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Validation of an alternative method of analysis by Laboratoire de Touraine
Application to food microbiology

Validation study conducted according to an adaptation of the NF EN ISO 16140-2 standard: 2016, part 1: comparative study

CHROMagar™ Enterobacteria Agar
for the enumeration of Enterobacteriaceae

Protocols for human food products

Quantitative method

CONFIDENTIAL

Tests conducted by: **Laboratoire de Touraine**
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This report consists of 23 pages and 4 appendices.

Version 0
05/07/2018

SUMMARY

1. Introduction

The CHROMagar™ company wishes to carry out a validation study of the chromogenic medium CHROMagar™ Enterobacteria for the detection and enumeration of Enterobacteriaceae, compared to the ISO 21528-2 reference method.

2. Methods protocol

2.1. Alternative method

2.1.1. Principle of the alternative method

CHROMagar™ Enterobacteria agar is a chromogenic medium for the detection and enumeration of Enterobacteriaceae. This selective medium inhibits the growth of many microorganisms, mainly gram positive bacteria.
The appearance of the colonies on the agar is as follows:

- E. *coli*: blue with or without blue halo
- *Proteus*: red with swarming
- Other enterobacteria: pink to red

Pseudomonas are mainly inhibited.

2.1.2. Protocol of the alternative method

The protocol of the method is presented in **Appendix 1**.

2.1.3. Field of application

The method applies to all human food products.

2.2. Reference method

The reference method used for the method comparison is NF ISO 21528-2 (June 2017): Horizontal method for the detection and enumeration of enterobacteriaceae - Part 2: Colony counting method. The protocol of this method is presented in **Appendix2.**

3. Comparative study

The comparative study of methods is the part of the validation process carried out within the organising laboratory. It has three parts.

- *A comparative study of the results of the reference method against the results of the alternative method, for a range of different samples of various matrices (naturally and/or artificially) contaminated (called sensitivity study).*
- *A comparative study of the results of the reference method against the results of the alternative method of artificially contaminated samples, using replicates of only one matrix per category. Data is analysed using the accuracy profile (AP) approach (called AP profile study).*
- *An inclusivity/exclusivity study of the alternative method. Inclusivity is the ability of the alternative method to detect the target analyte from a wide-range of strains. Exclusivity is the absence of interference by an appropriate range of non-target strains of the alternative method.*

The study was conducted on different samples and different strains frequently isolated from these samples, representative of food products. This is not an exhaustive list of the different matrices included in the field of application.

3.1. Sensitivity study

3.1.1. Number and nature of samples

94 samples were tested during the study, producing 85 exploitable results. Their distribution is presented in Table 1.

Table 1: Distribution of analysed samples

Category number	Category	Number of samples analysed	Number of samples interpretable
1	**Egg products/various products**	16	16
2	**Meat products**	23	23
3	**Plant products**	20	15
4	**Seafood products**	15	15
5	**Dairy products**	20	16
Total		94	85

3.1.2. Artificial contamination of samples

Artificial contaminations were performed, by seeding protocol: the contaminating strain is put back into cultivation and then diluted until the desired level of contamination is obtained. The food is then inoculated with a known volume of the chosen dilution, and then mixed to ensure good homogeneity. The food is then stored at 4 ° C for 48 to 72 hours before analysis. The same contaminating strain is never used more than 6 times.

A total of 39 samples were artificially contaminated and 55 samples were naturally contaminated.

3.1.3. Protocol applied during the study

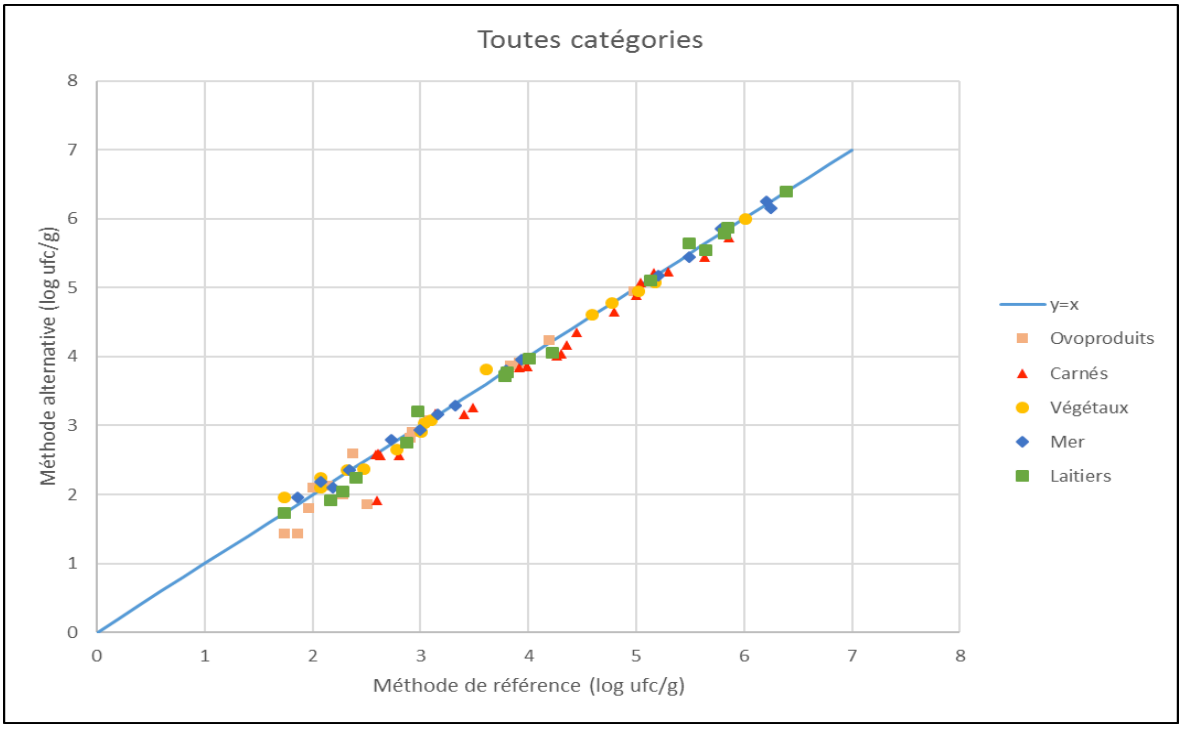
Tests were performed with an incubation period of 24 hours for CHROMagar^{TM} Enterobacteria plates. Confirmations of the alternative method were made from 5 characteristic colonies, applying the confirmation protocol of the reference method. The reference method was applied in its entirety.

3.1.4. Test results

The raw results are presented in **Appendix 3**.

The samples were analysed by the reference method and the alternative method, so as to have 15 minimum exploitable results per category.
The values obtained were exploited by tracing the dispersion of the results of the alternative method in relation to the results of the reference method. The identity line on which all the points are supposed to be if the 2 methods had given identical results for each sample analyzed is also represented (y=x).
Figure 1 shows this graphical representation for all categories.

Figure 1



Results with less than 4 colonies by the reference or alternative method and uninterpretable results (shown in grey in the table in Appendix 3) were not interpreted (in accordance with standard 7218).

The results observed are satisfactory for all categories tested.

3.2. Accuracy profile

3.2.1. Matrix

3 matrix/strain pairs were studied by the alternative and reference methods. 2 samples were contaminated at a low rate, 2 at an intermediate rate and 2 at a high rate. For each sample, 3 replicates were analysed. In the end, 18 samples were tested per category. Contaminations were carried out by seeding at 4°C for 48 hours.
The different matrix/strain pairs studied are presented in Table 3.

Table 3: matrix/strain pairs

Category	Matrix	Strain	Strain origin	Target contamination level
Meat products	Minced meat	*Escherichia coli* (I11)	Food	1: 300 2: 10 000 3: 1 000.000.
Seafood products	Smoked salmon	*Hafnia alvei* (I14)	Food	
Dairy products	Goat cheese	*Enterobacter cloacae* (I6)	Food	

3.2.2. Calculations and interpretation

The raw results are presented in **Appendix 4**.

The values obtained were exploited by tracing the dispersion of the results of the alternative method in relation to the results of the reference method. The identity line on which all the points are supposed to be if the 2 methods had given identical results for each sample analysed is also represented (y=x).
Figures 2, 3 and 4 show these graphical representations for each matrix/strain pair.

The results observed are satisfactory for each category.

Figure 2: Accuracy profiles, meat products

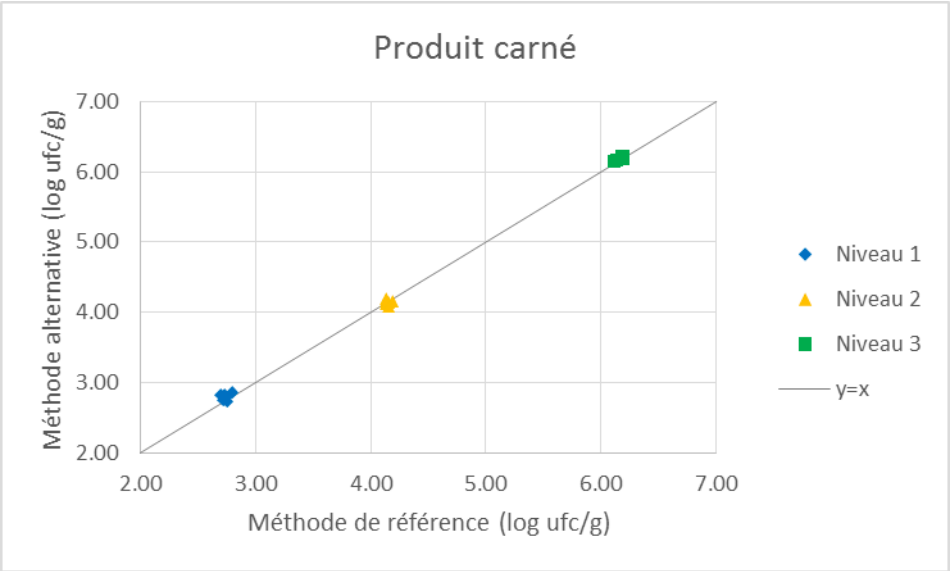


Figure 3: Accuracy profiles, sea product

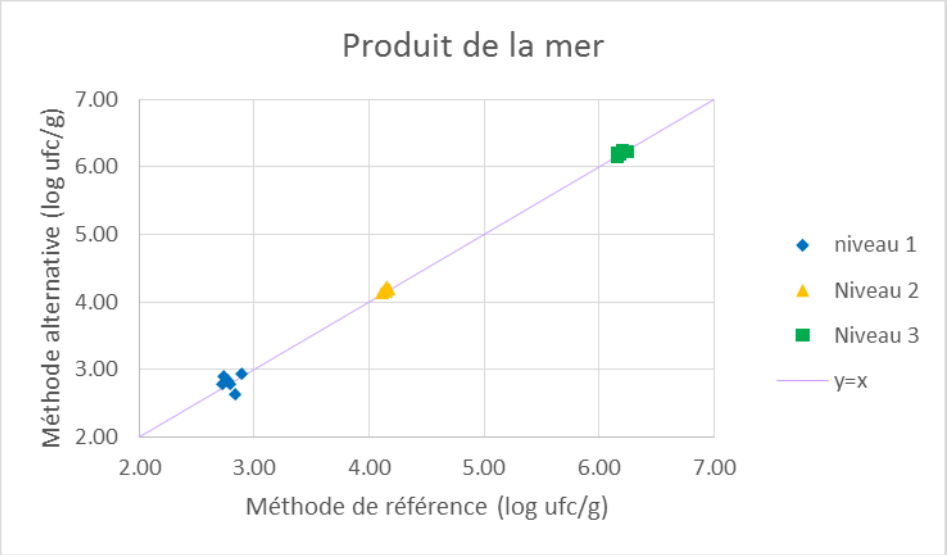
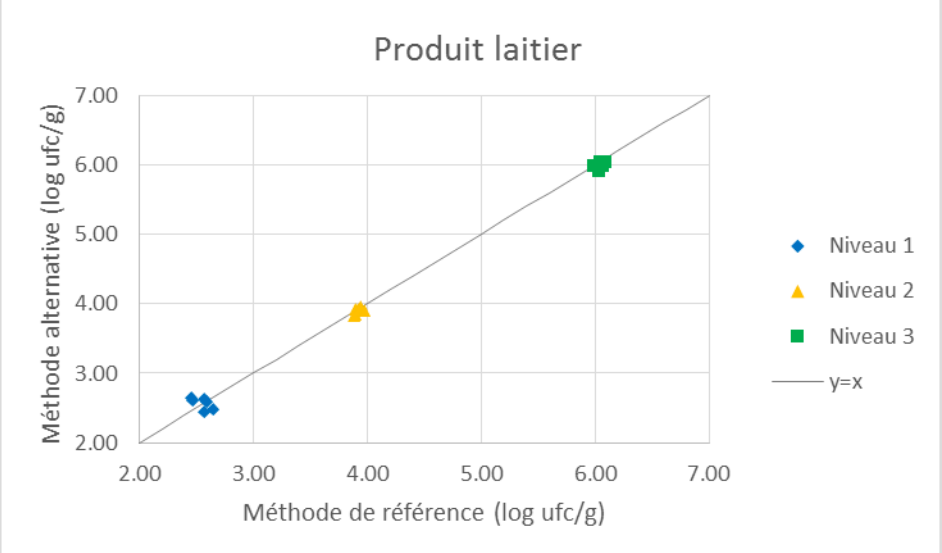


Figure 4: Accuracy profiles, dairy products



3.3. Inclusivity/Exclusivity study

3.3.1. Inclusivity

- **Protocol**

30 target strains were tested, each by the reference method, the alternative method and a non-specific agar (TSA). The inoculation rate was the same for each agar and provided a sufficient number of microorganisms to enumerate them. VRBG, CHROMagar™ Enterobacteria and TSA agars were incubated 24 hours before colony counting.

- **Results**

The results are presented in Table 4.

Table 4: Inclusivity study

INCLUSIVITE			Origine	Référence	TSA	VRBG	Chromagar
Souche							
1	*Citrobacter*	*freundii*	ATCC 43864	1822/22/40	83	96	84
2	*Citrobacter*	*koseri*	Lait de vache	109/1/28	44	26	27
3	*Citrobacter*	*rodentium*	Souche CIP	3187/39/28	56	59	60
4	*Cronobacter*	*sakasakii*	AD 940 (EIL Adria)	AFNOR 1/38	113	92	89
5	*Enterobacter*	*cloacae*	Gateau banane	18_IAA.2532.2	47	25	34
6	*Enterobacter*	*cloacae*	Tarte aux fraises	18_IAA.2538.1	53	74	52
7	*Enterobacter*	*sakazakii*	Poudre	2915/35/80	107	85	91
8	*Enterobacter*	*sakazakii*	Poudre riz	IAA82	34	33	20
9	*Escherichia*	*coli*	ATCC 8739	3089/38/11	47	30	11
10	*Escherichia*	*coli*	Produit laitier	IAA52	67	66	70
11	*Escherichia*	*coli*	Filet de poulet	18_IAA.2935.2 AFNOR 3/23	43	21	27
12	*Hafnia*	*alvei*	Cecalait lait	IAA72	87	100	84
13	*Hafnia*	*alvei*	Raclette au lait cru	bioMérieux Haf 1.9 AFNOR 2/42	72	70	73
14	*Hafnia*	*alvei*	Saucisse crue	18_IAA.2679.2	101	134	89
15	*Klebsiella*	*oxytoca*	Lait de vache	2164/26/58	66	66	66
16	*Klebsiella*	*pneumoniae*	ATCC 11298	1821/22/39	24	15	21
17	*Pantoea*	*sp*	Salade composée	18_IAA.2471.1	140	98	122
18	*Proteus*	*mirabilis*	Foie de veau	3185/39/26	99	98	94
19	*Proteus*	*mirabilis*	Œuf de poule	IAA61	86	96	96
20	*Providencia*	*stuartii*	Biomérieux	PRV3.1 AFNOR 1/41	37	10	19
21	*Raoultella*	*terrigena*	Eclair café	18_IAA.2537.1	111	106	106
22	*Rhanella*	*aquatilis*	Eclair café	18_IAA.3504.1	47	20	24
23	*Salmonella*	*Dublin*	Volaille	sal9.24 AFNOR 1/38	65	47	54
24	*Serratia*	*liquefaciens*	Alimentaire	2175/26/69	50	36	31
25	*Serratia*	*liquefaciens*	Bœuf	18_IAA.3583.3	37	21	19
26	*Shigella*	*sonnei*	ATCC 9290	2145/32/53	140	119	120
27	*Shigella*	*flexneri*	ATCC 12022	2646/32/54	71	56	45
28	*Yersinia*	*enterocolitica*	ATCC 23715	1820/22/38	116	76	87
29	*Yersinia*	*ruckeri*	Poisson	1681/20/61	78	37	68
30	*Yersinia*	*pseudotuberculosis*	Lait de vache	1342/16/46	74	71	80

The 30 strains tested were detected by the alternative method.

Observation: The *Shigella sonnei* strain has an atypical blue-green colour on CHROMagar™ Enterobacteria.

3.3.2. Exclusivity

- **Protocol**

20 non-target strains were tested exclusively, each by the reference method, the alternative method and a non-specific agar (TSA). The inoculation rate was the same for each agar and provided a sufficient number of microorganisms to enumerate them on TSA. VRBG, CHROMagar™Enterobacteria and TSA agars were incubated 24 hours before colony counting.

- **Results**

The results are presented in Table 5.

Table 5: Exclusivity study

EXCLUSIVITE Souche			Origine	Référence	TSA	VRBG	Chromagar	oxydase	gélose OF
1	*Alcaligenes*	*sp*	Urine de chien	1084/13/3	44	0	0	/	/
3	*Bacillus*	*cereus*	Eau	IAA45	17	0	0	/	/
4	*Bacillus*	*cereus*	Sauté de dinde	13_IAA.3520.1	36	0	0	/	/
5	*Bacillus*	*mycoïdes*	Radis bio	IAA3	52	0	0	/	/
6	*Brevibacillus*	*laterosporus*	Fromage de chèvre	13_IAA_698.1	20	0	0	/	/
7	*Enterococcus*	*faecalis*	Lait de vache	IAA8	56	0	0	/	/
8	*Enterococcus*	*faecium*	Découpe de canard	IAA9	140	0	0	/	/
9	*Lactobacillus*	*acidophilus*	Produit laitier	1415/17/38	63	0	0	/	/
10	*Lactobacillus*	*casei*	Produit laitier	1416/17/39	0	0	0		
11	*micrococcus*	*luteus*	Alimentaire	IAA29	52	0	0	/	/
12	*Pseudomonas*	*aeruginosa*	ATCC 27853	1792/22/10	34	0	0	/	/
13	*Pseudomonas*	*aeruginosa*	Oreille de chien	santé animal 750S	49	0	0	/	/
14	*Pseudomonas*	*fluorescens*	Urine de chien	1104/13/51	48	0	0	/	/
15	*Pseudomonas*	*luteola*	Volaille	IAA57	20	33	29 (col pourpres)	Négative	+
16	*Pseudomonas*	*putida*	Alfalfa	13_IAA.1579.1	46	36	51	Positive	/
17	*Staphylococcus*	aureus	Lait de vache	1310/16/15	79	0	0	/	/
2	*Staphylococcus*	*epidermidis*	Produit laitier	IAA53	40	0	0	/	/
18	*Staphylococcus*	intermidius	Alimentaire	1321/16/25	46	0	0	/	/
19	*Streptococcus*	*agalactiae*	Lait de vache	16/0/16	34	0	0	/	/
20	*Streptococcus*	*uberis*	Lait de vache	772/9/1943	57	0	0	/	/

/: test not conducted

Of the 20 strains tested, 18 did not yield culture by alternative method and by reference method.
2 strains gave characteristic colonies on CHROMagar™ Enterobacteria and on VRBG:
- *Pseudomonas putida*
- *Pseudomonas luteola*

These 2 strains were confirmed using the confirmation protocol of the reference method (oxidase and OF Glucose).

Pseudomonas putida did not give a typical result of confirmation of enterobacteriaceae (positive oxidase) but Pseudomonas luteola gave a negative oxidase and positive OF Glucose test for the alternative method and for the reference method. This atypical *Pseudomonas luteola* oxidase negative criterion is confirmed by the bibliography.

4. Conclusion

The observed results confirm the performance of the alternative method:

- 85 samples were tested during the sensitivity study, showing consistent results between the alternative method and the reference method for all categories tested.
- The accuracy profiles are satisfactory for each of the 3 categories tested.
- The inclusivity and exclusivity study showed satisfactory results. Only one non-target strain, *Pseudomonas luteola*, gave characteristic colonies on CHROMagar™ Enterobacteria agar and VRBG agar. It should also be noted that *Shigella sonnei* gives atypical blue-green colonies on CHROMagar™ Enterobacteria agar. The Indole Test permit the differenciation between *Shigella sonnei* which is Indole negative from *E.coli* which is Indole positive.

Done at TOURS, on the

05/07/2018

Stéphanie ROTILY-FORCIOLI
Head of Microbiology Department

APPENDICES

APPENDICE 1

Operating mode of the alternative method

CHROMagar™ Enterobacteria Protocol

Xg + 9X mL appropriate diluent

↓

Stomacher

↓

Decimal dilutions

↓

Inoculate 1mL per plate

↓

Add CHROMagar medium (about 15mL)

Solidification

Add a double layer (about 5mL)

↓

Incubate 24h at 37°C

↓

Count colonies with characteristics (blue and/or pink)

↓

Confirmations: Prick out 5 colonies per plate retained on TSA

↓

Incubate 24±2h at 37±1°C

↙ ↘

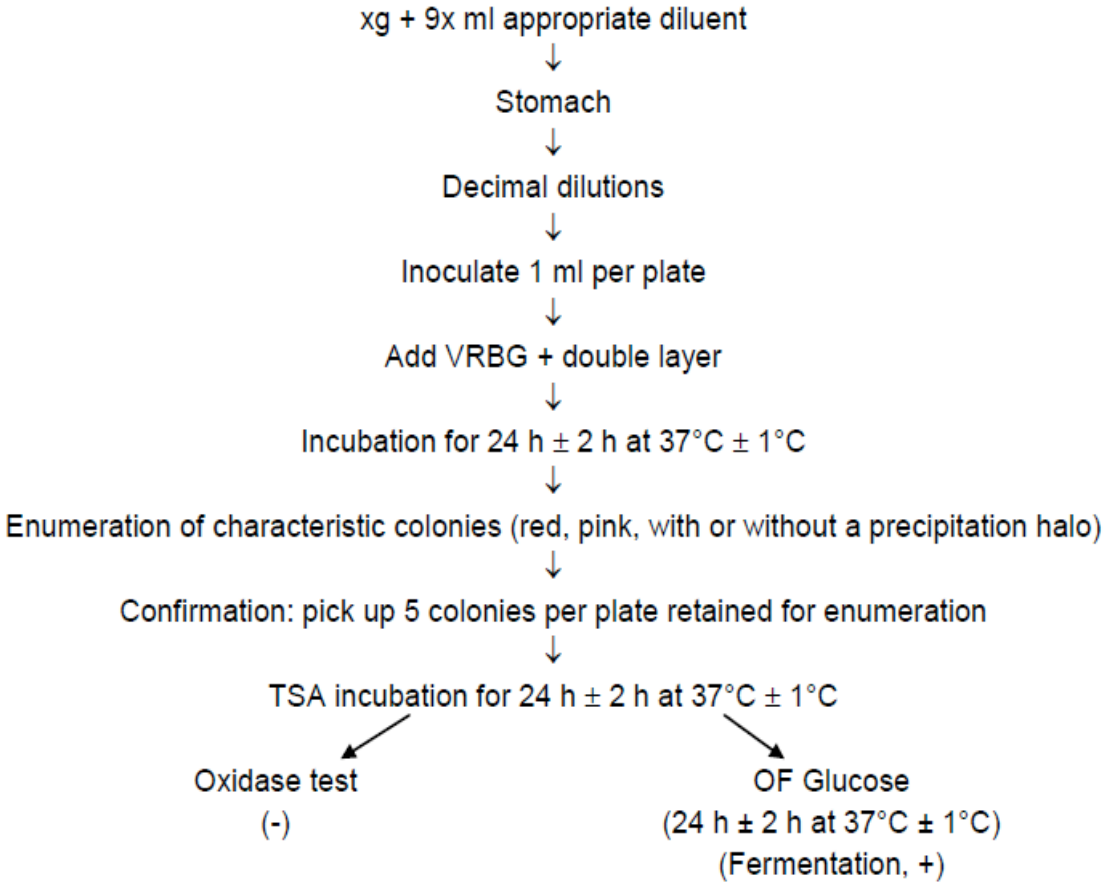
Oxydase test (-) **Agar OF 24±2h at 37±1°C**

(Fermentation +)

APPENDICE 2

Operating mode of the reference method

NF ISO 21528-2 (June 2017) - Microbiology of food and animal feeding stuffs - Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: colony-count method



APPENDICE 3

Raw results sensitivity study

Numéro	Référence interne	Catégorie	Matrice	conta naturelle (CN) ou conta artificielle (CA)	Souche (si identifiée)	MR: NF EN ISO 21528-2 VRBG#					MA: Chromagar				
						Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g	Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g
1	2471.1	Ovoproduits - Divers	œufs poché dinde salade	CN	*Pantoea spp2*	1 000	94	94	94545	4,98	1 000	87	87	87273	4,94
						10 000	10	10			10 000	9	9		
2	2538.1	Ovoproduits - Divers	Tarte aux fraises	CN	*Enterobacter cloacae*	10	6	6	55	1,74	10	3	3	27	1,44
						100	0	0			100	0	0		
3	2537.1	Ovoproduits - Divers	Eclair café	CN	*Raoultella terrigena*	10	31	31	318	2,50	10	7	7	73	1,86
						100	4	4			100	1	1		
4	2515.2	Ovoproduits - Divers	3 chocolats	CN	*Hafina alvei*	100	83	83	8273	3,92	100	81	81	8182	3,91
						1 000	8	8			1 000	9	9		
5	2479.1	Ovoproduits - Divers	Paris Brest	CN	*Klebsiella oxytoca*	10	9	9	91	1,96	10	5	5	64	1,80
						100	1	1			100	2	2		
6	2536.1	Ovoproduits - Divers	Eclair	CN		10	8	8	73	1,86	10	3	3	27	1,44
						100	0	0			100	0	0		
7	2480.1	Ovoproduits - Divers	Eclair chocolat	CN		10	15	15	136	2,13	10	13	13	136	2,13
						100	0	0			100	2	2		
8	18	Ovoproduits - Divers	Eclair café	CN	*Enterobacter cloacae*	10	19	19	191	2,28	10	11	11	100	2,00
						100	2	2			100	0	0		
9	22	Ovoproduits - Divers	Paella	CN		100	70	70	6818	3,83	100	76	76	7455	3,87
						1 000	5	5			1 000	6	6		
10	31	Ovoproduits - Divers	Mille feuille	CN		10	79	79	809	2,91	10	68	68	664	2,82
						100	10	10			100	5	5		
11	32	Ovoproduits - Divers	Religieuse au café	CN		10	120	120	1191	3,08	10	120	120	1191	3,08
						100	11	11			100	11	11		
12	38	Ovoproduits - Divers	Eclair au chocolat	CN		1 000	17	17	15455	4,19	1 000	15	15	17273	4,24
						10 000	0	0			10 000	4	4		
13	39	Ovoproduits - Divers	Passionata	CN		10	23	23	236	2,37	10	42	42	400	2,60
						100	3	3			100	2	2		
14	42	Ovoproduits - Divers	Piémontaise	CN		10	8	8	100	2,00	10	13	13	127	2,10
						100	3	3			100	1	1		
15	43	Ovoproduits - Divers	charlottes aux fraises	CN		10	84	84	836	2,92	10	80	80	800	2,90
						100	8	8			100	8	8		
16	44	Ovoproduits - Divers	eclair au café	CN		100	60	60	6182	3,79	100	50	50	5091	3,71
						1 000	8	8			1 000	6	6		
17	1572.2	Produits carnés	Bœuf cru	CN	*E.coli*	100	26	26	2545	3,41	100	15b	15b	1455	3,16
						1 000	2	2			1 000	1b	1b		
18	1372.2	Produits carnés	Sanglier	CN	*E.coli*	100	169	169	17636	4,25	100	18b 95r	18b 95r	11182	4,05
						1 000	25	25			1 000	0b 10r	0b 10r		
19	1650.1	Produits carnés	Foie Gras	CN	*E.coli*	100	99	99	9727	3,99	100	1b 74r	1b 74r	7273	3,86
						1 000	8	8			1 000	0b 5r	0b 5r		
20	2679.2	Produits carnés	Saucisses crues	CN	*Hafina alvei*	10	40	40	391	2,59	10	9	9	82	1,91
						100	3	3			100	0	0		
21	2933.2	Produits carnés	Chair à saucisses	CN	*Serratia liquefaciens*	100	182	182	18364	4,26	100	99	99	10273	4,01
						1 000	20	20			1 000	14	14		
22	13	Produits carnés	Saucisse crue	CN	*Klebsiella oxytoca*	10	39	39	400	2,60	10	41	41	391	2,59
						100	5	5			100	2	2		
23	15	Produits carnés	Veau	CN	*Serratia liquefaciens*	100	103	103	9727	3,99	100	72	72	7273	3,86
						1 000	4	4			1 000	8	8		
24	16	Produits carnés	Agneau	CN		10 000	21	21	200000	5,30	10 000	1b 17r	1b 17r	172727	5,24
						100 000	1	1			100 000	0b 1r	0b 1r		
25	17	Produits carnés	Bœuf	CN	*Serratia liquefaciens*	100	204	204	20364	4,31	100	109	109	11091	4,04
						1 000	20	20			1 000	13	13		
26	19	Produits carnés	Saucisse crue	CN		10	42	42	427	2,63	10	3b 34r	3b 34r	373	2,57
						100	5	5			100	0b 4r	0b 4r		
27	20	Produits carnés	Pâté de tête	CN	*Hafnia alvei*	10 000	42	42	427273	5,63	10 000	8b 20r	8b 20r	281818	5,45
						100 000	5	5			100 000	2b 1r	2b 1r		
28	21	Produits carnés	Boudin blanc	CN	*Serratia liquefaciens*	10 000	10	10	109091	5,04	10 000	13	13	118182	5,07
						100 000	2	2			100 000	0	0		
29	23	Produits carnés	Merguez	CN		1 000	23	23	22727	4,36	1 000	15	15	14545	4,16
						10 000	2	2			10 000	1	1		
30	24	Produits carnés	Merguez	CN		1 000	63	63	62727	4,80	1 000	43	43	44545	4,65
						10 000	6	6			10 000	6	6		
31	25	Produits carnés	Saucisse crue	CN		10 000	15	15	145455	5,16	10 000	17b	17	163636	5,21
						100 000	1	1			100 000	1b	1		
32	27	Produits carnés	Pâté de campagne	CN		10	139	139	1382	3,14	10	148	148	1491	3,17
						100	13	13			100	16	16		
33	28	Produits carnés	Bœuf	CN		1 000	29	29	28182	4,45	1 000	24	24	22727	4,36
						10 000	2	2			10 000	1	1		
34	30	Produits carnés	Poulet	CN		1 000	99	99	99091	5,00	1 000	78	78	78182	4,89
						10 000	10	10			10 000	8	8		
35	35	Produits carnés	cervelas	CN		10	37	37	382	2,58	10	39	39	382	2,58
						100	5	5			100	3	3		
36	36	Produits carnés	Boudin noir	CN		10	65	65	636	2,80	10	37	37	373	2,57
						100	5	5			100	4	4		
37	37	Produits carnés	Saucisse de campagne	CN		10 000	72	72	727273	5,86	10 000	2b 49r	2b 49r	527273	5,72
						100 000	8	8			100 000	0b 7r	0b 7r		
38	40	Produits carnés	Selle d'Agneau crue	CN		100	31	31	3091	3,49	100	18	18	1818	3,26
						1 000	3	3			1 000	2	2		
39	41	Produits carnés	Terrine de campagne	CN		100	82	82	8182	3,91	100	69	69	6909	3,84
						1 000	8	8			1 000	7	7		

Numéro	Référence interne	Catégorie	Matrice	conta naturelle (CN) ou conta artificielle (CA)	N° souche	Souche (si identifiée)	MR: NF EN ISO 21528-2 VRBG#					MA: Chromagar				
							Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g	Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g
40	2529.1	Végétaux	Céleri rémoulade	CN	/	*Rhanella aquatilis*	10	23	23	209	2,32	10	23	23	227	2,36
							100	0	0			100	2	2		
41	2469.3	Végétaux	Carottes rapées	CN	/	/	10	0	0	<10	<1	10	0	0	<10	<1
							100	0	0			100	0	0		
42	2685.1	Végétaux	Salade Egyptienne	CN	/	*Pantoea spp3*	1 000	159	159	148182	5,17	1 000	119	119	120000	5,08
							10 000	4	4			10 000	13	13		
43	2525.3	Végétaux	Légumes poelée	CN	/	/	10	0	0	<10	<1	10	0	0	<10	<1
							100	0	0			100	0	0		
44	9	Végétaux	Haricot mango	CA	INCLU n°17	*Pantoea spp* seeding 72h	10	Envahie Proteus	Envahie Proteus			10	Envahie Proteus	Envahie Proteus		
							100	Envahie Proteus	Envahie Proteus			100	Envahie Proteus	Envahie Proteus		
45	10	Végétaux	Carotte rapée	CA	INCLU n°17	*Pantoea spp* seeding 72h	10	131	131	1245	3,10	10	120	120	1182	3,07
							100	6	6			100	10	10		
46	11	Végétaux	Haricot vert	CA	INCLU n°17	*Pantoea spp* seeding 72h	1 000	105	105	105455	5,02	1 000	90	90	88182	4,95
							10 000	11	11			10 000	7	7		
47	12	Végétaux	Flageolets	CA	INCLU n°17	*Pantoea spp* seeding 72h	10	104	104	1018	3,01	10	87	87	809	2,91
							100	8	8			100	2	2		
48	14	Végétaux	Carottes rapés	CN	/	*Pantoea spp*	10	13	13	118	2,07	10	18	18	173	2,24
							100	0	0			100	1	1		
49	29	Végétaux	Vermicelle	CN	/	/	10	1	1	<10	<1	10	1	1	<10	<1
							100	0	0			100	0	0		
50	34	Végétaux	carottes rapées	CN	/	/	10	2	2	18	1,26	10	3	3	27	1,44
							100	0	0			100	0	0		
51	45	Végétaux	Tomates	CN	/	/	10 000	105	105	1036364	6,02	10 000	99	99	981818	5,99
							100 000	9	9			100 000	9	9		
52	65	Végétaux	Petits pois	CA	INCLU n°13	*Hafnia alvei* seeding 72h	10	30	30	300	2,48	10	26	26	236	2,37
							100	3	3			100	0	0		
53	66	Végétaux	Carottes rapé	CA	INCLU n°13	*Hafnia alvei* seeding 72h	10	13	13	118	2,07	10	14	14	127	2,10
							100	0	0			100	0	0		
54	67	Végétaux	Salade de lentilles	CA	INCLU n°13	*Hafnia alvei* seeding 72h	10	6	6	55	1,74	10	9	9	91	1,96
							100	0	0			100	1	1		
55	68	Végétaux	Comcombres celeri	CA	AFNOR n°188(3/26)	*Pantoea* seeding 72h	100	11	11	1091	3,04	100	11	11	1091	3,04
							1 000	1	1			1 000	1	1		
56	69	Végétaux	Courgettes	CA	AFNOR n°188(3/26)	*Pantoea* seeding 72h	100	44	44	4091	3,61	100	67	67	6455	3,81
							1 000	1	1			1 000	4	4		
57	70	Végétaux	Flageolets	CA	AFNOR n°188(3/26)	*Pantoea* seeding 72h	1 000	39	39	39091	4,59	1 000	44	44	40909	4,61
							10 000	4	4			10 000	1	1		
58	71	Végétaux	Fraises	CA	AFNOR n°188(3/26)	*Pantoea* seeding 72h	1 000	60	60	60000	4,78	1 000	60	60	60000	4,78
							10 000	6	6			10 000	6	6		
59	72	Végétaux	Betteraves	CA	AFNOR n°188(3/26)	*Pantoea* seeding 72h	10	59	59	600	2,78	10	46	46	455	2,66
							100	7	7			100	4	4		
60	2680.1	Produits de la mer	Encornets	CN	/	*Serratia liquefaciens*	**10 000**	**59**	**59**	618182	**5,79**	**10 000**	**71**	**71**	718182	**5,86**
							100 000	**9**	**9**			**100 000**	**8**	**8**		
61	2712.1	Produits de la mer	Langoustines	CN	/	*Pantoea spp2*	**10 000**	**33**	**33**	309091	**5,49**	**10 000**	**29**	**29**	281818	**5,45**
							100 000	**1**	**1**			**100 000**	**2**	**2**		
62	1	Produits de la mer	Brandade de morue	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	10	**149**	**149**	1436	**3,16**	10	**150**	**150**	1473	**3,17**
							100	**9**	**9**			100	**12**	**12**		
63	2	Produits de la mer	Saumon fumé	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	10	**15**	**15**	155	**2,19**	10	**10**	**10**	127	**2,10**
							100	**2**	**2**			100	**4**	**4**		
64	3	Produits de la mer	Sardine cuite	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	10	**205**	**205**	2100	**3,32**	10	**192**	**192**	1927	**3,28**
							100	**26**	**26**			100	**20**	**20**		
65	4	Produits de la mer	Poisson colin	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	**1 000**	**170**	**170**	160909	**5,21**	**1 000**	**160**	**160**	150909	**5,18**
							10 000	**7**	**7**			**10 000**	**6**	**6**		
66	26	Produits de la mer	Thon rouge fumé	CN	/	/	10	**100**	**100**	1000	**3,00**	10	**6b 82r**	**6b 82r**	864	**2,94**
							100	**10**	**10**			100	**0b 7r**	**0b 7r**		
67	33	Produits de la mer	Rillettes Poisson ecrevisses	CN	/	/	10	**10**	**10**	118	**2,07**	10	**2b 12r**	**2b 12r**	155	**2,19**
							100	**3**	**3**			100	**0b 3r**	**0b 3r**		
68	58	Produits de la mer	Thon salade	CA	INCLU n°26	*Shigella sonnei* seeding 72h	10	22	22	218	2,34	10	2b 20r	2b 20r	227	2,36
							100	2	2			100	2b 1r	2b 1r		
69	59	Produits de la mer	Filet de colin	CA	INCLU n°26	*Shigella sonnei* seeding 72h	10	7	7	73	1,86	10	9	9	91	1,96
							100	1	1			100	1	1		
70	60	Produits de la mer	encornets	CA	INCLU n°26	*Shigella sonnei* seeding 72h	10	53	53	536	2,73	10	64	64	627	2,80
							100	6	6			100	5	5		
71	61	Produits de la mer	brandade de morue	CA	INCLU n°26	*Shigella sonnei* seeding 72h	100	88	88	8545	3,93	100	92	92	8909	3,95
							1 000	6	6			1 000	6	6		
72	62	Produits de la mer	filet de colin	CA	INCLU n°26	*Shigella sonnei* seeding 72h	100	64	64	6182	3,79	100	63	63	6273	3,80
							1 000	4	4			1 000	6	6		
73	63	Produits de la mer	filet de colin	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	10 000	190	190	1754545	6,24	10 000	152	152	1418182	6,15
							100 000	3	3			100 000	4	4		
74	64	Produits de la mer	Brandade de morue	CA	INCLU n°29	*Yersinia ruckeri* seeding 72h	10 000	145	145	1600000	6,20	10 000	170	170	1763636	6,25
							100 000	31	31			100 000	24	24		

Numéro	Référence interne	Catégorie	Matrice	conta naturelle (CN) ou conta artificielle (CA)	N° souche	Souche (si identifiée)	MR: NF EN ISO 21528-2 VRBG[#]					MA: Chromagar				
							Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g	Dilution	ufc / boite	ufc confirmés	ufc / g	log ufc / g
75	1877.1	Produits Laitiers	Tomme lait cru de vache	CN	/	*E.coli*	10	94	94	936	2,97	10	5b 156r	5b 156r	1600	3,20
							100	9	9			100	1b 14r	1b 14r		
76	5	Produits Laitiers	Lait de vache	CA	INCLU n°15	*Klebsiella oxytoca* seeding 72h	10	Envahie Proteus	Envahie Proteus			10	Envahie Proteus	Envahie Proteus		
							100	Envahie Proteus	Envahie Proteus			100	Envahie Proteus	Envahie Proteus		
77	6	Produits Laitiers	Valençay	CA	INCLU n°15	*Klebsiella oxytoca* seeding 72h	100	162	162	16545	4,22	100	116	116	11455	4,06
							1 000	20	20			1 000	10	10		
78	7	Produits Laitiers	Cheddar	CA	INCLU n°15	*Klebsiella oxytoca* seeding 72h	10	28	28	255	2,41	10	18	18	173	2,24
							100	0	0			100	1	1		
79	8	Produits Laitiers	Fromage frais chèvre	CA	INCLU n°15	*Klebsiella oxytoca* seeding 72h	10	1	1	<10	<1	10	5	5	45	1,66
							100	0	0			100	0	0		
80	46	Produits Laitiers	yaourt lait de vache pêche	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	10	0	0	<10	<1	10	0	0	<10	<1
							100	0	0			100	0	0		
81	47	Produits Laitiers	buche de chèvre	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	10	21	21	191	2,28	10	11	11	109	2,04
							100	0	0			100	1	1		
82	48	Produits Laitiers	Fromage de chèvre	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	100	104	104	10091	4,00	100	95	95	9182	3,96
							1 000	7	7			1 000	6	6		
83	49	Produits Laitiers	fromage de chèvre	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	10	5	5	55	1,74	10	5	5	55	1,74
							100	1	1			100	1	1		
84	50	Produits Laitiers	Faisselle de chèvre	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	10	0	0	<10	<1	10	0	0	<10	<1
							100	0	0			100	0	0		
85	51	Produits Laitiers	caillé de chèvre	CA	INCLU n°2	*Citrobacter koseri* seeding 72h	10 000	248	248	2481818	6,39	10 000	240	240	2454545	6,39
							100 000	25	25			100 000	30	30		
86	52	Produits Laitiers	lait de chèvre	CA	INCLU n°10	*Escherichia coli* seeding 72h	10	15	15	145	2,16	10	9	9	82	1,91
							100	1	1			100	0	0		
87	53	Produits Laitiers	caillé de chèvre	CA	INCLU n°10	*Escherichia coli* seeding 72h	10 000	14	14	136364	5,13	10 000	1b 12r	1b 12r	127273	5,10
							100 000	1	1			100 000	0b 1r	0b 1r		
88	54	Produits Laitiers	Valençay	CA	INCLU n°10	*Escherichia coli* seeding 72h	10 000	45	45	445455	5,65	10 000	1b 32r	1b 32r	345455	5,54
							100 000	4	4			100 000	0b 5r	0b 5r		
89	55	Produits Laitiers	Fromage de chèvre	CA	INCLU n°10	*Escherichia coli* seeding 72h	10	70	70	745	2,87	10	52	52	564	2,75
							100	12	12			100	10	10		
90	56	Produits Laitiers	Yaourt 0% nature lait de vache	CA	INCLU n°10	*Escherichia coli* seeding 72h	100	59	59	6364	3,80	100	60	60	6000	3,78
							1 000	11	11			1 000	6	6		
91	57	Produits Laitiers	yaourt lait de vache	CA	INCLU n°10	*Escherichia coli* seeding 72h	100	58	58	6000	3,78	100	49	49	5182	3,71
							1 000	8	8			1 000	8	8		
92	73	Produits Laitiers	Fromage au lait cru	CN	/	/	10 000	64	64	654545	5,82	10 000	64	64	609091	5,78
							100 000	8	8			100 000	3	3		
93	74	Produits Laitiers	Fromage au lait cru	CN	/	/	10 000	33	33	309091	5,49	10 000	43	43	445455	5,65
							100 000	1	1			100 000	6	6		
94	75	Produits Laitiers	Fromage au lait cru	CN	/	/	10 000	67	67	700000	5,85	10 000	70	70	736364	5,87
							100 000	10	10			100 000	11	11		

APPENDICE 4

Raw results accuracy profiles

Profil d'exactitude

Matrice	Souche	Niveau de conta	n°LOT	Numéro échantillon	MR: NF EN ISO 21528-2 VRBG #				MA: Chromagar			
					Dilution	ufc / boite	ufc / g	log ufc / g	Dilution	ufc / boite	ufc / g	log ufc / g
Produits carnés : Viande haché	Escherichia coli	1	Lot 1	1	10	56	573	2,76	10	53	527	2,72
					100	7			100	5		
				2	10	53	527	2,72	10	54	545	2,74
					100	5			100	6		
				3	10	65	627	2,80	10	73	727	2,86
					100	4			100	7		
			Lot 2	4	10	53	509	2,71	10	66	655	2,82
					100	3			100	6		
				5	10	53	545	2,74	10	67	655	2,82
					100	7			100	5		
				6	10	54	555	2,74	10	62	591	2,77
					100	7			100	3		
		2	Lot 1	7	100	144	14455	4,16	100	134	13545	4,13
					1 000	15			1 000	15		
				8	100	134	13727	4,14	100	141	13818	4,14
					1 000	17			1 000	11		
				9	100	164	15727	4,20	100	138	14000	4,15
					1 000	9			1 000	16		
			Lot 2	10	100	140	13909	4,14	100	130	13000	4,11
					1 000	13			1 000	13		
				11	100	135	13727	4,14	100	154	15545	4,19
					1 000	16			1 000	17		
				12	100	140	14636	4,17	100	115	12000	4,08
					1 000	21			1000	17		
		3	Lot 1	13	10 000	139	1390909	6,14	10 000	144	1463636	6,17
					100 000	14			100 000	17		
				14	10 000	156	1536364	6,19	10 000	160	1627273	6,21
					100 000	13			100 000	19		
				15	10 000	130	1318182	6,12	10 000	134	1372727	6,14
					100 000	15			100 000	17		
			Lot 2	16	10 000	154	1554545	6,19	10 000	161	1618182	6,21
					100 000	17			100 000	17		
				17	10 000	139	1372727	6,14	10 000	141	1418182	6,15
					100 000	12			100 000	15		
				18	10 000	156	1563636	6,19	10 000	150	1509091	6,18
					100 000	16			100 000	16		

Profil d'exactitude

Matrice	Souche	Niveau de conta	n°LOT	Numéro échantillon	MR: NF EN ISO 21528-2 VRBG #				MA: Chromagar			
					Dilution	ufc / boite	ufc / g	log ufc / g	Dilution	ufc / boite	ufc / g	log ufc / g
Produits de la mer : Saumon fumé	Hafnia alvei	1	Lot 1	1	10	51	545	2,74	10	77	773	2,89
					100	9			100	8		
				2	10	75	782	2,89	10	89	836	2,92
					100	11			100	3		
				3	10	61	618	2,79	10	62	609	2,78
					100	7			100	5		
			Lot 2	4	10	55	527	2,72	10	56	591	2,77
					100	3			100	9		
				5	10	64	682	2,83	10	40	418	2,62
					100	11			100	6		
				6	10	53	591	2,77	10	67	673	2,83
					100	12			100	7		
		2	Lot 1	7	100	143	14273	4,15	100	136	13818	4,14
					1 000	14			1 000	16		
				8	100	147	15091	4,18	100	154	14909	4,17
					1 000	19			1 000	10		
				9	100	138	13636	4,13	100	144	14818	4,17
					1 000	12			1 000	19		
			Lot 2	10	100	140	14182	4,15	100	161	16455	4,22
					1 000	16			1 000	20		
				11	100	129	12909	4,11	100	136	13545	4,13
					1 000	13			1 000	13		
				12	100	133	13273	4,12	100	130	13182	4,12
					1 000	13			1000	15		
		3	Lot 1	13	10 000	162	1627273	6,21	10 000	171	1736364	6,24
					100 000	17			100 000	20		
				14	10 000	154	1536364	6,19	10 000	157	1536364	6,19
					100 000	15			100 000	12		
				15	10 000	164	1627273	6,21	10 000	168	1663636	6,22
					100 000	15			100 000	15		
			Lot 2	16	10 000	149	1454545	6,16	10 000	159	1600000	6,20
					100 000	11			100 000	17		
				17	10 000	178	1809091	6,26	10 000	164	1654545	6,22
					100 000	21			100 000	18		
				18	10 000	148	1463636	6,17	10 000	142	1390909	6,14
					100 000	13			100 000	11		

Profil d'exactitude

Matrice	Souche	Niveau de conta	n°LOT	Numéro échantillon	MR: NF EN ISO 21528-2 VRBG #				MA: Chromagar			
					Dilution	ufc / boite	ufc / g	log ufc / g	Dilution	ufc / boite	ufc / g	log ufc / g
Produits laitiers : fromage de chèvre au lait cru	Enterobacter cloacae	1	Lot 1	1	10	40	400	2,60	10	42	391	2,59
					100	4			100	1		
				2	10	38	373	2,57	10	30	273	2,44
					100	3			100	0		
				3	10	30	291	2,46	10	44	427	2,63
					100	2			100	3		
			Lot 2	4	10	47	455	2,66	10	30	300	2,48
					100	3			100	3		
				5	10	25	300	2,48	10	39	400	2,60
					100	8			100	5		
				6	10	40	373	2,57	10	43	409	2,61
					100	1			100	2		
		2	Lot 1	7	100	85	8364	3,92	100	85	8182	3,91
					1 000	7			1 000	5		
				8	100	81	7909	3,90	100	69	6818	3,83
					1 000	6			1 000	6		
				9	100	80	7727	3,89	100	67	6545	3,82
					1 000	5			1 000	5		
			Lot 2	10	100	79	8000	3,90	100	80	8091	3,91
					1 000	9			1 000	9		
				11	100	95	8909	3,95	100	88	8818	3,95
					1 000	3			1 000	9		
				12	100	99	9636	3,98	100	79	7818	3,89
					1 000	7			1000	7		
		3	Lot 1	13	10 000	127	1236364	6,09	10 000	105	1081818	6,03
					100 000	9			100 000	14		
				14	10 000	118	1127273	6,05	10 000	105	1072727	6,03
					100 000	6			100 000	13		
				15	10 000	103	981818	5,99	10 000	97	963636	5,98
					100 000	5			100 000	9		
			Lot 2	16	10 000	110	1090909	6,04	10 000	89	890909	5,95
					100 000	10			100 000	9		
				17	10 000	115	1181818	6,07	10 000	92	954545	5,98
					100 000	15			100 000	13		
				18	10 000	106	1081818	6,03	10 000	82	818182	5,91
					100 000	13			100 000	8		