

NF VALIDATION
Validation of alternative analytical methods
Application in food microbiology

Summary report

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

TEMPO® CAM method
for the enumeration of thermotolerant *Campylobacter*
in raw poultry and ready-to-cook poultry products

Quantitative method

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This report consists of 50 pages, including 11 appendices.

Only copies including the totality of this report are authorised.

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

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

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

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

Validation protocols	<ul style="list-style-type: none"> ▪ ISO 16140-1 (2016): Microbiology of the food chain - Method validation - <i>Part 1: Vocabulary</i> ▪ ISO 16140-2 (2016): Microbiology of the food chain - Method validation - <i>Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method</i> ▪ AFNOR Technical Rules (PR Revision 7)
Reference method[♦]	<p>ISO 10272-2:2017 - Microbiology of the food chain - Horizontal method for detection and enumeration of <i>Campylobacter</i> spp. - Part 2: Colony-count technique</p> <p>ISO 10272-2:2017 / Amd 1: 2023- Microbiology of the food chain - Horizontal method for detection and enumeration of <i>Campylobacter</i> spp. - Part 2: Colony-count technique – Amendment 1: Inclusion of methods for molecular confirmation and identification of thermotolerant <i>Campylobacter</i> spp. and correction of the performance testing of the media</p>
Alternative method	TEMPO® CAM for the enumeration of thermotolerant <i>Campylobacter</i>
Scope	Raw poultry and ready-to-cook poultry products
Certification organism	AFNOR Certification (http://nf-validation.afnor.org/)

[♦] Analyses performed according to the COFRAC accreditation

1 INTRODUCTION

The **TEMPO® CAM method** was validated in March 2020 (certificate number BIO 12/43-04/20) for the enumeration of thermotolerant *Campylobacter* in raw poultry and ready-to-cook poultry products according to the ISO 16140-2:2016.

The method was renewed in February 2024.

2 METHOD PROTOCOLS

2.1 Alternative method

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

2.1.1 Principle

The TEMPO® CAM test consists of a vial of culture medium and a card which are specific to this test.

The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred by the TEMPO® Filler instrument into the card containing 48 wells of three different volumes. The card contains 3 sets of 16 wells (small, medium and large wells) with a one log difference in volume for each set of wells. The card is designed to simulate the Most Probable Number (MPN) method. The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling.

The card is incubated in a microaerobic atmosphere as recommended for *Campylobacter* growth. The microorganisms present in the card reduce the substrate in the culture medium during incubation and cause a fluorescent signal to appear, which is detected by the TEMPO® Reader instrument. Depending on the number and type of the positive wells, the TEMPO® system calculates the number of microorganisms present in the original sample according to a calculation based on the MPN method.

2.1.2 Protocol

- Preparation of primary dilution in Buffered Peptone Water (BPW) in a TEMPO® bag filter;

- Preparation of subsequent dilutions if necessary using BPW;
- Reconstitution of the culture medium (TEMPO® CAM culture medium) by dispensing 3 mL or 3.9 mL of secondary diluent (sterile water) per vial; mix using a vortex;
- Transfer of 1 mL of primary dilution or 0.1 mL of subsequent dilutions into the vial containing the reconstituted culture medium; mix gently without using vortex in order to avoid oxygenation of the media;
- Fill and seal the TEMPO® cards using the TEMPO® Filler;
- Incubation of the cards for 44 - 48 h at 41.5°C ± 1°C in jars in microaerobic atmosphere;
- Read the cards using the TEMPO® Reader. No confirmation is needed.

2.1.3 Restrictions

Campylobacter upsaliensis is excluded from the scope of the validation.

2.2 Reference method♦

The reference method corresponds to the:

- ISO 10272-2 (2017) - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique (See **Appendix 2**).
- -ISO 10272-2:2017 / Amd 1: 2023- Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique – Amendment 1: Inclusion of methods for molecular confirmation and identification of thermotolerant *Campylobacter* spp. and correction of the performnace testing of the media

Changes linked to this Amendment are considered as minor and thus with no impact on the alternative method validations.

3 METHOD COMPARISON STUDY

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

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

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

3.1 Relative trueness study

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

3.1.1 Number and nature of the samples

One food category was tested. The repartition per type is provided in **Table 1**.

Table 1 – Category and types

Category	Type	Number of analysed samples	Number of interpretable results
Raw poultry and ready-to-cook poultry products	Raw non-processed poultry meat	12	5
	Poultry neck skin	12	6
	Seasoned poultry	9	6
Total		33	17

33 samples were analysed, leading to 17 exploitable results by both methods. The TEMPO Cards were read after 44 h incubation time at 41.5°C, stored for 48 h at 5°C ± 3°C and read again.

3.1.2 Artificial and natural contamination of the samples

Artificial contaminations were realized by seeding protocols. The inoculated strains, the contamination protocols, the injured protocols of the inoculated cells are provided in **Appendix 3**.

5 samples were naturally contaminated, and 12 samples were artificially contaminated, they all gave interpretable results.

29.4 % of the samples giving exploitable results by both methods were naturally contaminated.

3.1.3 Raw data

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

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

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

- Interpretable results with the reference and the alternative methods.
- Results with less than 4 colonies per plate with the reference (indicated with “*” in the data) in order to have a more precise result. These results are not included in the calculation.
- Results below or above the quantification limit: according to the ISO 16140-2:2016, if any result (either reference or alternative method) is below the quantification limit, the data should be plotted using a substituted value of 1 log₁₀ units less than the observed value in case of a lower than value. Similarly, any value greater than the upper limit should be amended by adding 1 log unit. These results are not included in the calculations but also appear on the graphs.
- Samples with no result (ND).

Table 2 - Classification of the data

Category	Type	Number of samples analysed	Number of samples with <4 CFU/plate	Number of samples below or above the detection limit	Number of samples with interpretable results	
Raw poultry and ready-to-cook poultry products	a	Raw non-processed poultry meat	12	0	7	5
	b	Poultry neck skin	12	3	3	6
	c	Seasoned poultry	9	0	3	6
Total		33	3	13	17	

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

Table 3 - Samples which were not used in the calculations

Year of analysis	N° Sample	Product	ISO 10272-2*	TEMPO CAM 44 h at 41.5°C	TEMPO CAM 44 h at 41.5°C + 48 h 5°C	Type
			Log CFU/g	Log CFU/g	Log CFU/g	
2019	4984	Raw cockerel	1,30*	<1,00	<1,00	a
2019	4985	Raw guinea fowl fillet	<1,00	1,85	1,85	a
2019	4986	Raw chicken garlic	<1,00	<1,00	<1,00	a
2019	4987	Raw guinea fowl leg	<1,00	<1,00	<1,00	a
2019	4988	Raw turkey cutlet	<1,00	<1,00	<1,00	a
2019	4989	Turkey brochette	<1,00	<1,00	<1,00	a
2019	4990	Minced turkey	<1,00	1,00	1,00	a
2019	5625	Chicken neck skin	<2,00	2,08	2,08	b
2019	5626	Chicken neck skin	1,48*	2,00	2,28	b
2019	5627	Chicken neck skin	1,48*	1,52	1,65	b
2019	5628	Chicken neck skin	<1,00	<1,00	1,00	b
2019	5629	Chicken neck skin	1,30*	1,64	1,93	b
2019	5750	Pigeon neck skin	<1,00	<1,00	<1,00	b
2019	4991	Seasoned chicken leg	<1,00	<1,00	<1,00	c
2019	4992	Marinated chicken	<1,00	<1,00	<1,00	c
2019	4993	Seasoned minced duck	<1,00	<1,00	<1,00	c

*: results obtained with less than 4 colonies on the plate

3.1.4 Statistical interpretation

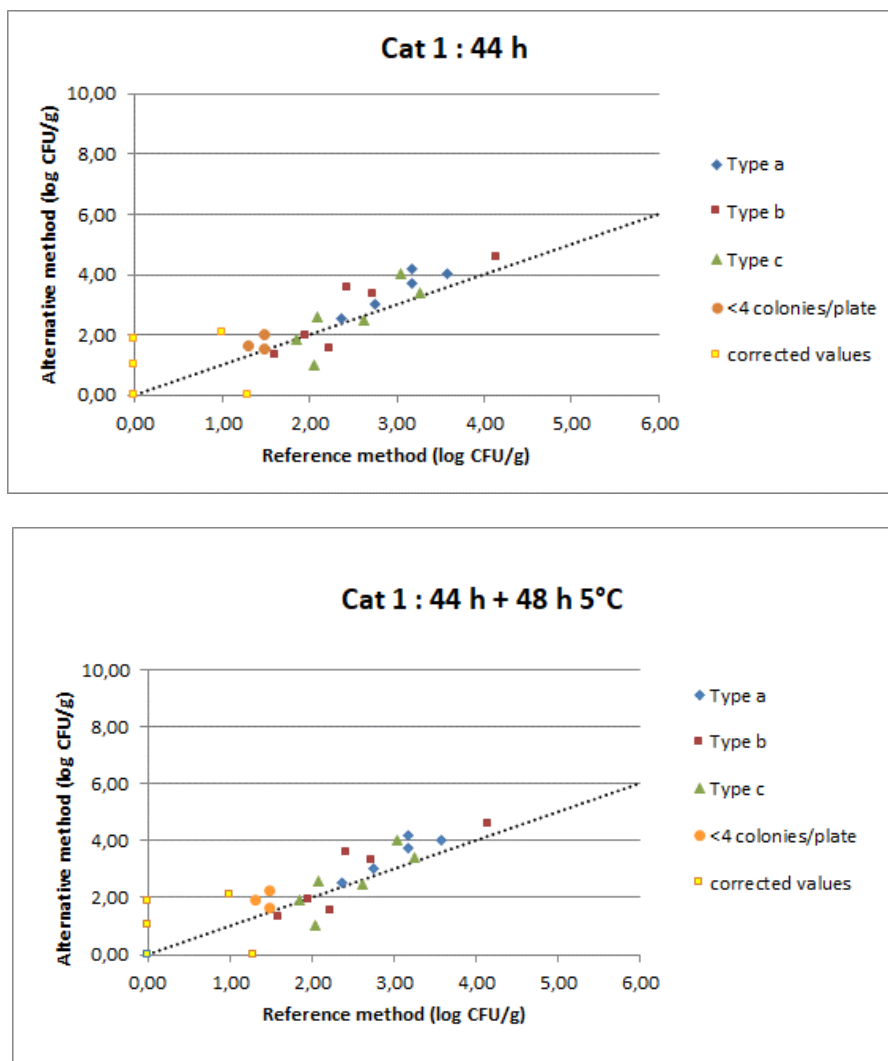
The calculations are provided in **Appendix 5**.

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

The **Figure 1** shows the data plotted for the Raw poultry and ready-to-cook poultry products category after incubation of the cards and after storage of the cards for 48 h at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.

♦ Analyses performed according to the COFRAC accreditation

Figure 1 - Data plotted for the Raw poultry and ready-to-cook poultry products



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

Table 4 - Calculated values

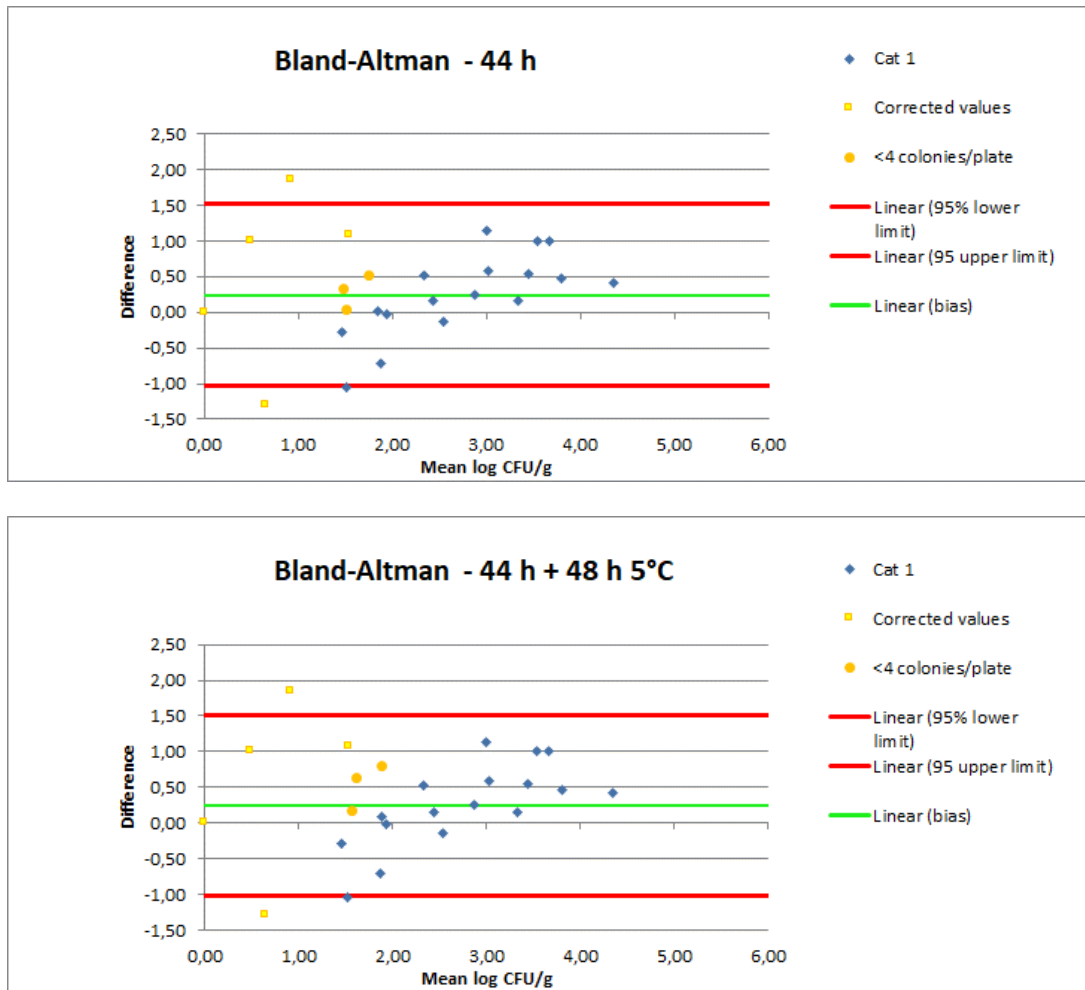
Category		Incubation time	n	\bar{D}	SD	95% lower limit	95% upper limit
1	Raw poultry and ready to cook poultry products	44 h at 41.5°C	17	0,24	0,58	-1,03	1,51
		44 h at 41.5°C + 48 h 5°C	17	0,24	0,58	-1,02	1,51

\bar{D} : Average difference

SD: Standard deviation of differences

The Bland-Altman difference plot for all the samples is given **Figure 2**.

Figure 2 – Bland-Altman difference plot



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

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

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

TEMPO CAM for 44 h at 41.5°C and for 44 h at 41.5°C + 48 h at 5°C ± 3°C										
Classification of the data	Category	Type	N° Sample	Product	Reference method	Alternative method	Values before correction (Reference or/and alternative method)	Mean	Difference	LCL / UCL
< or > quantification limit	1	a	4984	Raw cockerel	1,30	0,00	1,00	0,65	-1,30	-1,03
		a	4985	Raw guinea fowl fillet	0,00	1,85	1,00	0,93	1,85	/ 1,51

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

3.1.5 Discordant results

The number of samples outside the 95 % confidence limit is given in **Table 6**.

Table 6 - Number of samples outside the 95 % Confidence Limit

		Number of samples	
		44 h	44 h + 48 h
Interpretable results by both methods	< LCL	0	0
	> UCL	0	0
	Total	0	0
<4 CFU/plate	< LCL	0	0
	> UCL	0	0
	Total	0	0
< or > the quantification limit	< LCL	1	1
	> UCL	1	1
	Total	2	2
Total < LCL		1	1
Total >UCL		1	1
Total		2	2

For some samples, enumeration differences were observed between the reference and the TEMPO CAM methods. The samples concerned are listed in **Table 7**.

Table 7 – Samples with discordant results

N° sample	Product	ISO 10272-2*	TEMPO CAM
4985	Raw guinea fowl fillet	<1,00	1,85
5563	Guinea fowl leg	3,18	4,18
5567	Chicken pieces meat with paprika	3,04	4,04
5631	Chicken neck skin	2,73	3,32
5751	Quail neck skin	2,43	3,57
5625	Chicken neck skin	<2,00	2,08

In order to confirm that the positive results were linked to the presence of *Campylobacter* strains in the wells, a VIDAS CAM test was performed on each of the positive well in the TEMPO card. Presence of *Campylobacter* was confirmed in all the wells except for sample 4985 (confirmation done more than 2 months after the enumeration) indicating that the TEMPO CAM better recovered the *Campylobacter* strains.

3.1.6 Conclusion

The relative trueness study of the alternative method is satisfying.

3.2 Accuracy profile study

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

3.2.1 Matrices

One matrix was tested with three contamination levels and five test portions per level. The tested category, type, matrix and inoculated strain are provided in **Table 8**.

Table 8 – Category, type and matrix

Category	Type	Matrix	Strain	Target inoculation level (CFU/g)
Raw poultry and ready-to-cook poultry products	a	Chicken breast	<i>Campylobacter jejuni</i> Ad1015	100
				1000
				10000

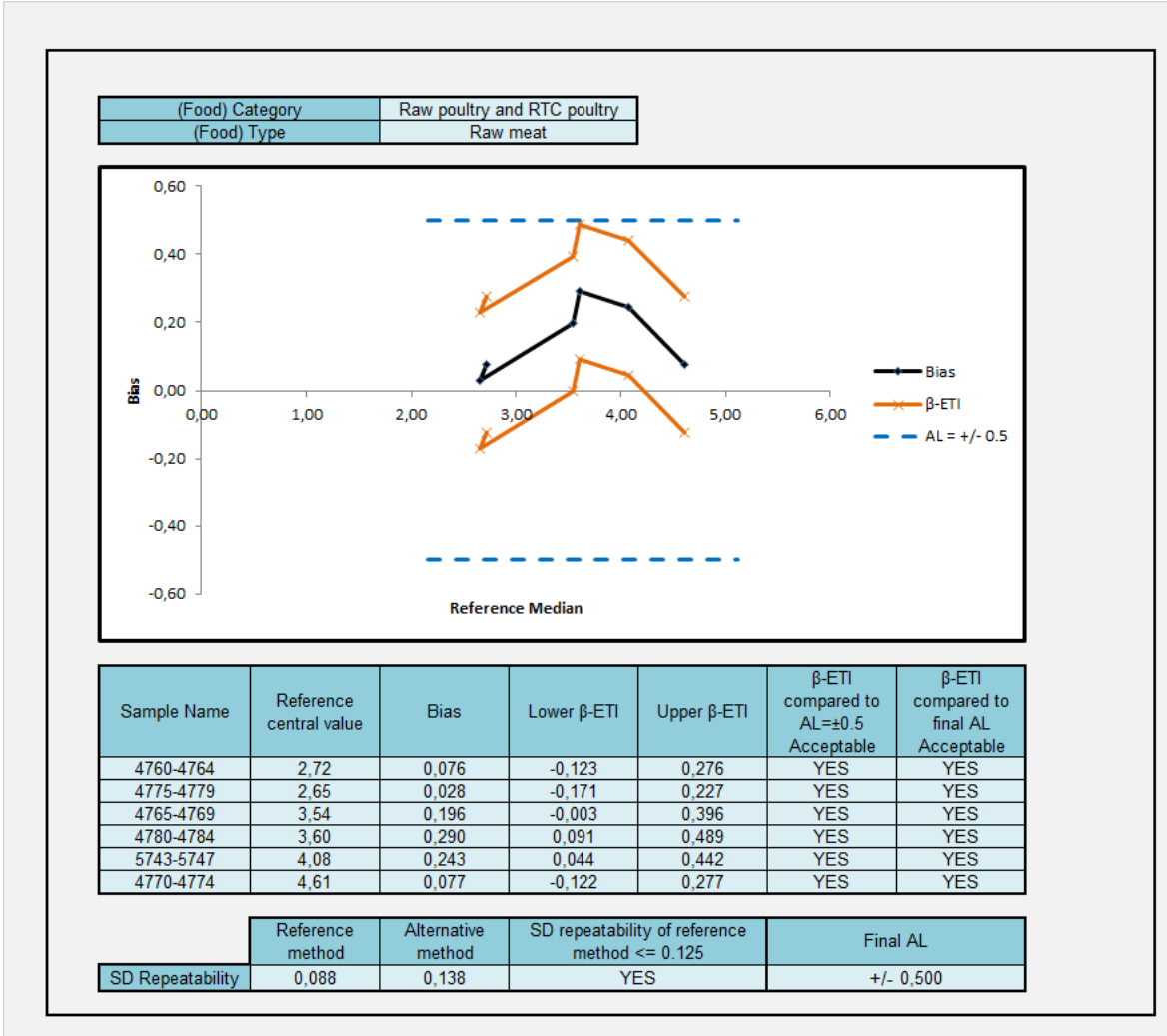
* Analyses performed according to the COFRAC accreditation

3.2.2 Calculation and interpretation

The raw data are provided in **Appendix 6**. The summary tables (in log CFU/g) and calculations are provided in **Appendix 7**. The statistical results and the accuracy profiles are provided **Figure 3**.

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

Figure 3 – Accuracy profile



3.2.3 Conclusion

The observed profile is comprised within the AL. The accuracy profile fulfils the performance criteria.

3.3 Quantification limits (LOQ)

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

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

3.3.1 Experimental design

Blank samples were tested for chicken breast (same matrix as used for the accuracy profile). These blank samples were used to verify the limit of quantification of the alternative method. 10 test portions from the same sample were analysed.

3.3.2 Calculation and interpretation

The threshold standard deviation S_0 was calculated as followed:

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

where:

n = the total number of test portions used

y_j = the log transformed result of test portion j

y = the average log transformed result of all test portions

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

3.3.3 Results

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

Table 9 - Quantification limits per tested matrix

Matrix	S_0	LOQ
Chicken breast	0	0

3.4 Inclusivity and exclusivity studies

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.

3.4.1 Protocol

After growth according to appropriate conditions (CFB broth 20 h to 24 h at 41.5°C in microaerobic atmosphere for target strains), decimal dilutions were realised and enumerated once by the TEMPO® CAM method, onto mCCDA plates and onto Columbia Blood Agar plates or appropriate non-selective agar plate for non-target strains.

3.4.2 Results

The raw data are provided in **Appendix 9**.

> **Inclusivity**

The 50 target strains were enumerated with the TEMPO® CAM method giving generally higher enumeration than with CBA (Columbia Blood Agar) and mCCDA plates. The recovery of the *Campylobacter* cells seems better with TEMPO® CAM than onto plates.

> **Exclusivity**

30 non-target strains were tested. One strain was enumerated with the TEMPO® CAM method: *Ralstonia mannitolityca* Ad1059.

Two other strains have been tested: *Ralstonia mannitolityca* DSM17512 was also enumerated with the TEMPO® CAM method but not the strain *Ralstonia sp.* Ad2504.

3.5 Practicability

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

<i>Storage conditions, shelf-life and modalities of utilisation after first use</i>	The storage temperatures are between 2 and 25°C (room temperature before analysis) depending on the materials and the reagents.		
<i>Time to result</i>	Steps	Reference method	Alternative method
	Negative samples		
	Sampling	Day 0	Day 0
	Reading	Day 2	Day 2
	Presumptive positive or positive results		
	Sampling	Day 0	Day 0
	Reading	Day 2	Day 2
	Confirmation	Day 5 - Day 6	/
<i>Common step with the reference method</i>	Initial suspension		

The results are available in 2 days with the TEMPO® CAM method (positive and negative). The negative results are available in 2 days for negative samples with the reference method and in 5 - 6 days for the presumptive and positive results.

4 INTER-LABORATORY STUDY

The inter-laboratory 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.

4.1 Study organisation

Samples were sent to 13 laboratories; 2 collaborators were involved in two labs (A and F). Minced poultry meat was inoculated by *Campylobacter jejuni* Ad1000, isolated from poultry.

Samples were prepared and inoculated on Monday 20th January 2020, as described below:

- 7 minced poultry meat codified samples (10 g) for enumeration of *Campylobacter* spp. by the ISO 10272-2 reference method and TEMPO CAM method,
- 1 sample 10 g (labelled “Sample for Total Count enumeration”) for Aerobic mesophilic flora enumeration by the ISO 4833-1 method,
- 1 water flask labelled “Temperature Control” with a temperature probe.

The targeted inoculation levels were the following:

- Level 0: < 10 CFU/g,
- Level 1: 100 - 1000 CFU/g,
- Level 2: 1000 – 10000 CFU/g,
- Level 3: 10000 – 100000 CFU/g.

Labelling and shipping

Blind coded samples were placed in isothermal boxes, which contained cooling blocks, and express-shipped to the different laboratories.

A temperature control flask containing a sensor was added to the package in order to register the temperature profile during the transport, and storage until analyses.

Samples were shipped in 24 h to the involved laboratories. The temperature conditions had to stay lower or equal to 8°C during transport, and between 0°C and 8°C in the labs.

Collaborative study laboratories and the expert laboratory carried out the analyses on Wednesday 22nd January 2020 with the alternative and reference methods. **The analyses by the reference method and the alternative method were performed on the same day.**

4.2 Experimental parameters controls

4.2.1 Sample stability

In order to evaluate the *Campylobacter jejuni* strain behaviour during transport, bacterial counts were done at different time, i.e. inoculation time, after 24 h and 48 h of storage at 2°C. Results are reported in **Table 10**.

Table 10 – Stability of the strain in the matrix

Day of analysis	Inoculation level	Reference method: ISO 10272-2	Alternative method: TEMPO CAM
Day 0	Level 1	1400	810
		1100	2300
		1100	1200
	Level 2	12 000	15000
		11 000	12000
		10 000	21 000
	Level 3	110000	68000
		120000	250000
		150 000	170 000
Day 1	Level 1	1200	900
		920	1000
		870	530
	Level 2	5200	15000
		1900	6000
		6 300	7 800
	Level 3	57000	170000
		62000	91000
		4 2000	50000
Day 2	Level 1	1100	580
		780	640
	Level 2	5100	6000
		8800	6800
	Level 3	120000	40000
		130000	60000

No evolution was observed during 48 h storage at 3°C ± 2°C.

4.2.2 Logistic conditions

The temperatures measured at reception by the Labs, the temperatures registered by the thermo-probe, and the receipt dates are given in **Table 11**.

Table 11 - Sample temperatures at receipt

Collaborators	Temperature measured by the probe (°C)	Temperature measured at receipt (°C)	Receipt date and time		Analysis date
A1	0.0	1.1	21/01/2020	10 h 10	22/01/2020
A2	0.5	1.6	21/01/2020	10 h 10	22/01/2020
B	1.0	1.1	21/01/2020	14 h 00	22/01/2020
C	1.5	5.8	21/01/2020	7 h 25	22/01/2020
D	1.0	1.4	21/01/2020	11 h 30	22/01/2020
E	2.0	2.8	21/01/2020	13 h 00	22/01/2020
F1	1.0	3.0	21/01/2020	9 h 30	22/01/2020
F2	1.0	2.0	21/01/2020	9 h 30	22/01/2020
G	0.5	3.8	21/01/2020	10 h 45	22/01/2020
H	2.0	4.6	21/01/2020	11 h 00	22/01/2020
I	1.5	6.0	21/01/2020	8 h 00	22/01/2020
J	1.0	3.4	21/01/2020	8 h 45	22/01/2020
K	1.5	1.0	21/01/2020	17 h 00	22/01/2020
L	1.5	4.0	21/01/2020	13 h 00	22/01/2020
M	1.0	4.1	21/01/2020	13 h 00	22/01/2020

No problem was encountered during the transport or at receipt for the 15 collaborators. All the samples were delivered on time and in appropriate conditions. Temperatures during shipment and at receipt were all correct.

4.2.3 Homogeneity of inoculation

Homogeneity tests were conducted according to the ISO/TS 22117. Ten samples per inoculation level were analysed in duplicate by the reference method. The results are provided in **Appendix 10**. The test concluded that the samples were sufficiently homogeneous for the three contamination levels.

4.3 Result analysis

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

4.3.1 *Aerobic mesophilic enumeration*

The aerobic mesophilic enumeration varied from 1.5×10^6 CFU/g (Lab H) to 2.3×10^7 CFU/g (Lab K).

4.3.2 *Results obtained by the expert Lab.*

The results obtained by the expert Lab. are the following (See **Table 12**).

Table 12 – Results obtained by the expert Lab.

Level	Reference method	Alternative method
L0	< 10	< 10
L1	1100	580
	780	640
L2	5100	6000
	8 800	6 800
L3	120000	40000
	130 000	60 000

The enumeration results correspond to the target inoculation levels.

4.3.3 *Results obtained by the collaborators*

Samples were sent to 15 collaborators.

A summary of the test results is given in **Table 13** (CFU/g) and **Table 14** (log CFU/g).

Table 13 - Summary of data (CFU/g)

Collaborator	Level 0		Level 1				Level 2				Level 3			
	Reference method	TEMPO CAM method	Reference method		TEMPO CAM method		Reference method		TEMPO CAM method		Reference method		TEMPO CAM method	
	Replicate 1	Replicate 1	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
A1	<10	<10	1100	950	930	1300	8500	6400	7800	3700	87000	90000	74000	56000
A2	<10	<10	990	1100	1300	570	7300	6700	3700	6800	120000	72000	110000	49000
B	<10	<10	870	1100	440	400	5500	5800	2800	3400	64000	66000	48000	43000
C	<10	<10	260	470	820	430	1200	1100	6800	21000	120000	36000	78000	91000
D	<10	<10	660	770	830	710	4900	2100	4400	9100	18000	32000	74000	37000
E	<10	<10	1200	1400	1400	730	9000	8800	7400	12000	99000	60000	120000	68000
F1	<10	<10	780	640	950	1300	5100	4100	4400	6800	8000	110000	44000	67000
F2	<10	<10	600	700	830	400	5400	9900	6700	8200	76000	68000	78000	44000
G	<10	<10	830	670	510	260	8500	6300	5500	7800	77000	73000	37000	31000
H	<10	<10	970	960	730	630	8400	9200	7800	5500	95000	91000	50000	110000
I	<10	<10	760	930	1000	480	2800	8000	3700	11000	110000	20000	50000	53000
J	<10	<10	560	680	1100	640	2900	3500	6700	5300	50000	25000	110000	49000
K	<10	<10	310	380	430	1400	3200	3400	5600	5300	18000	14000	30000	49000
L	<10	<10	250	160	570	760	1000	2800	11000	6000	4000	20000	68000	49000
M	<10	<10	1000	860	950	930	7700	9000	4000	5500	130000	100000	120000	78000

Table 14 - Summary of data (log CFU/g)

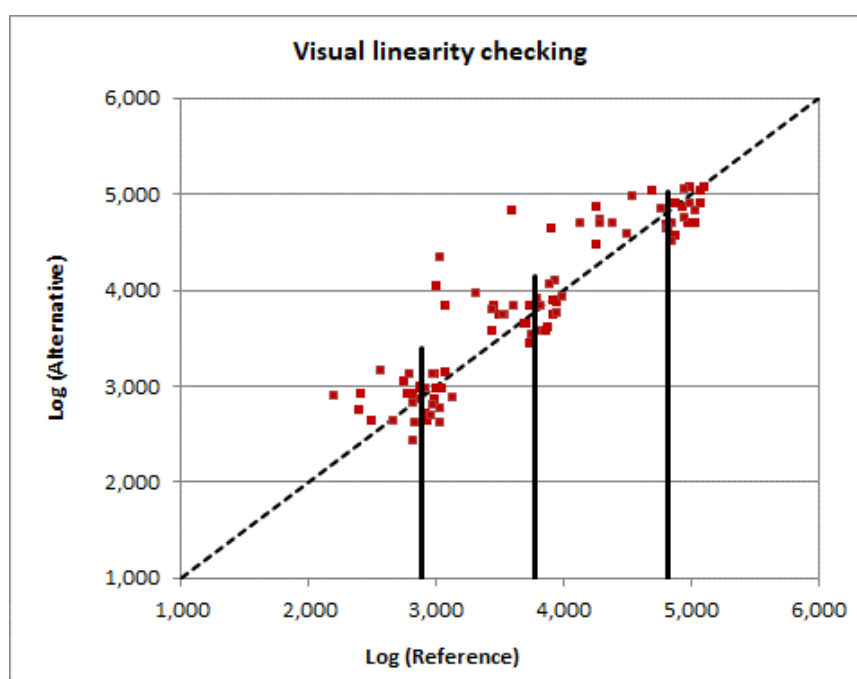
Collaborator	Level 0		Level 1				Level 2				Level 3			
	Reference method	TEMPO CAM method	Reference method		TEMPO CAM method		Reference method		TEMPO CAM method		Reference method		TEMPO CAM method	
	Replicate 1	Replicate 1	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
A1	<1,00	<1,00	3,04	2,98	2,97	3,11	3,93	3,81	3,89	3,57	4,94	4,95	4,87	4,75
A2	<1,00	<1,00	3,00	3,04	3,11	2,76	3,86	3,83	3,57	3,83	5,08	4,86	5,04	4,69
B	<1,00	<1,00	2,94	3,04	2,64	2,60	3,74	3,76	3,45	3,53	4,81	4,82	4,68	4,63
C	<1,00	<1,00	2,41	2,67	2,91	2,63	3,08	3,04	3,83	4,32	5,08	4,56	4,89	4,96
D	<1,00	<1,00	2,82	2,89	2,92	2,85	3,69	3,32	3,64	3,96	4,26	4,51	4,87	4,57
E	<1,00	<1,00	3,08	3,15	3,15	2,86	3,95	3,94	3,87	4,08	5,00	4,78	5,08	4,83
F1	<1,00	<1,00	2,89	2,81	2,98	3,11	3,71	3,61	3,64	3,83	3,90	5,04	4,64	4,83
F2	<1,00	<1,00	2,78	2,85	2,92	2,60	3,73	4,00	3,83	3,91	4,88	4,83	4,89	4,64
G	<1,00	<1,00	2,92	2,83	2,71	2,41	3,93	3,80	3,74	3,89	4,89	4,86	4,57	4,49
H	<1,00	<1,00	2,99	2,98	2,86	2,80	3,92	3,96	3,89	3,74	4,98	4,96	4,70	5,04
I	<1,00	<1,00	2,88	2,97	3,00	2,68	3,45	3,90	3,57	4,04	5,04	4,30	4,70	4,72
J	<1,00	<1,00	2,75	2,83	3,04	2,81	3,46	3,54	3,83	3,72	4,70	4,40	5,04	4,69
K	<1,00	<1,00	2,49	2,58	2,63	3,15	3,51	3,53	3,75	3,72	4,26	4,15	4,48	4,69
L	<1,00	<1,00	2,40	2,20	2,76	2,88	3,00	3,45	4,04	3,78	3,60	4,30	4,83	4,69
M	<1,00	<1,00	3,00	2,93	2,98	2,97	3,89	3,95	3,60	3,74	5,11	5,00	5,08	4,89

4.4 Calculation and interpretation

4.4.1 Visual linearity checking

The **Figure 4** shows the data points after \log_{10} transformation. The visual inspection shows that the alternative method gives results, which are proportional to those of the reference method. The data are distributed closely to the first bisecting lines with a slope equal to 1.

Figure 4 - Visual linearity checking



The dispersion of the points on the graph is mostly related to the heterogeneity of the results observed with the reference method within an inoculation level, with low enumeration observed for some samples. *Examples:*

- Level 1 (target value: 2.8 log): Sample L5: 2.20 log
- Level 2 (target value: 3.7 log): Sample C1: 3.08 log
Sample C7: 3.04 log
Sample L1: 3.00 log
- Level 3 (target value: 4.7 log): Sample L2: 3.60 log

These data confirm the tendency of the reference method to undercount.

4.4.2 Accuracy profile calculation

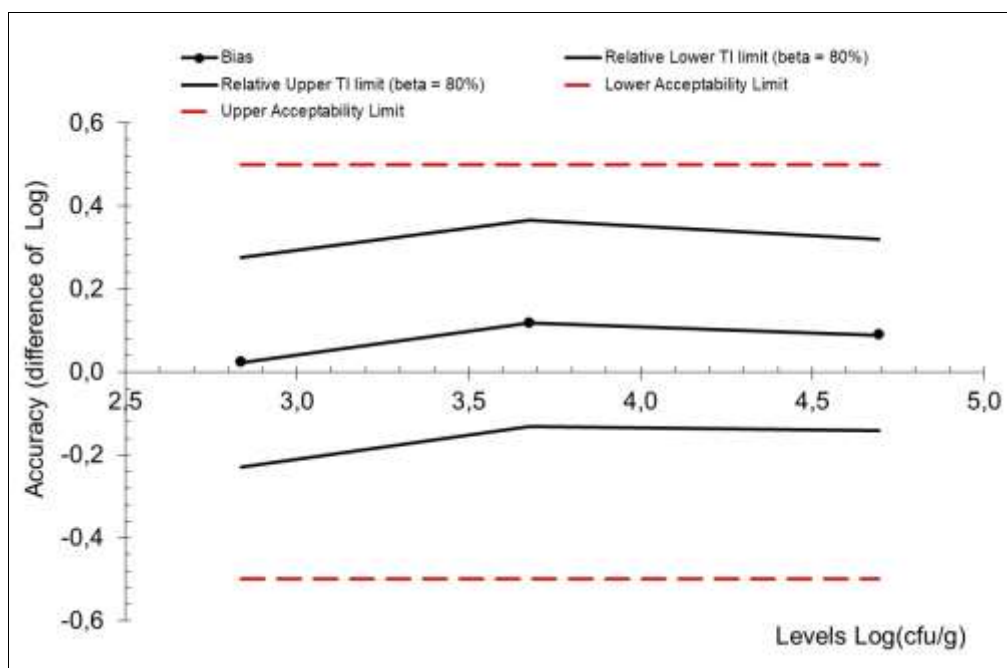
Statistical calculations were done according to the Excel spreadsheet available on <http://standards.iso.org/ISO/16140>. A summary of the statistical test is provided in **Table 15**.

Table 15 - Summary of statistical tests

Accuracy profile		0,5					
Study Name	TEMPO CAM						Application of clause 6.2.3 Step 8: If any of the values for the β -ETI fall outside the acceptability limits, calculate the pooled average reproducibility standard deviation of the reference method. Step 9: Calculate new acceptability limits as a function of this standard deviation.
Date	January 2020						
Coordinator	ADRIA Développement						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	0,50	0,50	0,50				
Levels	Alternative method			Reference method			
	Low	Medium	High	Low	Medium	High	
Target value	2,838	3,677	4,694				
Number of participants (K)	15	15	15	15	15	15	
Average for alternative method	2,860	3,794	4,783	2,838	3,677	4,694	
Repeatability standard deviation (sr)	0,179	0,181	0,157	0,077	0,150	0,310	
Between-labs standard deviation (sL)	0,062	0,048	0,072	0,213	0,248	0,233	
Reproducibility standard deviation (sR)	0,189	0,187	0,173	0,227	0,289	0,388	
Corrected number of dof	28,418	28,708	27,791	15,701	18,242	25,059	
Coverage factor	1,336	1,335	1,338				
Interpolated Student t	1,312	1,312	1,313				
Tolerance interval standard deviation	0,1928	0,1902	0,1761				
Lower TI limit	2,608	3,545	4,552				
Upper TI limit	3,113	4,043	5,014				
Bias	0,023	0,117	0,089				
Relative Lower TI limit (beta = 80%)	-0,230	-0,132	-0,143				
Relative Upper TI limit (beta = 80%)	0,276	0,367	0,320				
Lower Acceptability Limit	-0,50	-0,50	-0,50				
Upper Acceptability Limit	0,50	0,50	0,50				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0,309						

These values are collected in a graphical representation together with the acceptability limits (AL). This representation is given **Figure 5**.

Figure 5 - Accuracy profile



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

The alternative method is considered equivalent to the reference method as β ETI values meet the Acceptability limits fixed at ± 0.5 log for all levels of contamination.

A summary of the obtained values is given **Table 16**.

Table 16 – Summary of the obtained values

	Dataset		
	15		
	Low level	Medium level	High level
Target value	2,838	3,677	4,694
Bias	0,023	0,117	0,089
β .ETI lower (80 %)	-0,230	-0,132	-0,143
β .ETI upper (80 %)	0,276	0,367	0,320
Lower AL	-0,500		
Upper AL	0,500		

4.4.3 Conclusion

The alternative method is equivalent to the reference method for all inoculation levels.

5 CONCLUSION

The **method comparison study conclusions** are:

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

- ☒ **33 samples were tested in the relative trueness study**, which clearly satisfied the required criteria for quantitative method comparison per ISO 16140-2; **this study confirms the possibility as well to store TEMPO cards for 48 h at 5°C ± 3°C.**
- ☒ **The observed profile is comprised within the AL set at 0.5 Log CFU/g in the EN ISO 16140-2:2016.**
- ☒ **A positive bias (0.24 log CFU/g) was observed compared to the ISO 10272-2 standard, due to a better resuscitation of *Campylobacter*.**
- ☒ **The inclusivity and exclusivity testing show satisfying results.**

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

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

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

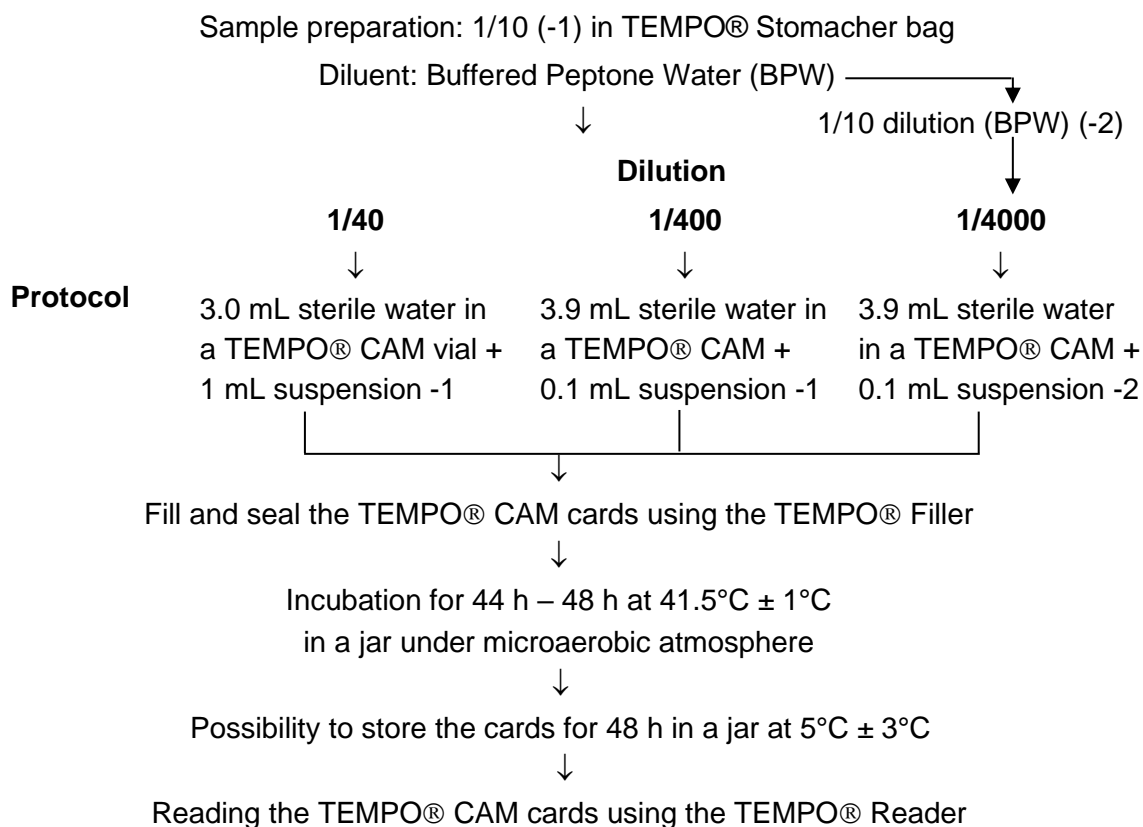
Quimper, 23 February 2024

Astrid CARIOU
Deputy Manager
Validation of Alternative methods

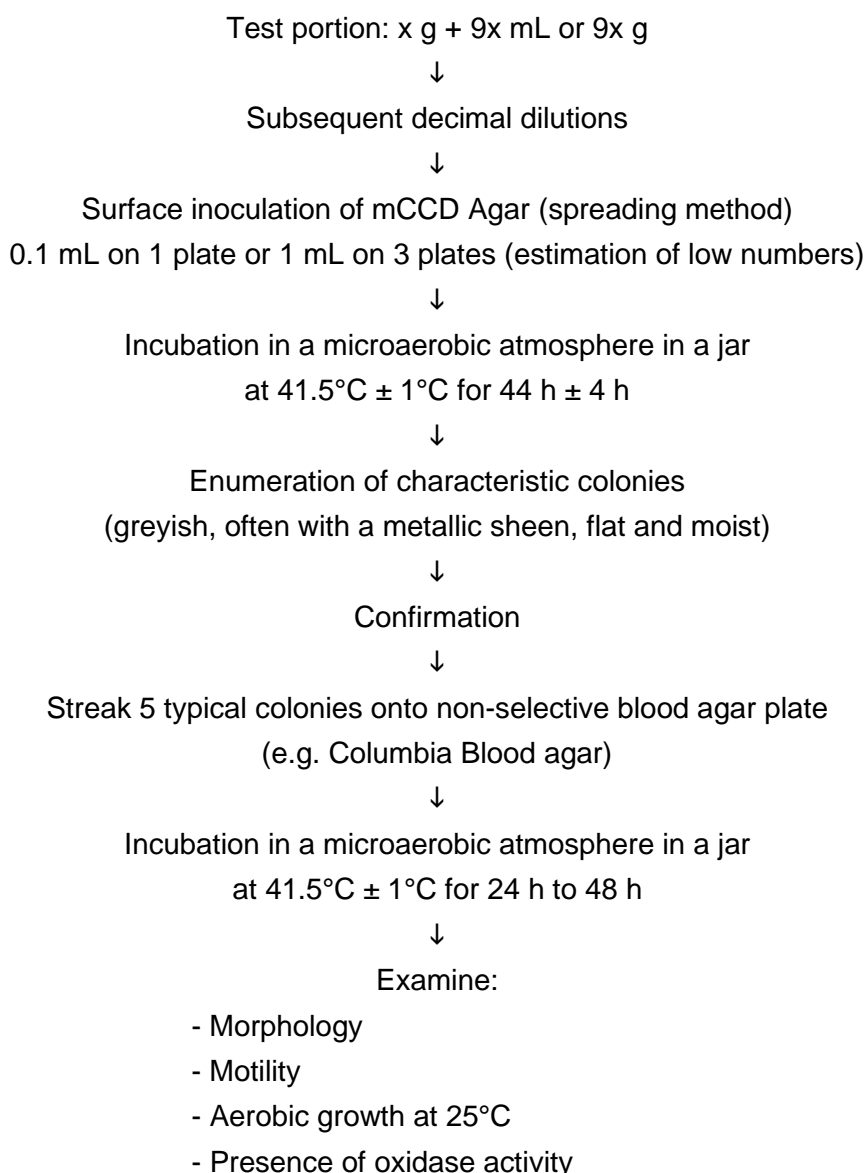


I hereby attest to the validation of the verification of the conformity of the report (opinion and interpretation).

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



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

Appendix 2 – Flow diagram of the reference method**ISO 10272-2 (2017) - Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique****ISO 10272-2:2017 / Amd 1: 2023- Microbiology of the food chain - Horizontal method for detection and enumeration of *Campylobacter* spp. - Part 2: Colony-count technique – Amendment 1: Inclusion of methods for molecular confirmation and identification of thermotolerant *Campylobacter* spp. and correction of the performance testing of the media**

Appendix 3 – Artificial contaminations of samples

Year of analysis	N° Sample	Product	Artificial contaminations			Type
			Strain	Origin	Injury protocol	
2019	5560	Turkey meat	<i>C. coli</i> Ad1072	Poultry neck skin	Seeding 48 h 3±2°C vacuum packaged	a
2019	5561	Turkey meat	<i>C. coli</i> Ad1914	Raw poultry	Seeding 48 h 3±2°C vacuum packaged	a
2019	5562	White cockerel	<i>C. coli</i> Ad1914	Raw poultry	Seeding 48 h 3±2°C vacuum packaged	a
2019	5563	Guinea fowl leg	<i>C. jejuni</i> Ad1951	Poultry	Seeding 48 h 3±2°C vacuum packaged	a
2019	5564	Marinated turkey meat	<i>C. jejuni</i> Ad1951	Poultry	Seeding 48 h 3±2°C vacuum packaged	c
2019	5565	Seasoned chicken wings	<i>C. jejuni</i> Ad1937	Poultry	Seeding 48 h 3±2°C vacuum packaged	c
2019	5566	Seasoned chicken skewers	<i>C. jejuni</i> Ad1937	Poultry	Seeding 48 h 3±2°C vacuum packaged	c
2019	5567	Chicken pieces meat with paprika	<i>C. coli</i> Ad1914	Raw poultry	Seeding 48 h 3±2°C vacuum packaged	c
2019	5568	Chicken pieces meat with paprika	<i>C. jejuni</i> Ad1951	Poultry	Seeding 48 h 3±2°C vacuum packaged	c
2019	5821	Guinea fowl neck skin	<i>C. coli</i> Ad1905	Poultry	Seeding 48 h 3±2°C vacuum packaged	b
2019	5822	Quail leg	<i>C. jejuni</i> Ad1903	Poultry	Seeding 48 h 3±2°C vacuum packaged	a
2019	5823	Chicken wings with paprika	<i>C. jejuni</i> Ad1089	Poultry	Seeding 48 h 3±2°C vacuum packaged	c

Appendix 4 - Relative trueness study: raw data

RAW POULTRY AND READY-TO-COOK POULTRY PRODUCTS																	
Year of analysis	N° Sample	Product	Reference method: ISO 10272-2*						Alternative method: TEMPO CAM								Type
			Dilution	Before confirmation	After confirmation			Log CFU/g	44 h at 41.5°C microaerobic atmosphere				44 h at 41.5°C microaerobic atmosphere + 48 h 4°C microaerobic atmosphere				
				CFU/plate	CFU/plate	CFU/g	CFU/g (rounded)		d 1/40	d 1/4000	Result	Log CFU/g	d 1/40	d 1/4000	Result	Log CFU/g	
2019	4984	Raw cockerel	10	2	2	20	20	1,30*	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	a
			100	0	0												
2019	4985	Raw guinea fowl fillet	10	0	0	<10	<10	<1,00	71	<1000	71	1,85	71	<1000	71	1,85	a
			100	0	0												
2019	4986	Raw chicken garlic	10	1d	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	a
			100	0	0												
2019	4987	Raw guinea fowl leg	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	a
			100	0	0												
2019	4988	Raw turkey cutlet	10	1d	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	a
			100	0	0												
2019	4989	Turkey brochette	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	a
			100	0	0												
2019	4990	Minced turkey	10	0	0	<10	<10	<1,00	10	<1000	10	1,00	10	<1000	10	1,00	a
			100	0	0												
2019	4991	Seasoned chicken leg	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	c
			100	0	0												
2019	4992	Marinated chicken	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	c
			100	0	0												
2019	4993	Seasoned minced duck	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	c
			100	0	0												
2019	5560	Turkey meat	10	25	25	227	230	2,36	330	<1000	330	2,52	330	<1000	330	2,52	a
			100	0	0												
2019	5561	Turkey meat	10	61	61	573	570	2,76	1000	1000	1000	3,00	1000	1000	1000	3,00	a
			100	2	2												
2019	5562	White cockerel	100	14	14	1545	1500	3,18	5300	11000	5300	3,72	5300	11000	5300	3,72	a
			1000	3	3												
2019	5563	Guinea fowl leg	100	15	15	1545	1500	3,18	15000	7100	15000	4,18	15000	7100	15000	4,18	a
			1000	2	2												
2019	5564	Marinated turkey meat	10	13	13	118	120	2,08	400	<1000	400	2,60	400	<1000	400	2,60	c
			100	0	0												
2019	5565	Seasoned chicken wings	10	41	41	410	410	2,61 N'	300	<1000	300	2,48	300	<1000	300	2,48	c
			100	0	0												
2019	5566	Seasoned chicken skewers	100	20	20	1818	1800	3,26	2600	4500	2600	3,41	2600	4500	2600	3,41	c
			1000	0	0												
2019	5567	Chicken pieces meat with paprika	100	12	12	1091	1100	3,04	11000	5700	11000	4,04	11000	8300	11000	4,04	c
			1000	0	0												
2019	5568	Chicken pieces meat with paprika	10	12	12	109	110	2,04	10	<1000	10	1,00	10	<1000	10	1,00	c
			100	0	0												
2019	5625	Chicken neck skin	100	14	0	<100	<100	<2,00	120	<1000	120	2,08	120	<1000	120	2,08	b
			1000	2	0												

* Analyses performed according to the COFRAC accreditation
 ADRIA
 Summary report (Version 0)
 TEMPO CAM

RAW POULTRY AND READY-TO-COOK POULTRY PRODUCTS																	
Year of analysis	N° Sample	Product	Reference method: ISO 10272-2*						Alternative method: TEMPO CAM								Type
			Dilution	Before confirmation	After confirmation			Log CFU/g	44 h at 41.5°C microaerobic atmosphere				44 h at 41.5°C microaerobic atmosphere + 48 h 4°C microaerobic atmosphere				
				CFU/plate	CFU/plate	CFU/g	CFU/g (rounded)		d 1/40	d 1/4000	Result	Log CFU/g	d 1/40	d 1/4000	Result	Log CFU/g	
2019	5626	Chicken neck skin	10	7	3	30	30	1,48*	100	<1000	100	2,00	190	<1000	190	2,28	b
			100	1	0												
2019	5627	Chicken neck skin	10	3	3	30	30	1,48*	33	<1000	33	1,52	45	<1000	45	1,65	b
			100	0	0												
2019	5628	Chicken neck skin	10	0	0	<10	<10	<1,00	<10	<1000	<10	<1,00	10	<1000	10	1,00	b
			100	0	0												
2019	5629	Chicken neck skin	10	4	2	20	20	1,30*	44	<1000	44	1,64	86	<1000	86	1,93	b
			100	2	1												
2019	5630	Chicken neck skin	10	6	4	40	40	1,60 Ne	21	<1000	21	1,32	21	<1000	21	1,32	b
			100	0	0												
2019	5631	Chicken neck skin	10	63	53	536	540	2,73	2100	<1000	2100	3,32	2100	<1000	2100	3,32	b
			100	6	6												
2019	5748	Chicken neck skin	10	10	10	91	90	1,95	86	<1000	86	1,93	86	<1000	86	1,93	b
			100	0	0												
2019	5749	Guinea fowl neck skin	10	18	18	173	170	2,23	33	1000	33	1,52	33	1000	33	1,52	b
			100	1	1												
2019	5750	Pigeon neck skin	10	25	0	<10	<10	<1,00	<10	<1000	<10	<1,00	<10	<1000	<10	<1,00	b
			100	2	0												
2019	5751	Quail neck skin	10	25	25	273	270	2,43	3700	<1000	3700	3,57	3700	<1000	3700	3,57	b
			100	5	5												
2019	5821	Guinea fowl neck skin	1000	15	15	13636	14000	4,15	37000	26000	37000	4,57	37000	26000	37000	4,57	b
			10000	0	0												
2019	5822	Quail leg	100	35	35	3727	3700	3,57	11000	10000	11000	4,04	11000	10000	11000	4,04	a
			1000	6	6												
2019	5823	Chicken wings with paprika	10	7	7	70	70	1,85 Ne	71	<1000	71	1,85	86	<1000	86	1,93	c
			100	1	1												

Appendix 5 - Relative trueness study: calculations

TEMPO CAM 44 h												
Category	Sample N°	Product	Log CFU/g		Average	Difference	Data not included in the interpretation		Average <4 CFU/plate	Difference <4 CFU/plate	Average corrected values	Difference Corrected values
			Reference method	Alternative method			<4 CFU/plate	<or> threshold corrected values				
1	4984	Raw cockerel	1,30		#N/A			0,00	#N/A		0,65	-1,30
	4985	Raw guinea fowl fillet	0,00		#N/A			1,85	#N/A		0,93	1,85
	4986	Raw chicken garlic	0,00		#N/A			0,00	#N/A		0,00	0,00
	4987	Raw guinea fowl leg	0,00		#N/A			0,00	#N/A		0,00	0,00
	4988	Raw turkey cutlet	0,00		#N/A			0,00	#N/A		0,00	0,00
	4989	Turkey brochette	0,00		#N/A			0,00	#N/A		0,00	0,00
	4990	Minced turkey	0,00		#N/A			1,00	#N/A		0,50	1,00
	5560	Turkey meat	2,36	2,52	2,44	0,16			#N/A		#N/A	
	5561	Turkey meat	2,76	3,00	2,88	0,24			#N/A		#N/A	
	5562	White cockerel	3,18	3,72	3,45	0,55			#N/A		#N/A	
	5563	Guinea fowl leg	3,18	4,18	3,68	1,00			#N/A		#N/A	
	5822	Quail leg	3,57	4,04	3,80	0,47			#N/A		#N/A	
	5625	Chicken neck skin	1,00		#N/A			2,08	#N/A		1,54	1,08
	5626	Chicken neck skin	1,48		#N/A		2,00		1,74	0,52	#N/A	
	5627	Chicken neck skin	1,48		#N/A		1,52		1,50	0,0	#N/A	
	5628	Chicken neck skin	0,00		#N/A			0,00	#N/A		0,00	0,00
	5629	Chicken neck skin	1,30		#N/A		1,64		1,47	0,34	#N/A	
	5630	Chicken neck skin	1,60	1,32	1,46	-0,28			#N/A		#N/A	
	5631	Chicken neck skin	2,73	3,32	3,03	0,59			#N/A		#N/A	
	5748	Chicken neck skin	1,95	1,93	1,94	-0,02			#N/A		#N/A	
	5749	Guinea fowl neck skin	2,23	1,52	1,87	-0,71			#N/A		#N/A	
	5750	Pigeon neck skin	0,00		#N/A			0,00	#N/A		0,00	0,00
	5751	Quail neck skin	2,43	3,57	3,00	1,14			#N/A		#N/A	
	5821	Guinea fowl neck skin	4,15	4,57	4,36	0,42			#N/A		#N/A	
	4991	Seasoned chicken leg	0,00		#N/A			0,00	#N/A		0,00	0,00
	4992	Marinated chicken	0,00		#N/A			0,00	#N/A		0,00	0,00
	4993	Seasoned minced duck	0,00		#N/A			0,00	#N/A		0,00	0,00
	5564	Marinated turkey meat	2,08	2,60	2,34	0,52			#N/A		#N/A	
	5565	Seasoned chicken wings	2,61	2,48	2,54	-0,14			#N/A		#N/A	
	5566	Seasoned chicken skewers	3,26	3,41	3,34	0,16			#N/A		#N/A	
5567	Chicken pieces meat with paprika	3,04	4,04	3,54	1,00			#N/A		#N/A		
5568	Chicken pieces meat with paprika	2,04	1,00	1,52	-1,04			#N/A		#N/A		
5823	Chicken wings with paprika	1,85	1,85	1,85	0,01			#N/A		#N/A		
Average category 1						0,24						
Standard deviation of differences category 1						0,58						
Average all categories					Dall	0,24						
Standard deviation of differences all categories					SDAll	0,58						

β=95%	n all	17					
	T(0,05;70)=	2,119905299					
		1,271880086	Upper limit	Lower limit	Linear		
	Average (minimal value)	0,00	1,51	-1,03	0,24		
	Average (maximal value)	10,00	1,51	-1,03	0,24		
Category	n	T(0,05;70)=	SD	ISO formula	Bias	Lower limit (95%)	Upper limit (95%)
1	17	2,12	0,58	1,27	0,24	-1,03	1,51
All categories	17	2,12	0,58	1,27	0,24	-1,03	1,51

TEMPO CAM 44 h + 48 h 5°C													
Category	Sample N°	Product	Log CFU/g		Average	Difference	Data not included in the interpretation		Average <4 CFU/plate	Difference <4 CFU/plate	Average corrected values	Difference Corrected values	
			Reference method	Alternative method			<4 CFU/plate	<or> threshold corrected values					
1	4984	Raw cockerel	1,30		#N/A			0,00	#N/A		0,65	-1,30	
	4985	Raw guinea fowl fillet	0,00		#N/A			1,85	#N/A		0,93	1,85	
	4986	Raw chicken garlic	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4987	Raw guinea fowl leg	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4988	Raw turkey cutlet	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4989	Turkey brochette	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4990	Minced turkey	0,00		#N/A			1,00	#N/A		0,50	1,00	
	5560	Turkey meat	2,36	2,52	2,44	0,16			#N/A		#N/A		
	5561	Turkey meat	2,76	3,00	2,88	0,24			#N/A		#N/A		
	5562	White cockerel	3,18	3,72	3,45	0,55			#N/A		#N/A		
	5563	Guinea fowl leg	3,18	4,18	3,68	1,00			#N/A		#N/A		
	5822	Quail leg	3,57	4,04	3,80	0,47			#N/A		#N/A		
	5625	Chicken neck skin	1,00		#N/A			2,08	#N/A		1,54	1,08	
	5626	Chicken neck skin	1,48		#N/A		2,28		1,88	0,80	#N/A		
	5627	Chicken neck skin	1,48		#N/A		1,65		1,57	0,17	#N/A		
	5628	Chicken neck skin	0,00		#N/A			1,00	#N/A		0,50	1,00	
	5629	Chicken neck skin	1,30		#N/A		1,93		1,62	0,63	#N/A		
	5630	Chicken neck skin	1,60	1,32	1,46	-0,28			#N/A		#N/A		
	5631	Chicken neck skin	2,73	3,32	3,03	0,59			#N/A		#N/A		
	5748	Chicken neck skin	1,95	1,93	1,94	-0,02			#N/A		#N/A		
	5749	Guinea fowl neck skin	2,23	1,52	1,87	-0,71			#N/A		#N/A		
	5750	Pigeon neck skin	0,00		#N/A			0,00	#N/A		0,00	0,00	
	5751	Quail neck skin	2,43	3,57	3,00	1,14			#N/A		#N/A		
	5821	Guinea fowl neck skin	4,15	4,57	4,36	0,42			#N/A		#N/A		
	4991	Seasoned chicken leg	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4992	Marinated chicken	0,00		#N/A			0,00	#N/A		0,00	0,00	
	4993	Seasoned minced duck	0,00		#N/A			0,00	#N/A		0,00	0,00	
	5564	Marinated turkey meat	2,08	2,60	2,34	0,52			#N/A		#N/A		
	5565	Seasoned chicken wings	2,61	2,48	2,54	-0,14			#N/A		#N/A		
	5566	Seasoned chicken skewers	3,26	3,41	3,34	0,16			#N/A		#N/A		
	5567	Chicken pieces meat with paprika	3,04	4,04	3,54	1,00			#N/A		#N/A		
	5568	Chicken pieces meat with paprika	2,04	1,00	1,52	-1,04			#N/A		#N/A		
	5823	Chicken wings with paprika	1,85	1,93	1,89	0,09			#N/A		#N/A		
Average category 1						0,24							
Standard deviation of differences category 1						0,58							
Average all categories				Dall		0,24							
Standard deviation of differences all categories				SDAll		0,58							

β=95%	n all	17					
	T(0,05;70)=	2,119905299					
	Average (minimal value)	0,00	Upper limit	Lower limit	Linear		
	Average (maximal value)	10,00	1,51	-1,02	0,24		
			1,51	-1,02	0,24		
Category	n	T(0,05;70)=	SD	ISO formula	Bias	Lower limit (95%)	Upper limit (95%)
1	17	2,12	0,58	1,27	0,24	-1,02	1,51
All categories	17	2,12	0,58	1,27	0,24	-1,02	1,51

Appendix 6 - Accuracy profile study: raw data

Matrix	Strain	Level	Sample N°	Reference method: ISO 10272-2*					Alternative method: TEMPO CAM 44 h at 41.5°C microaerobic atmosphere			
				Dilution	cfu/plate	cfu/g	cfu/g (rounded)	log cfu/g	Dilution 1/40	Dilution 1/400	cfu/g	log cfu/g
Chicken breast-Batch 1 Aerobic mesophilic flora : 5,8.10 ³ CFU/g	Campylobacter jejuni Ad1015	1	4760	10	51	509	510	2,71	630	710	630	2,80
				100	5							
			4761	10	60	609	610	2,79	630	730	630	2,80
				100	7							
			4762	10	53	518	520	2,72	330	450	330	2,52
				100	4							
		4763	10	49	509	510	2,71	620	730	620	2,79	
			100	7								
		4764	10	54	536	540	2,73	430	330	430	2,63	
			100	5								
		2	4765	100	31	3000	3000	3,48	7800	12000	7800	3,89
				1000	2							
			4766	100	46	4364	4400	3,64	5500	6900	5500	3,74
				1000	2							
			4767	100	31	3000	3000	3,48	4500	5700	4500	3,65
				1000	2							
		4768	100	34	3545	3500	3,54	7800	5100	7800	3,89	
			1000	5								
		3	4769	100	44	4400	4400	3,64 N'	4500	9300	4500	3,65
				1000	0							
			4770	1000	43	43636	44000	4,64	>49000	60000	60000	4,78
10000	5											
4771	1000		40	40000	40000	4,60	49000	50000	49000	4,69		
	10000		4									
4772	1000	33	30909	31000	4,49	>49000	110000	110000	5,04			
	10000	1										
4773	1000	40	40909	41000	4,61	49000	45000	49000	4,69			
	10000	5										
4774	1000	49	49000	49000	4,69 N'	>49000	34000	34000	4,53			
	10000	0										
Chicken breast-Batch 2 Aerobic mesophilic flora : 8,0.10 ⁴ CFU/g	Campylobacter jejuni Ad1015	1	4775	10	41	409	410	2,61	500	450	500	2,70
				100	4							
			4776	10	54	518	520	2,72	480	210	480	2,68
				100	3							
			4777	10	47	445	450	2,65	480	330	480	2,68
				100	2							
		4778	10	48	445	450	2,65	730	450	730	2,86	
			100	1								
		4779	10	51	500	500	2,70	480	590	480	2,68	
			100	4								
		2	4780	100	21	2000	2000	3,30	7800	5200	7800	3,89
				1000	1							
			4781	100	35	3545	3500	3,54	7800	3800	7800	3,89
				1000	4							
			4782	100	50	4818	4800	3,68	7800	3300	7800	3,89
				1000	3							
		4783	100	42	4182	4200	3,62	5000	4000	5000	3,70	
			1000	4								
4784	100	41	4000	4000	3,60	3700	4300	3700	3,57			
	1000	3										
Chicken breast-Batch 2 Aerobic mesophilic flora : 3,9.10 ³ CFU/g	Campylobacter jejuni Ad1015	3	5743	100	104	10455	10000	4,00	15000	17000	15000	4,18
				1000	11							
			5744	100	119	11909	12000	4,08	25000	12000	25000	4,40
				1000	12							
			5745	100	153	15364	15000	4,18	25000	37000	25000	4,40
				1000	16							
		5746	100	90	8636	8600	3,93	12000	16000	12000	4,08	
			1000	5								
		5747	100	130	13000	13000	4,11	21000	34000	21000	4,32	
			1000	13								

* Analyses performed according to the COFRAC accreditation

Appendix 7 - Accuracy profile study: summarized results

(Food) Category 1		Raw poultry and RTC poultry products										
(Food) Type 1		Raw meat										
			Reference method result					Alternative method result				
Sample Name	(Food) item	Level	rep 1	rep 2	rep 3	rep 4	rep 5	rep 1	rep 2	rep 3	rep 4	rep 5
4760-4764	Chicken breast	1	510	610	520	510	540	630	630	330	620	430
4775-4779	Chicken breast	1	410	520	450	450	500	500	480	480	730	480
4765-4769	Chicken breast	2	3000	4400	3000	3500	4400	7800	5500	4500	7800	4500
4780-4784	Chicken breast	2	2000	3500	4800	4200	4000	7800	7800	7800	5000	3700
5743-5747	Chicken breast	3	10000	12000	15000	8600	13000	15000	25000	25000	12000	21000
4770-4774	Chicken breast	3	44000	40000	31000	41000	49000	60000	49000	110000	49000	34000

Appendix 8 – Quantification limits (LOQ): raw data

Sample N°	Matrix	Alternative method: TEMPO CAM					
		Observed value	Value for calculation	Yi	y	So	LOQ
4790	Chicken breast	<10	10	1,00	1,00	0	0
4791		<10	10	1,00			
4792		<10	10	1,00			
4793		<10	10	1,00			
4794		<10	10	1,00			
4795		<10	10	1,00			
4796		<10	10	1,00			
4797		<10	10	1,00			
4798		<10	10	1,00			
4799		<10	10	1,00			

Appendix 9 – Inclusivity / Exclusivity: raw data

INCLUSIVITY												
N°	Strain		Reference	Origin	Dilution	COS (CFU/plate)	Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM			Log TEMPO CAM - log 10272-2
							mCCDA (CFU/plate)	Result	TEMPO CAM		Result	
									Dilution	CFU/ml		
1	<i>Campylobacter</i>	<i>coli</i>	Ad1914	Poultry	-6	77	37	3,70E+07	-6	71	7,10E+07	0,28
2	<i>Campylobacter</i>	<i>coli</i>	Ad1004	Poultry	-5	41	14	1,40E+06	-5	39	3,90E+06	0,44
3	<i>Campylobacter</i>	<i>coli</i>	Ad1005	Poultry	-5	4	7	7,00E+05	-5	14	1,40E+06	0,30
4	<i>Campylobacter</i>	<i>coli</i>	Ad1006	Poultry	-6	121	116	1,20E+08	-6	280	2,80E+08	0,37
5	<i>Campylobacter</i>	<i>coli</i>	Ad1007	Poultry	-6	72	76	7,60E+07	-6	250	2,50E+08	0,52
6	<i>Campylobacter</i>	<i>coli</i>	Ad1008	Poultry	-5	60	13	1,30E+06	-5	98	9,80E+06	0,88
7	<i>Campylobacter</i>	<i>coli</i>	Ad1009	Poultry	-6	71	48	4,80E+07	-6	220	2,20E+08	0,66
8	<i>Campylobacter</i>	<i>coli</i>	Ad1010	Poultry	-6	>150	>150	>1,5E+08	-6	600	6,00E+08	<0,60
9	<i>Campylobacter</i>	<i>coli</i>	Ad1011	Poultry	-5	6	28	2,80E+06	-5	43	4,30E+06	0,19
10	<i>Campylobacter</i>	<i>coli</i>	Ad1012	Poultry	-6	24	>150	>1,5E+08	-6	450	4,50E+08	<0,48
11	<i>Campylobacter</i>	<i>coli</i>	Ad1024	Poultry	-6	16	73	7,30E+07	-6	130	1,30E+08	0,25
12	<i>Campylobacter</i>	<i>coli</i>	Ad1025	Poultry	-6	71	43	4,30E+07	-6	310	3,10E+08	0,86
13	<i>Campylobacter</i>	<i>coli</i>	Ad1018	Poultry	-6	13	19	1,90E+07	-6	240	2,40E+08	1,10
14	<i>Campylobacter</i>	<i>coli</i>	Ad1019	Poultry	-6	23	60	6,00E+07	-6	210	2,10E+08	0,54
15	<i>Campylobacter</i>	<i>coli</i>	Ad1982	River water	-5	8	6	6,00E+05	-5	26	2,60E+06	0,64
16	<i>Campylobacter</i>	<i>coli</i>	Ad1125	Poultry	-6	26	11	1,10E+07	-6	63	6,30E+07	0,76
17	<i>Campylobacter</i>	<i>coli</i>	Ad1485	Faeces	-4	18	19	1,90E+05	-4	30	3,00E+05	0,20
18	<i>Campylobacter</i>	<i>coli</i>	Ad1997	Environment	-6	6	121	1,21E+08	-6	370	3,70E+08	0,49
19	<i>Campylobacter</i>	<i>coli</i>	Ad1121	Porcine faeces	-6	42	36	3,60E+07	-5	600	6,00E+07	0,22
20	<i>Campylobacter</i>	<i>coli</i>	Ad1122	Porcine faeces	-6	63	50	5,00E+07	-5	780	7,80E+07	0,19
21	<i>Campylobacter</i>	<i>coli</i>	Ad1123	Porcine meat	-4	16	17	1,70E+05	-4	57	5,70E+05	0,53
22	<i>Campylobacter</i>	<i>jejuni</i>	Ad1000	Poultry	-5	10	22	2,20E+06	-5	53	5,30E+06	0,38
23	<i>Campylobacter</i>	<i>jejuni</i>	Ad1002	Poultry	-6	52	33	3,30E+07	-5	910	9,10E+07	0,44

* Analyses performed according to the COFRAC accreditation

INCLUSIVITY												
N°	Strain		Reference	Origin	Dilution	COS (CFU/plate)	Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM			Log TEMPO CAM - log 10272-2
							mCCDA (CFU/plate)	Result	TEMPO CAM		Result	
									Dilution	CFU/ml		
24	<i>Campylobacter</i>	<i>jejuni</i>	Ad1003	Poultry	-5	40	17	1,70E+06	-5	51	5,10E+06	0,48
25	<i>Campylobacter</i>	<i>jejuni</i>	Ad1013	Poultry	-6	57	54	5,40E+07	-6	120	1,20E+08	0,35
26	<i>Campylobacter</i>	<i>jejuni</i>	Ad1014	Poultry	-6	20	16	1,60E+07	-5	780	7,80E+07	0,69
27	<i>Campylobacter</i>	<i>jejuni</i>	Ad1015	Poultry	-6	82	13	1,30E+07	-6	240	2,40E+08	1,27
28	<i>Campylobacter</i>	<i>jejuni</i>	Ad1016	Poultry	-6	62	80	8,00E+07	-6	170	1,70E+08	0,33
29	<i>Campylobacter</i>	<i>jejuni</i>	Ad1021	Poultry	-6	83	42	4,30E+07	-6	170	1,70E+08	0,60
30	<i>Campylobacter</i>	<i>jejuni</i>	Ad1023	Poultry	-6	22	14	1,40E+07	-6	52	5,20E+07	0,57
31	<i>Campylobacter</i>	<i>jejuni</i>	Ad1076	Poultry	-6	31	11	1,10E+07	-6	130	1,30E+08	1,07
32	<i>Campylobacter</i>	<i>jejuni</i>	Ad1078	Poultry	-6	20	21	2,10E+07	-6	73	7,30E+07	0,54
33	<i>Campylobacter</i>	<i>jejuni</i>	Ad1079	Poultry	-6	47	38	3,80E+07	-5	1100	1,10E+08	0,46
34	<i>Campylobacter</i>	<i>jejuni</i>	Ad1080	Poultry	-6	19	30	3,00E+07	-6	69	6,90E+07	0,36
35	<i>Campylobacter</i>	<i>jejuni</i>	Ad1081	Poultry	-5	82	23	2,30E+06	-5	270	2,70E+07	1,07
36	<i>Campylobacter</i>	<i>jejuni</i>	Ad1082	Poultry	-7	2	4	4,00E+07	-5	3700	3,70E+08	0,97
37	<i>Campylobacter</i>	<i>jejuni</i>	CIP 70.2 ^T (ATCC33560)	Bovine faeces	-6	39	44	4,40E+07	-6	260	2,60E+08	0,77
38	<i>Campylobacter</i>	<i>jejuni</i>	Ad1083	Poultry	-6	40	9	9,00E+06	-6	390	3,90E+08	1,64
39	<i>Campylobacter</i>	<i>jejuni</i>	Ad1084	Poultry	-6	63	>150	>1,5E+08	-6	340	3,40E+08	<0,36
40	<i>Campylobacter</i>	<i>jejuni</i>	Ad1085	Poultry	-6	83	30	3,00E+07	-6	600	6,00E+08	1,30
41	<i>Campylobacter</i>	<i>lari</i>	Ad1067	Poultry	-6	6	8	8,00E+06	-6	36	3,60E+07	0,65
42	<i>Campylobacter</i>	<i>lari</i>	CIP 102722 ^T (ATCC35221)	Gull cloacal swab	-5	7	7	7,00E+05	-6	36	3,60E+07	1,71
43	<i>Campylobacter</i>	<i>lari</i>	ATCC35222	unknown	-6	49	26	2,60E+07	-5	520	5,20E+07	0,30
44	<i>Campylobacter</i>	<i>lari</i>	Ad1130	unknown	-6	6	10	1,00E+07	-6	40	4,00E+07	0,60
45	<i>Campylobacter</i>	<i>jejuni</i>	Ad1993	Wild crow	-6	18	21	2,10E+07	-6	44	4,40E+07	0,32
46	<i>Campylobacter</i>	<i>jejuni</i>	Ad1951	Turkey meat	-6	15	6	6,00E+06	-6	69	6,90E+07	1,06
47	<i>Campylobacter</i>	<i>jejuni</i>	Ad1936	Chicken	-6	78	51	5,10E+07	-6	370	3,70E+08	0,86
48	<i>Campylobacter</i>	<i>jejuni</i>	Ad1892	Guinea fowl	-6	3	12	1,20E+07	-6	13	1,30E+07	0,03
49	<i>Campylobacter</i>	<i>jejuni</i>	Ad1908	Chicken leg	-6	31	>150	>1,5E+08	-6	1100	1,10E+09	<0,87

INCLUSIVITY												
N°	Strain		Reference	Origin	Dilution	COS (CFU/plate)	Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM			Log TEMPO CAM - log 10272-2
							mCCDA (CFU/plate)	Result	TEMPO CAM		Result	
									Dilution	CFU/ml		
50	<i>Campylobacter</i>	<i>jejuni</i>	Ad1998	Mechanically deboned poultry meat	-5	49	21	2,10E+06	-5	33	3,30E+06	0,20

EXCLUSIVITY											
N°	Strain		Reference	Origin	Control media Incubation temperature	Control media		Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM	
						Dilution	CFU/plate	Dilution	CFU/plate	Dilution	CFU/plate
1	<i>Acinetobacter</i>	<i>baumanii</i>	Ad 1070	Poultry	TSA	-6	52	-3	28	-3	<1
					30°C	-7	6	-4	0	-4	<1
2	<i>Arcobacter</i>	<i>butzleri</i>	Ad1881	Swab feather	COS	-6	42	-3	0	-3	<1
					30°C	-7	5	-4	0	-4	<1
3	<i>Arcobacter</i>	<i>butzleri</i>	Ad1126	Chicken	COS	-7	29	-3	0	-3	<1
					30°C	-8	7	-4	0	-4	<1
4	<i>Arcobacter</i>	<i>butzleri</i>	CIP 103493	Unknown	COS	-7	35	-3	0	-3	<1
					37°C	-8	1	-4	0	-4	<1
5	<i>Acinetobacter</i>	<i>calcoaceticus</i>	Ad 1090	Poultry	PCA	-6	119	-4	>150	-3	<1
					30°C	-7	9	-5	±500	-4	<1
6	<i>Acinetobacter</i>	<i>johnsonii</i>	Ad1317	Egg	PCA	-6	177	-3	0	-3	<1
					30°C	-7	8	-4	0	-4	<1
7	<i>Aeromonas</i>	<i>hydrophila</i>	CIP 5750	Unknown	PCA	-7	54	-3	0	-3	<1
					30°C	-8	3	-4	0	-4	<1
8	<i>Arcobacter</i>	<i>cryaerophilus</i>	CIP 104014	Unknown	COS	-6	93	-3	0	-3	<1
					30°C	-7	15	-4	0	-4	<1
9	<i>Arcobacter</i>	<i>cryoaerophilus</i>	Ad1124	Chicken	COS	-7	40	-3	0	-3	<1
					30°C	-8	2	-4	0	-4	<1
10	<i>Arcobacter</i>	<i>skirrowii</i>	ATCC 51132	Faeces	COS	-6	82	-3	0	-3	<1
					30°C	-7	6	-4	0	-4	<1
11	<i>Campylobacter</i>	<i>fetus</i>	Ad1069	Poultry	COS	-4	>150 (µ)	-4	>150 (µ)	-3	<1
					41,5°C	-5	50 (µ)	-5	50 (µ)	-4	<1
12	<i>Campylobacter</i>	<i>fetus</i>	Ad1068	Poultry	COS	-5	>150 (µ)	-2/-7	Impossible to enumerate (µ)	-3	<1
					25°C	-6	59 (µ)			-4	<1
13	<i>Citrobacter</i>	<i>freundii</i>	Ad 173	Poultry	TSA	-7	54	-3	0	-3	<1
					37°C	-8	10	-4	0	-4	<1
14	<i>Campylobacter</i>	<i>upsaliensis</i>	ATCC 49816	Faeces	COS	-5	>150	-5	131	-3	<1
					41,5°C	-6	32	-6	9	-4	<1

* Analyses performed according to the COFRAC accreditation

EXCLUSIVITY											
N°	Strain		Reference	Origin	Control media Incubation temperature	Control media		Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM	
						Dilution	CFU/plate	Dilution	CFU/plate	Dilution	CFU/plate
15	<i>Campylobacter</i>	<i>upsaliensis</i>	Ad1139	Faeces	COS 41,5°C	-5	>150	-5	>150	-3	<1
						-6	96	-6	48	-4	<1
16	<i>Enterobacter</i>	<i>fergusoni</i>	2876	Environment	TSA 37°C	-7	27	-3	0	-3	<1
						-8	3	-4	0	-4	<1
17	<i>Escherichia</i>	<i>coli</i>	Ad 241	Poultry	TSA 37°C	-7	75	-3	0	-3	<1
						-8	9	-4	0	-4	<1
18	<i>Escherichia</i>	<i>coli</i> ESBL	CIP103982	Unknown	TSA 37°C	-6	182	-3	0	-3	<1
						-7	38	-4	0	-4	<1
19	<i>Enterococcus</i>	<i>durans</i>	Ad148	Ham	TSA 37°C	-7	11	-3	0	-3	<1
						-8	2	-4	0	-4	<1
20	<i>Enterococcus</i>	<i>durans</i>	Ad175	Egg product	TSA 37°C	-6	80	-3	0	-3	<1
						-7	11	-4	0	-4	<1
21	<i>Klebsellia</i>	<i>pneumoniae</i>	133	Beef	TSA 37°C	-6	175	-3	0	-3	<1
						-7	12	-4	0	-4	<1
22	<i>Lactobacillus</i>	<i>curvatus</i>	Ad 379	Pork meat	MRS 30°C anaer	-6	191	-3	0	-3	<1
						-7	15	-4	0	-4	<1
23	<i>Macrococcus</i>	<i>cohnii</i>	Ad 156	Poultry	PCA 37°C	-6	15	-3	0	-3	<1
						-7	7	-4	0	-4	<1
24	<i>Proteus</i>	<i>vulgaris</i>	Ad 984	Meat product (pork/beef mix)	TSA 37°C	-6	ND	-3	0	-3	<1
						-7	ND	-4	0	-4	<1
25	<i>Providencia</i>	<i>stuartii</i>	46	Food product	TSA 37°C	-7	35	-3	0	-3	<1
						-8	2	-4	0	-4	<1
26	<i>Pseudomonas</i>	<i>aeruginosa</i>	20	Raw milk	PCA 37°C	-7	31	-3	0	-3	<1
						-8	3	-4	0	-4	<1
27	<i>Pseudomonas</i>	<i>putida</i>	4	Poultry	PCA 30°C	-6	85	-3	0	-3	<1
						-7	9	-4	0	-4	<1
28	<i>Ralstonia</i>	<i>mannitolityca</i>	Ad 1059	Poultry	PCA 30°C	-6	164	-3/-5	ND (µ)	-3	>4900
						-7	17			-4	>4900
28bis	<i>Ralstonia</i>	<i>mannitolityca</i>	DSM17512	Blood	PCA 30°C	-6	76	-3	0	-3	3300
						-7	8	-4	0	-4	2500

EXCLUSIVITY											
N°	Strain		Reference	Origin	Control media Incubation temperature	Control media		Reference method NF EN ISO 10272-2*		Alternative method TEMPO CAM	
						Dilution	CFU/plate	Dilution	CFU/plate	Dilution	CFU/plate
28ter	<i>Ralstonia</i>	sp.	Ad2504	Unknown	PCA	-6	91	-3	0	-3	<1
					30°C	-7	7	-4	0	-4	<1
29	<i>Saccharomyces</i>	<i>cerevisiae</i>	Ad 999	Dairy product	Sabouraud	-6	12	-3	0	-3	<1
					25°C	-7	2	-4	0	-4	<1
30	<i>Staphylococcus</i>	<i>aureus</i>	Ad 157	Poultry skin	PCA	-7	21	-3	0	-3	<1
					37°C	-8	3	-4	0	-4	<1

Appendix 10 - Homogeneity of inoculation

Low level							
Sample	Analysis 1	Analysis 2	Log Analysis 1	Log Analysis 2	D	S	D ²
1	1200	1400	3,079	3,146	0,067	6,225	0,004
2	1200	1200	3,079	3,079	0,000	6,158	0,000
3	1100	1300	3,041	3,114	0,073	6,155	0,005
4	1200	1200	3,079	3,079	0,000	6,158	0,000
5	1300	1200	3,114	3,079	-0,035	6,193	0,001
6	1300	1200	3,114	3,079	-0,035	6,193	0,001
7	1200	1600	3,079	3,204	0,125	6,283	0,016
8	1500	1300	3,176	3,114	-0,062	6,290	0,004
9	1300	1300	3,114	3,114	0,000	6,228	0,000
10	1100	1200	3,041	3,079	0,038	6,121	0,001
sum	12400	12900	30,917	31,088	0,171	62,005	0,033

S _w	0,00165	San ²	0,00165	F1	1,88
S _b	0,0016	Ssam ²	-0,000040	F2	1,01
Target standard deviation to apply		0,25	Test value		0,01224

Test value > Ssam²: the test material is sufficiently uniform.

Medium level							
Sample	Analysis 1	Analysis 2	Log Analysis 1	Log Analysis 2	D	S	D ²
11	17000	5100	4,230	3,708	-0,523	7,938	0,273
12	5400	3800	3,732	3,580	-0,153	7,312	0,023
13	10000	13000	4,000	4,114	0,114	8,114	0,013
14	12000	9400	4,079	3,973	-0,106	8,052	0,011
15	11000	9900	4,041	3,996	-0,046	8,037	0,002
16	11000	6400	4,041	3,806	-0,235	7,848	0,055
17	9100	6200	3,959	3,792	-0,167	7,751	0,028
18	5500	3300	3,740	3,519	-0,222	7,259	0,049
19	3300	3000	3,519	3,477	-0,041	6,996	0,002
20	3800	4300	3,580	3,633	0,054	7,213	0,003
sum	88100	64400	38,923	37,598	-1,325	76,520	0,460

S _w	0,02300	San ²	0,02300	F1	1,88
S _b	0,0858	Ssam ²	0,031423	F2	1,01
Target standard deviation to apply		0,25	Test value		0,03380

Test value > Ssam²: the test material is sufficiently uniform.

High level							
Sample	Analysis 1	Analysis 2	Log Analysis 1	Log Analysis 2	D	S	D ²
21	100000	140000	5,000	5,146	0,146	10,146	0,021
22	74000	130000	4,869	5,114	0,245	9,983	0,060
23	20000	120000	4,301	5,079	0,778	9,380	0,606
24	66000	68000	4,820	4,833	0,013	9,652	0,000
25	110000	120000	5,041	5,079	0,038	10,121	0,001
26	130000	130000	5,114	5,114	0,000	10,228	0,000
27	140000	95000	5,146	4,978	-0,168	10,124	0,028
28	55000	52000	4,740	4,716	-0,024	9,456	0,001
29	120000	48000	5,079	4,681	-0,398	9,760	0,158
30	110000	140000	5,041	5,146	0,105	10,188	0,011
sum	925000	1043000	49,152	49,886	0,734	99,038	0,887

S _w	0,04433	San ²	0,04433	F1	1,88
S _b	0,0502	Ssam ²	0,002952	F2	1,01
Target standard deviation to apply		0,25	Test value		0,05535

Test value > Ssam²: the test material is sufficiently uniform.

**Appendix 11 - Results obtained by the collaborative laboratories
and the expert laboratory**

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
A1 Aerobic mesophilic flora : 3,7.10 ⁶ CFU/g	4	10	2	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	107	107	1064	1100	3,04	930	860	1000	930	2,97
		100	10	10								
	5	10	95	95	945	950	2,98	1300	440	1000	1300	3,11
		100	9	9								
	1	100	86	86	8455	8500	3,93	7800	5800	3300	7800	3,89
		1000	7	7								
	7	100	62	62	6364	6400	3,81	3700	5700	8900	3700	3,57
		1000	8	8								
	2	1000	85	85	87273	87000	4,94	>49000	74000	170000	74000	4,87
		10000	11	11								
	6	1000	91	91	90000	90000	4,95	>49000	56000	73000	56000	4,75
		10000	8	8								
A2 Aerobic mesophilic flora : 3,2.10 ⁶ CFU/g	13	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	8	10	106	106	991	990	3,00	1300	710	1000	1300	3,11
		100	3	3								
	14	10	117	117	1145	1100	3,04	570	590	<1000	570	2,76
		100	9	9								
	9	100	70	70	7273	7300	3,86	3700	7100	5700	3700	3,57
		1000	10	10								
	11	100	70	70	6727	6700	3,83	6800	6400	7100	6800	3,83
		1000	4	4								
	10	1000	124	124	122727	120000	5,08	>49000	110000	43000	110000	5,04
		10000	11	11								
	12	1000	72	72	71818	72000	4,86	49000	91000	57000	49000	4,69
		10000	7	7								

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
B Aerobic mesophilic flora : $4,0 \cdot 10^6$ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	84	84	873	870	2,94	440	570	1000	440	2,64
		100	12	12								
	5	10	109	109	1055	1100	3,04	400	330	<1000	400	2,60
		100	7	7								
	1	100	54	54	5455	5500	3,74	2800	3600	5900	2800	3,45
		1000	6	6								
	7	100	57	57	5818	5800	3,76	3400	8300	7100	3400	3,53
		1000	7	7								
	2	1000	63	63	63636	64000	4,81	2400 ⁽¹⁾	48000	33000	48000	4,68
		10000	7	7								
6	1000	64	64	66364	66000	4,82	1200 ⁽¹⁾	43000	36000	43000	4,63	
	10000	9	9									
C Aerobic mesophilic flora : $5,3 \cdot 10^6$ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	30	24	264	260	2,41	820	1000	<1000	820	2,91
		100	5	5								
	5	10	48	48	473	470	2,67	430	1200	1000	430	2,63
		100	4	4								
	1	100	11	11	1182	1200	3,08	6800	6400	11000	6800	3,83
		1000	2	2								
	7	100	11	11	1091	1100	3,04	21000	8200	4500	21000	4,32
		1000	1	1								
	2	1000	126	126	123636	120000	5,08	>49000	78000	120000	78000	4,89
		10000	10	10								
6	1000	30	30	36364	36000	4,56	>49000	91000	160000	91000	4,96	
	10000	10	10									

(1): inconsistent result, 1/400 dilution taken into account for calculation

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
D Aerobic mesophilic flora : 3,1.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	65	65	664	660	2,82	830	800	<1000	830	2,92
		100	8	8								
	5	10	80	80	773	770	2,89	710	450	1000	710	2,85
		100	5	5								
	1	100	52	52	4909	4900	3,69	4400	4000	8900	4400	3,64
		1000	2	2								
	7	100	21	21	2091	2100	3,32	9100	6300	4500	9100	3,96
		1000	2	2								
2	1000	19	19	18182	18000	4,26	>49000	74000	71000	74000	4,87	
	10000	1	1									
6	1000	31	31	31818	32000	4,51	37000	45000	64000	37000	4,57	
	10000	4	4									
E Aerobic mesophilic flora : 3,2.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	100	12	12	1182	1200	3,08	1400	570	<1000	1400	3,15
		1000	1	1								
	5	100	14	14	1364	1400	3,15	730	1000	<1000	730	2,86
		1000	1	1								
	1	100	95	95	9000	9000	3,95	7400	5000	8600	7400	3,87
		1000	4	4								
	7	100	91	91	8818	8800	3,94	12000	9300	5900	12000	4,08
		1000	6	6								
	2	1000	94	94	99091	99000	5,00	>49000	120000	68000	120000	5,08
		10000	15	15								
	6	1000	56	56	60000	60000	4,78	>49000	68000	69000	68000	4,83
10000		10	10									

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
F1 Aerobic mesophilic flora : 3,1.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	80	80	782	780	2,89	950	1000	<1000	950	2,98
		100	6	6								
	5	10	64	64	640	640	2,81 N'	1300	330	1000	1300	3,11
		100	0	0								
	1	100	51	51	5091	5100	3,71	4400	3600	4400	4400	3,64
		1000	5	5								
	7	100	40	40	4091	4100	3,61	6800	5600	8600	6800	3,83
		1000	5	5								
	2	1000	8	8	8000	8000	3,90 Ne	>49000	44000	120000	44000	4,64
		10000	4	/								
6	10000	11	11	109091	110000	5,04	>49000	67000	83000	67000	4,83	
	100000	1	1									
F2 Aerobic mesophilic flora : 5,7.10 ⁶ CFU/g	13	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	8	10	56	56	600	600	2,78	830	710	2100	830	2,92
		100	10	10								
	14	10	70	70	700	700	2,85	400	890	1000	400	2,60
		100	7	7								
	9	100	51	51	5364	5400	3,73	6700	10000	7100	6700	3,83
		1000	8	8								
	11	100	100	100	9909	9900	4,00	8200	4800	7300	8200	3,91
		1000	9	9								
	10	1000	78	78	76364	76000	4,88	>49000	78000	53000	78000	4,89
		10000	6	6								
	12	1000	69	69	68182	68000	4,83	>49000	44000	40000	44000	4,64
		10000	6	6								

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
G Aerobic mesophilic flora : 2,1.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	84	84	827	830	2,92	510	<100	<1000	510	2,71
		100	7	7								
	5	10	72	72	673	670	2,83	260	100	<1000	260	2,41
		100	2	2								
	1	100	83	83	8545	8500	3,93	5500	210	<1000	5500	3,74
		1000	11	11								
	7	100	64	64	6273	6300	3,80	7800	2400	4500	7800	3,89
		1000	5	5								
	2	1000	80	80	77273	77000	4,89	37000	1700	2100	37000	4,57
		10000	5	5								
	6	1000	74	74	72727	73000	4,86	>49000	31000	64000	31000	4,49
		10000	6	6								
H Aerobic mesophilic flora: 1,5.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	96	96	973	970	2,99	730	890	<1000	730	2,86
		100	11	11								
	5	10	94	94	955	960	2,98	630	210	<1000	630	2,80
		100	11	11								
	1	100	83	83	8364	8400	3,92	7800	570	1000	7800	3,89
		1000	9	9								
	7	100	96	96	9182	9200	3,96	5500	3300	2100	5500	3,74
		1000	5	5								
	2	1000	94	94	94545	95000	4,98	>49000	50000	53000	50000	4,70
		10000	10	10								
	6	1000	89	89	90909	91000	4,96	>49000	110000	45000	110000	5,04
		10000	11	11								

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
I Aerobic mesophilic flora : 3,7.10 ⁶ CFU/g	4	10	1	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	71	71	755	760	2,88	1000	590	<1000	1000	3,00
		100	12	12								
	5	10	98	98	927	930	2,97	480	1000	<1000	480	2,68
		100	5	4								
	1	100	26	26	2818	2800	3,45	3700	7100	4500	3700	3,57
		1000	5	5								
	7	100	84	84	8000	8000	3,90	11000	7300	8900	11000	4,04
		1000	4	4								
	2	1000	106	106	109091	110000	5,04	>49000	50000	44000	50000	4,70
		10000	14	14								
	6	1000	17	17	20000	20000	4,30	>49000	53000	47000	53000	4,72
		10000	5	5								
J Aerobic mesophilic flora : 2,9.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	60	60	564	560	2,75	1100	590	1000	1100	3,04
		100	2	2								
	5	10	69	69	682	680	2,83	640	1700	<1000	640	2,81
		100	7	6								
	1	100	29	29	2909	2900	3,46	6700	3900	3200	6700	3,83
		1000	3	3								
	7	100	37	37	3455	3500	3,54	5300	3000	4400	5300	3,72
		1000	1	1								
	2	1000	48	48	50000	50000	4,70	>49000	110000	100000	110000	5,04
		10000	7	7								
	6	1000	23	23	24545	25000	4,40	49000	91000	51000	49000	4,69
		10000	4	4								

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
K Aerobic mesophilic flora : 2,3.10 ⁷ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	32	32	309	310	2,49	430	590	1000	430	2,63
		100	2	2								
	5	10	36	36	382	380	2,58	1400	890	<1000	1400	3,15
		100	6	6								
	1	100	30	30	3182	3200	3,51	5600	4500	4900	5600	3,75
		1000	5	5								
	7	100	33	33	3364	3400	3,53	<1000	5300	2400	5300	3,72
		1000	4	4								
	2	1000	17	17	18182	18000	4,26	30000	44000	40000	30000	4,48
		10000	3	3								
	6	1000	15	15	13636	14000	4,15	49000	37000	44000	49000	4,69
		10000	0	0								
L Aerobic mesophilic flora :1,2.10 ⁷ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	25	25	245	250	2,40	570	590	900	570	2,76
		100	2	2								
	5	10	18	18	164	160	2,20	760	730	1000	760	2,88
		100	0	0								
	1	100	11	11	1000	1000	3,00	11000	4800	4400	11000	4,04
		1000	0	0								
	7	100	28	28	2818	2800	3,45	6000	14000	11000	6000	3,78
		1000	3	3								
	2	1000	4	4	4000	4000	3,60 Ne	>49000	68000	110000	68000	4,83
		10000	0	0								
	6	1000	20	20	20000	20000	4,30	49000	78000	67000	49000	4,69
		10000	2	2								

Laboratory	Sample N°	Reference method: ISO 10272-2						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
M Aerobic mesophilic flora : 4,1.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	98	98	1018	1000	3,00	950	890	1000	950	2,98
		100	14	14								
	5	10	89	89	864	860	2,93	930	590	<1000	930	2,97
		100	6	6								
	1	100	76	76	7727	7700	3,89	4000	4300	5700	4000	3,60
		1000	9	9								
	7	100	96	96	9000	9000	3,95	5500	83300	3300	5500	3,74
		1000	3	3								
	2	1000	130	130	125455	130000	5,11	>49000	120000	95000	120000	5,08
		10000	8	8								
	6	1000	110	110	103636	100000	5,00	>49000	78000	83000	78000	4,89
		10000	4	4								

Laboratory	Sample N°	Reference method: ISO 10272-2*						Alternative method: TEMPO CAM				
		Dilution	CFU/ plate	confirmed CFU/plate	CFU/g	CFU/g rounded	log CFU/g	Dilution 1/40	Dilution 1/400	Dilution 1/4000	CFU/g	log CFU/g
N=ADRIA Aerobic mesophilic flora : 6,7.10 ⁶ CFU/g	4	10	0	0	<10	<10	<1,00	<10	<100	<1000	<10	<1,00
		100	0	0								
	3	10	108	108	1064	1100	3,04	580	570	1000	580	2,76
		100	9	9								
	5	10	79	79	773	780	2,89	640	710	<1000	640	2,81
		100	6	6								
	1	100	51	51	5100	5100	3,71 N'	6000	2800	3200	6000	3,78
		1000	15	15								
	7	100	88	88	8818	8800	3,94	6800	15000	2100	6800	3,83
		1000	9	9								
	2	1000	121	121	119091	120000	5,08	>49000	40000	36000	40000	4,60
		10000	10	10								
	6	1000	139	139	134545	130000	5,11	>49000	60000	93000	60000	4,78
		10000	9	9								

* Analyses performed according to the COFRAC accreditation