000		
					-7	2	100%								-7	2				-7	2				
	1691986	Dill	No	a	-6	nc	100%						440000000		-6	nc		330000000		-6	nc		410000000		
					-7	44	100%								-7	33				-7	41				
	1692001	Country-style vegetables pan	No	b	-4	65	100%						660000		-4	39		450000		-4	89		860000		
					-5	8	100%								-5	10				-5	6				
	1692022	Chives	No	a	-4	35	100%						360000		-4	25		270000		-4	40		360000		
					-5	5	100%								-5	5				-5	0				
	1692030	Mung beans	No	a	-6	47	100%						47000000		-6	20		19000000		-6	28		28000000		
				-7	5	100%								-7	1				-7	3					
1692031	Lettuce mix	No	b	-2	70	100%						7700		-2	34		4100		-2	60		6100			
				-3	15	100%								-3	11				-3	7					
1692026	Unseasoned grated carrots	No	b	-5	87	100%						8900000		-5	18		1700000		-5	70		7500000			
				-6	11	100%								-6	1				-6	12					
1692003	Vegetables pan	No	b	-1	29	100%						300		-1	42		420		-1	43		420			
				-2	4	100%								-2	4				-2	3					
1729344	Lamb's lettuce and beet mix	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	Meat for animal feeding	No	c	-3	110	100%	120	100%	115	100%	130	100%	110000	120000	-3	91	94	97000	97000	-3	92	90	95000	88000
					-4	9	100%	12	100%	6	100%	15	100%			-4	16	13			-4	13	7		
	RD 1273	Meat for animal feeding	No	c	-3	>150		>150		>150		>150		360000	220000	-3	>150	>150	280000	280000	-3	>150	>150	200000	230000
					-4	35	100%	36	100%	25	100%	19	100%			-4	28	28			-4	20	23		
	P 2743	Bone meal	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	Bone meal	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	Bone meal	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	Bone meal	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	Lamb terrine for cat	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	Beef poultry slices for cat	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	Duck lamb slices for cat	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		0				-4	1	3			-4	4	4			
RD 1277	Rabbit liver slices for cat	Yes	a	-4	10	100%	18	100%	10	100%	4	100%	140000	70000	-4	20	42	210000	430000	-4	28	38	310000	360000	
				-5	2	100%	1	100%	2	100%	1	100%			-5	3	6			-5	6	2			
RD 1275	Chicken turkey slices for cat	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		0				-3	2	2			-3	2	3			
RD 1270	Trout cod terrine for cat	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	Oat pellets	No	b	-1	8	100%						80		-1	9		90		-1	9		90		
					-2	0									-2	0				-2	0				
	1714584	Rapeseed oilcake	No	b	-2	>150							70000		-2	>150		74000		-2	>150		61000		
					-3	70	100%								-3	74				-3	61				
	1692071	Raw meat for animal feeding	No	c	-3	>150							1100000		-3	>150		810000		-3	>150		1100000		
					-4	106	100%								-4	81				-4	110				
	1726637	Pellets for porks	No	c	-1	5	100%						50		-1	9		90		-1	7		70		
					-2	0									-2	0				-2	0				
	1726638	Pellets for piglets	No	c	-1	12	100%						120		-1	9		90		-1	10		100		
				-2	1	100%								-2	0				-2	1					
1726639	Soy	No	c	-1	57	100%						560		-1	54		550		-1	61		620			
				-2	5	100%								-2	6				-2	7					
1729342	Feed for laying hen	Yes	b	-2	120	100%						12000		-2	101		10000		-2	108		11000			
				-3	13	100%								-3	9				-3	11					
1729343	Feed for rabbit	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					
						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	Cheese salad	No	a	-4	43	100%	40	100%	23	100%	29	100%	400000	3100000	-4	48	37	460000	3400000	-4	41	39	410000	3900000
					-5	3	100%	3	100%	1	100%	6	100%			-5	3	0			-5	4	4		
	S 9939	Asian-style chicken salad	NC	a	-6	34	100%	60	100%	36	100%	33	100%	46000000	33000000	-6	30	40	31000000	39000000	-6	20	36	20000000	35000000
					-7	2	100%	5	100%	3	100%	1	100%			-7	4	3			-7	2	2		
	S 9940	Ham salad	NC	a	-5	>150		>150		>150		>150		66000000	74500000	-5	>150	>150	72000000	90000000	-5	>150	>150	50000000	65000000
					-6	77	100%	54	100%	84	100%	65	100%			-6	72	90			-6	50	65		
	DJ 3253	Pork and shrimps brioche	NC	a	-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 9942	Ocean-style salad	No	a	-6	>150		>150		>150		>150		300000000	230000000	-6	>150	>150	200000000	310000000	-6	>150	>150	100000000	200000000
					-7	19	100%	40	100%	24	100%	21	100%			-7	20	31			-7	10	20		
MV 2234	Tabbouleh of the sea	No	a	-1	0		0		0		0		<10	<10	-1	0	0	<10	<10	-1	0	0	<10	<10	
				-2	0		0		0		0				-2	0	0			-2	0	0			
S 10132	Crayfish salad	No	a	-2	>150		>150		>150		>150		36000	26000	-2	>150	>150	57000	40000	-2	>150	>150	40000	22000	
				-3	28	100%	43	100%	22	100%	30	100%			-3	57	40			-3	40	22			
MV 2163	Tomato mozzarella salad	No	a	-1	40	100%	34	100%	45	100%	33	100%	370	400	-2	3	2	300	270	-1	28	27	280	260	
				-2	5	100%	3	100%	7	100%	3	100%			-3	0	1			-2	3	2			
S 9937	Mixed salad	No	a	-6	18	100%	19	100%	17	100%	7	100%	20000000	12000000	-6	13	45	12000000	42000000	-6	17	28	19000000	25000000	
				-7	3	100%	4	100%	1	100%	1	100%			-7	0	1			-7	4	0			
VR 4497	Potatoes salad	No	a	-3	40	100%	60	100%	59	100%	48	100%	51000	52000	-3	58	54	55000	57000	-3	48	57	46000	56000	
				-4	5	100%	8	100%	1	100%	6	100%			-4	3	9			-4	3	5			
Renewal study	1714575	Ham pizza	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1714576	Calf sweetbread bites	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1714578	Potée	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1714581	Quiche lorraine	No	b	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1698389	Broccoli pudding	Yes	b	-1	nc							15000		-1	nc		13000		-1	nc		15000		
					-2	148	100%								-2	130				-2	150				
	1714577	Sliced smoked duck breast	No	c	-1	0							<10		-1	0		<10		-1	0		<10		
					-2	0									-2	0				-2	0				
	1698384	Cooked seafood in a shell	Yes	b	-1	7	100%						70		-1	4		40		-1	9		90		
				-2	1	100%								-2	0				-2	1					
1692052	Tomate farcie	Yes	b	-1	nc	100%						2400		-1	nc		2600		-1	nc		3400			
				-2	24	100%								-2	26				-2	34					
1692056	Tartiflette	Yes	b	-1	6	100%						60		-1	3		30		-1	5		50			
				-2	0									-2	0				-2	0					
1698387	Spinach pie	Yes	b	-1	104	100%						1000		-1	65		650		-1	120		1200			
				-2	10	100%								-2	7				-2	11					
1692059	Vegetables quiche	Yes	b	-1	2	100%						20		-1	3		30		-1	2		20			
				-2	0									-2	0				-2	0					
1692061	Chorizo soufflé	Yes	b	-1	1	100%						10		-1	2		20		-1	5		50			
				-2	0									-2	0				-2	0					
1692068	Bouchée à la reine	Yes	b	-1	3	100%						30		-1	4		40		-1	1		10			
				-2	0									-2	0				-2	0					

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Summary report - v0  
REBECCA+EB - Enterobacteriaceae

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		
Renewal study	1698396	Sliced smoked duck breast	Yes	c	-1	37	100%							370		-1	28			280		-1	44			420	
					-2	4	100%									-2	3					-2	2				
	1698399	Rollmops	Yes	c	-1	60	100%							600		-1	40			400		-1	60			600	
					-2	6	100%									-2	4					-2	6				
	1698386	Smoked mackerel	Yes	c	-1	2	100%							20		-1	0			<10		-1	2			20	
					-2	0										-2	0					-2	0				
	1698392	Venetian-style pizza	Yes	b	-1	17	100%							160		-1	15			150		-1	20			190	
					-2	1	100%									-2	1					-2	1				
	1698394	Paëlla	Yes	b	-2	44	100%							4400		-2	33			3300		-2	49			4900	
					-3	4	100%									-3	3					-3	5				
	1726634	Smoked salmon	No	c	-1	0								<10		-1	0			<10		-1	0			<10	
					-2	0										-2	0					-2	0				
1726635	Smoked herring	No	c	-1	0								<10		-1	0			<10		-1	0			<10		
				-2	0										-2	0					-2	0					
1726636	Marinated salmon	No	c	-2	60	100%							6100		-2	53			5500		-2	64			6400		
				-3	7	100%									-3	8					-3	6					
1729337	Smoked salmon	Yes	c	-1	9	100%							90		-1	8			80		-1	9			90		
				-2	0	100%									-2	0					-2	0					
1729338	Smoked herring	Yes	c	-2	77	100%							7500		-2	87			8700		-2	66			6700		
				-3	5	100%									-3	9					-3	8					

## **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>-0,01</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>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
	b	1692011	4,93	4,97	4,95	0,04
		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
	c	1729339	2,08	2,18	2,13	0,10
		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

### Inclusivity - Raw results

#	Code	Strain	Origin	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>	Dairy industry	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>	Milk powder	15	12	31	30
8	R123	<i>Enterobacter sakazakii</i>	CIP 103183	86	79	126	125
9	I2	<i>Escherichia coli</i>	Grated carrots	101	119	63	59
10	I23	<i>Escherichia coli</i>	Dairy industry	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>	Tabbouleh	29	33	58	53
15	R14	<i>Hafnia alvei</i>	CNRZ 713	31	37	7	17
16	I17	<i>Klebsiella oxytoca</i>	Soy salad	38	46	53	53
17	I6	<i>Klebsiella pneumoniae</i>	Pastry	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	Dairy industry	84	79	77	73
27	S7	<i>Salmonella</i> Hadar	Merguez	36	40	52	35
28	S65	<i>Salmonella</i> Javiana	Dried mushrooms	65	50	76	92
29	P35	<i>Salmonella</i> Typhimurium	Pork throat	31	33	47	30
30	R120	<i>Escherichia coli</i> O:157.H7	CIP 105917	14	12	11	19

#	Code	Strain	Origin	REBECCA+EB		EN ISO 21528-2	TSA
				Pour plate	Surface		
31	SWS017	<i>Ewingella americana</i>	DSMZ 4581	9	8	8	9
32	VRY654	<i>Providencia burhododranaria</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>	Food	55	50	53	56
35	RKL458	<i>Yersinia enterocolitica</i>	Whipped cream pastry	24	20	25	30
36	WDP406	<i>Enterobacter hormachaei</i>	DSMZ 101093	28	31	32	64
37	GAR051	<i>Escherichia coli</i>	Andouillette	56	61	69	87
38	GBL293	<i>Escherichia coli</i>	Uncooked chocolate biscuit	37	40	42	85
39	DBP642	<i>Enterobacter cloacae</i>	Vanilla ice-cream	16	14	14	18
40	AER835	<i>Serratia liquefaciens</i>	Raw rib of beef	63	76	9	66
41	AAY895	<i>Enterobacter asburiae</i>	Powdered infant formula	33	40	38	41
42	DEC391	<i>Klebsiella pneumonia</i>	Strawberry bavaroise mousse	26	14	19	55
43	VBT249	<i>Enterobacter cloacae</i>	Lactoserum	43	34	40	50
44	NAB548	<i>Proteus</i> sp.	Egg product industry environment	56	44	54	45
45	QAJ806	<i>Escherichia vulneris</i>	Mulberry extract	18	17	<1	62
46	RAX819	<i>Citrobacter youngae</i>	Ground beef	31	13	36	56
47	ARP296	<i>Citrobacter brakii</i>	Raw marinated pork meat	9	11	8	46
48	FAT267	<i>Leclercia adecarboxylata</i>	Cooked seafood preparation	7	9	4	23
49	EZN508	<i>Escherichia coli</i>	Ground beef	18	19	19	38
50	XAL298	<i>Serratia odorifera</i>	Marinated salmon	15	17	<1	40

## APPENDIX G

### Exclusivity - Raw results

#	Code	Strain	Origin	REBECCA+ EB	EN ISO 21528-2
1	I5	<i>Acinetobacter baumannii</i>	Cheese sandwich	<1	<1
2	I24	<i>Alcaligenes xyloxydans</i>	Dairy industry	<1	<1
3	R200	<i>Enterococcus faecalis</i>	ATCC 33186	<1	<1
4	I29	<i>Enterococcus faecium</i>	Dairy industry	<1	<1
5	I16	<i>Pseudomonas aeruginosa</i>	Cheese omelet	<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>	Dairy industry	<1	<1
10	I35	<i>Brevibacterium casei</i>	Dairy product	<1	<1
11	I31	<i>Hansenula anomala</i>	Dairy industry	<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>	Dairy product	<1	<1
15	P51	<i>Pseudomonas aeruginosa</i>	Ground beef	<1	<1
16	P52	<i>Pseudomonas aeruginosa</i>	Beef fillet	<1	<1
17	I30	<i>Micrococcus luteus</i>	Dairy industry	<1	<1
18	I21	<i>Bacillus circulans</i>	Dairy industry	<1	<1
19	I22	<i>Bacillus subtilis</i>	Pudding	<1	<1
20	I36	<i>Aeromonas aerophila</i>	Smoked salmon	<1	<1

#	Code	Strain	Origin	TSA	EN ISO 21528-2	R+EB pour plate	R+EB surface
21	SAE286	<i>Acinetobacter baumannii</i>	Powdered infant formula	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>	Marinated salmon	610	<10	<10	<10
25	AAZ671	<i>Pseudomonas fragi</i>	Red berries crémeux	510	<10	<10	<10
26	BDK055	<i>Pseudomonas fluorescens</i>	Meat product	600	<10	<10	<10
27	DAR118	<i>Rhodococcus erythropolis</i>	Unpeeled cucumber dices	610	<10	<10	<10
28	ANW492	<i>Lactobacillus paracasei</i>	Dairy product (ACTALIA)	500	<10	<10	<10
29	BHL731	<i>Stenotrophomonas maltophilia</i>	St-Nectaire cheese rind (ACTALIA)	1000	<10	<10	<10
30	YFJ492	<i>Carnobacterium divergens</i>	Salmon steak	560	<10	<10	<10

\*: very small pink colonies

**Raw results - Enterobacteriaceae**

**Level 0**

Laboratories	Reference method (ISO 21528-2)													
	Sample 2							Sample 8						
	-1		-2		-3		Results (UFC/mL)	-1		-2		-3		Results (UFC/mL)
	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	
A	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
B	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
C	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
D	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
F	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
G	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
H	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
I	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
J	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10
Expert laboratory	<1	<1	<1	<1	<1	<1	<10	<1	<1	<1	<1	<1	<1	<10

Laboratories	Alternative method (REBECCA + EB - blue to purple colonies + pink to red colonies)							
	Sample 2				Sample 8			
	-1	-2	-3	Results (UFC/mL)	-1	-2	-3	Results (UFC/mL)
A	<1	<1	<1	<10	<1	<1	<1	<10
B	<1	<1	<1	<10	<1	<1	<1	<10
C	<1	<1	<1	<10	<1	<1	<1	<10
D	<1	<1	<1	<10	<1	<1	<1	<10
F	<1	<1	<1	<10	<1	<1	<1	<10
G	<1	<1	<1	<10	<1	<1	<1	<10
H	<1	<1	<1	<10	<1	<1	<1	<10
I	<1	<1	<1	<10	<1	<1	<1	<10
J	<1	<1	<1	<10	<1	<1	<1	<10
Expert laboratory	<1	<1	<1	<10	<1	<1	<1	<10

**Raw results - Enterobacteriaceae**

**Level 1**

Initial contamination: 62 Enterobacteriaceae per mL

Laboratories	Reference method (ISO 21528-2)													
	Sample 4							Sample 7						
	-1		-2		-3		Results (UFC/mL)	-1		-2		-3		Results (UFC/mL)
	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	
A	5 <sup>a</sup>	4	<1	<1	<1	<1	45	7 <sup>a</sup>	2	2	<1	<1	<1	45
B	10	8	1	<1	<1	<1	90	7	5	1	<1	<1	1	60
C	4	2	<1	1	<1	<1	30	6	8	<1	2	<1	<1	70
D	4	2	<1	<1	<1	<1	30	6	1	<1	2	<1	<1	35
F	6	6	2	1	<1	<1	60	2	3	1	2	<1	<1	25
G	8	5	1	1	<1	<1	65	3	7	<1	<1	<1	<1	50
H	3	4	1	<1	<1	<1	35	4	4	1	<1	<1	<1	40
I	5	2	1	<1	<1	<1	35	11	4	<1	<1	<1	<1	75
J	4	1	1	<1	<1	<1	25	6	5	<1	<1	<1	<1	55
Expert laboratory	4	3	<1	<1	<1	<1	35	6	5	<1	<1	<1	<1	55

Laboratories	Alternative method (REBECCA + EB - blue to purple colonies + pink to red colonies)									
	Sample 4					Sample 7				
	-1	-2	-3	Results (UFC/mL)	-1	-2	-3	Results (UFC/mL)		
	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1			
A	3 <sup>a</sup>	1	<1	30	8 <sup>a</sup>	1	<1	80		
B	1	<1	<1	10	2	<1	<1	20		
C	4	1	<1	40	6	<1	<1	60		
D	6	<1	<1	60	4	1	<1	40		
F	5	<1	<1	50	6	<1	<1	60		
G	5	<1	<1	50	6	<1	<1	60		
H	10	1	<1	100	4	<1	<1	40		
I	5	<1	<1	50	4	<1	<1	40		
J	1	<1	<1	10	4	<1	<1	40		
Expert laboratory	2	1	<1	20	4	<1	<1	40		

a: estimated number

**Raw results - Enterobacteriaceae**

**Level 2**

Initial contamination: 840 Enterobacteriaceae per mL

Laboratories	Reference method (ISO 21528-2)													
	Sample 5							Sample 6						
	-1		-2		-3		Results (UFC/mL)	-1		-2		-3		Results (UFC/mL)
	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	
A	46	49	8	6	1	1	500	53	43	8	5	<1	<1	500
B	48	62	9	10	1	<1	590	46	48	7	9	<1	1	500
C	61	56	7	3	<1	<1	580	44	39	3	6	<1	<1	420
D	50	59	7	5	2	<1	550	51	64	7	7	2	<1	590
F	60	58	10	2	1	<1	590	55	49	10	3	1	<1	530
G	50	61	9	6	<1	<1	570	56	56	3	6	1	<1	550
H	59	66	5	6	<1	<1	620	54	52	8	3	1	<1	530
I	42	52	5	3	<1	<1	460	46	43	4	4	1	<1	440
J	38	35	3	3	1	<1	360	47	40	5	4	1	<1	440
Expert laboratory	51	51	4	4	2	2	500	41	54	8	4	1	<1	490

Laboratories	Alternative method (REBECCA + EB - blue to purple colonies + pink to red colonies)							
	Sample 5				Sample 6			
	-1	-2	-3	Results (UFC/mL)	-1	-2	-3	Results (UFC/mL)
	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1	
A	50	8	1	530	44	6	<1	460
B	44	2	<1	420	35	3	<1	350
C	32	8	1	360	33	1	<1	310
D	63	<1	1	570	55	<1	<1	500
F	58	5	<1	570	41	4	<1	410
G	50	4	<1	490	47	6	<1	480
H	42	8	<1	460	48	6	1	490
I	37	4	<1	370	36	11	1	430
J	38	3	<1	370	41	3	<1	400
Expert laboratory	51	4	<1	500	45	3	<1	440

**Raw results - Enterobacteriaceae**

**Level 3**

Initial contamination: 8300 Enterobacteriaceae per mL

Laboratories	Reference method (ISO 21528-2)													
	Sample 1							Sample 3						
	-1		-2		-3		Results (UFC/mL)	-1		-2		-3		Results (UFC/mL)
	Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2		Plate 1	Plate 2	Plate 1	Plate 2	Plate 1	Plate 2	
A	>150	>150	59	54	6	4	5600	>150	>150	54	26	7	5	4200
B	>150	>150	48	48	6	7	5000	>150	>150	53	39	2	3	4400
C	>150	>150	77	32	1	9	5400	>150	>150	53	54	5	8	5500
D	>150	>150	48	72	8	7	6100	>150	>150	65	71	6	4	6600
F	>150	>150	58	53	9	2	5600	>150	>150	60	49	12	3	5600
G	>150	>150	57	56	4	4	5500	>150	>150	58	51	7	12	5800
H	>150	>150	58	51	7	7	5600	>150	>150	48	50	16	6	5500
I	>150	>150	51	37	4	7	4500	>150	>150	41	51	10	8	5000
J	>150	>150	52	52	7	4	5200	>150	>150	43	55	4	4	4800
Expert laboratory	>150	>150	60	57	5	3	5700	>150	>150	53	46	6	6	5100

Laboratories	Alternative method (REBECCA + EB - blue to purple colonies + pink to red colonies)							
	Sample 1				Sample 3			
	-1	-2	-3	Results (UFC/mL)	-1	-2	-3	Results (UFC/mL)
	Boîte 1	Boîte 1	Boîte 1		Boîte 1	Boîte 1	Boîte 1	
A	>150	43	2	4100	>150	56	8	5800
B	>150	42	8	4600	>150	47	1	4400
C	>150	38	1	3600	>150	53	3	5100
D	>150	46	4	4600	>150	55	6	5600
F	>150	51	5	5100	>150	42	6	4400
G	>150	62	5	6100	>150	44	5	4500
H	>150	61	4	5900	>150	53	3	4100
I	>150	51	6	5200	>150	50	3	4800
J	>150	42	2	4000	>150	36	3	3600
Expert laboratory	>150	40	6	4200	>150	39	3	3800