Preamble

- **Protocols of validation :**

- **EN ISO 16140-1 and NF EN ISO 16140-2 (September 2016): Microbiology of the food chain — Method validation**
  Part 1: Vocabulary.
  Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

- **Reference method:**

- **NF ISO 6888-2 (September 2021):** Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 2: Method using rabbit plasma fibrinogen agar media.

- **Application scope:**

- **All human food products** by a validation testing of different categories of foods, including:
  - meat products,
  - dairy products,
  - seafood products,
  - vegetal products,
  - composite foods.

- **Animal feed products.**

Definitions

- **Method comparison study**

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

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

- **Relative trueness study**

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

- **Accuracy profile study**

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

- **Inclusivity and exclusivity study**

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

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Appendices

1. Introduction

CHROMagar™ Staph aureus is a selective chromogenic culture media intended for use in the qualitative direct detection, differentiation, and presumptive identification of *Staphylococcus aureus* to aid in the diagnosis of *Staphylococcus aureus* colonization. The medium can be used as an early warning indicator for diagnostic tests of infections to signal the possible presence of *Staphylococcus aureus*.
CHROMagar™ Staph aureus can also be used in the detection and enumeration of *Staphylococcus aureus* and other coagulase positive staphylococci in the analyses of food products for human consumption, animal feed and in environmental samples.

The aim of this study is to verify the performance of the CHROMagar™ Staph aureus agar media versus the Baird-Parker + RPF agar media with pure strains and food products associated with coagulase positive staphylococci. These food matrices represent a broad range of foods grouped into different categories.

The results set out in this report were produced during tests carried out by Laboratory MICROSEPT in 2022.

2. Protocols of the methods

2.1. Alternative method

2.1.1. Principle of the method

The alternative method tested is the CHROMagar™ Staph aureus method for the isolation, enumeration and direct differentiation of Staphylococcus aureus in food and feed samples without confirmation.

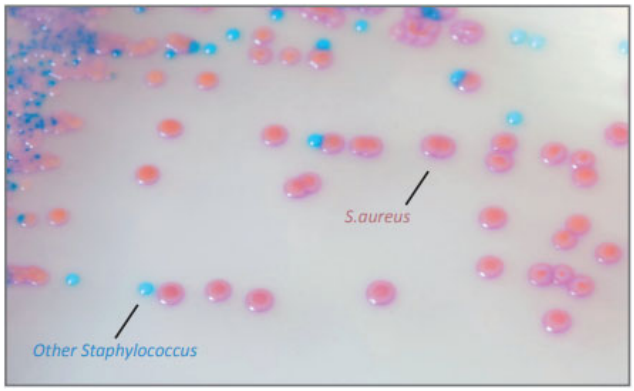
2.1.2. Protocol of the method

The diagram summarizing the method is shown in Appendix A.

10 g of sample in 90 mL of Buffered Peptone Water according to ISO 6887 standards.
Two modalities of inoculation are available:

- Surface spreading protocol: inoculate 1 mL across three CHROMagar™ Staph aureus plates and incubate for 18–24 hours at 37±2°C in aerobic conditions.
- Poured plate protocol: inoculate 1 mL in a Petri dish and add 20±2 mL of CHROMagar™ Staph aureus agar media and incubate 18–24 hours at 37±2°C in aerobic conditions.

After 18 to 24 hours at 35-37°C, the characteristic colonies of *S. aureus* and other coagulase-positive staphylococci appear pink to purple in color.



2.1.3. Restrictions

There are no restrictions on the use of the CHROMagar™ Staph aureus method.

2.2. Reference method

The reference method (RM) to which the alternative method (AM) will be compared is that described in ISO 6888-2 (09/2021): Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) — Part 2: Method using rabbit plasma fibrinogen agar media.

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

3. Relative trueness study

3.1. Number and categories of food and feed samples

In this study, 81 samples were analyzed giving 50 exploitable results for surface spreading inoculation and 51 exploitable results for poured plate inoculation.
The distribution of the samples per category and inoculation technique is given in table 2.

Table 2: number and nature of the samples analyzed for the relative trueness study

Category	Samples analyzed	Interpretable results	
		Surface	Poured plate
❶ **Meat products**	18	9	9
❷ **Dairy products**	15	10	10
❸ **Seafood products**	11	7	8
❹ **Vegetal products**	10	10	9
❺ **Composite foods**	24	11	12
❻ **Animal feed**	3	3	3
Total	**81**	**50**	**51**

3.1.1. Artificial contaminations

Naturally contaminated samples were analyzed preferably.
However, artificially contaminated samples were still analyzed, using seeding or spiking protocols as described in the standard NF EN ISO 16140-2:2016.

Among the interpretable results for surface spreading protocol, fifteen correspond to naturally contaminated samples and thirty-five to artificially contaminated samples. Among the interpretable results for poured plate protocol, sixteen correspond to naturally contaminated samples and thirty-five to artificially contaminated samples.
The artificial contaminations performed are presented in the Appendix C.

3.1.2. Protocols used during the study

The samples were analyzed by the reference method ISO 6888-2 using Baird-Parker + RPF and by the CHROMagar method using CHROMagar™ Staph aureus media.
For the CHROMagar™ Staph aureus media, the minimum incubation time of the Petri dishes was applied, namely 18 hours. After incubation of the CHROMagar™ Staph aureus and the Baird- Parker plates, a count was be made, and the characteristic colonies observed on the CHROMagar™ Staph aureus media were confirmed by the coagulase test.

3.1.3. Results

Raw results are shown in Appendix D.

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

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

All results are presented in scatter plots per category in figure 1 for surface spreading inoculation and in figure 2 for poured plate inoculation. Figures 3 and 4 present the results for all categories.

3.1.4. Calculation and interpretation of relative trueness study

The results obtained are analyzed using the Bland-Altman method.
Statistical calculations are presented in Appendix E, as well as the results excluded from the statistical analysis per category, type and modality of inoculation.

Table 3 presents the summary of the average differences and standard deviation differences per category and for all categories with surface spreading inoculation.

Table 3: values for the Bland-Altman difference plot with surface spreading inoculation

Category	N	Average difference	Standard deviation differences	Bias	Lower Confidence Limit	Upper Confidence Limit
Meat products	9	0.10	0.20	0.10	-0.39	0.58
Dairy products	10	-0.07	0.18	-0.07	-0.50	0.37
Seafood products	7	0.12	0.19	0.12	-0.37	0.60
Vegetal products	10	-0.04	0.16	-0.04	-0.41	0.34
Composite foods	11	0.04	0.26	0.04	-0.56	0.65
Feed products	3	0.07	0.25	0.07	-1.17	1.30
All categories	**50**	**0.03**	**0.21**	**0.03**	**-0.39**	**0.45**

Overall, the average difference is equal to 0.03, showing a weak positive bias between the CHROMagar™ Staph aureus method and the reference method with surface spreading inoculation.

Table 4 presents the summary of the average differences and standard deviation differences per category and for all categories with poured plate inoculation.

Table 4: values for the Bland-Altman difference plot with poured plate inoculation

Category	n	Average difference	Standard deviation differences	Bias	Lower Confidence Limit	Upper Confidence Limit
Meat products (MP)	9	-0.04	0.28	-0.04	-0.73	0.64
Dairy products (DP)	10	-0.13	0.26	-0.13	-0.74	0.48
Seafood products (SFP)	7	0.01	0.19	0.01	-0.47	0.49
Vegetal products (VP)	10	0.00	0.18	0.00	-0.43	0.43
Composite foods (CF)	11	-0.04	0.30	-0.04	-0.71	0.69
Feed products (FP)	3	0.03	0.25	0.03	-1.23	1.29
All categories	**51**	**-0.03**	**0.25**	**-0.03**	**-0.53**	**0.47**

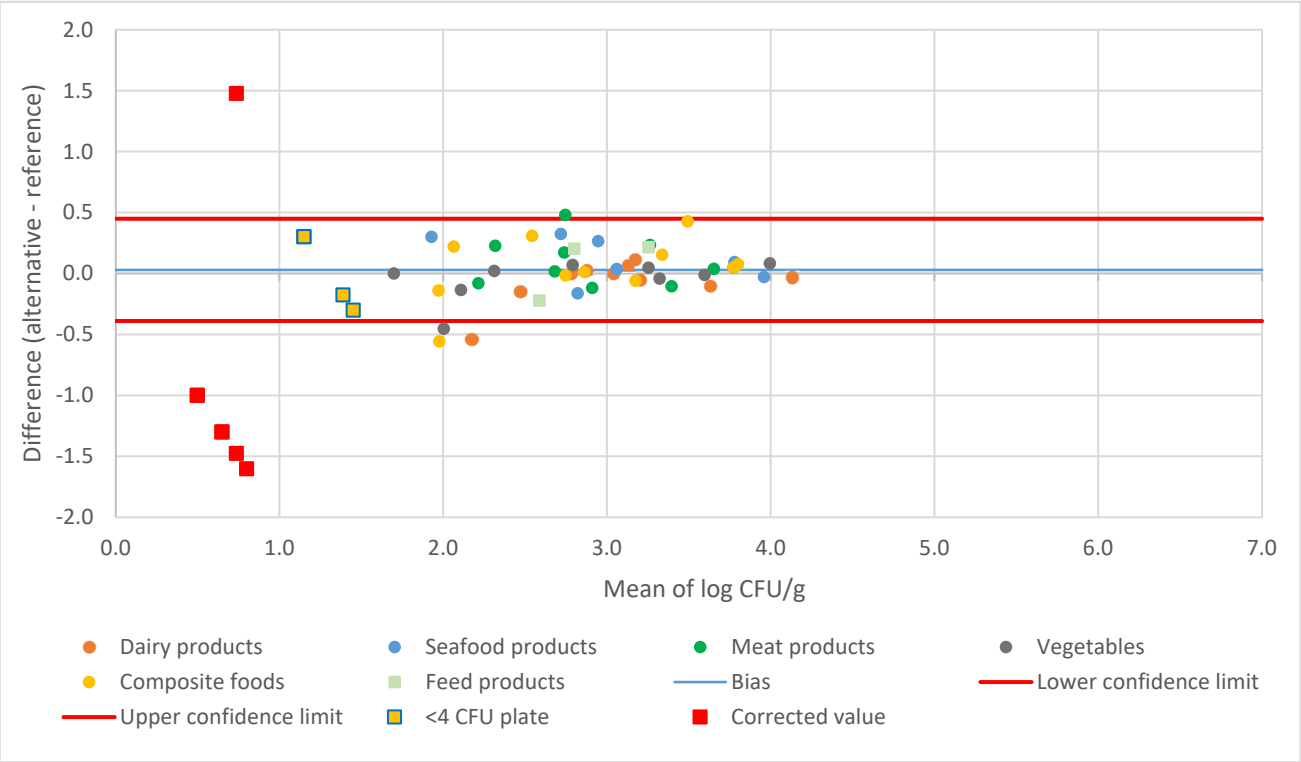
Overall, the average difference is equal to -0.03, showing a weak negative bias between the CHROMagar™ Staph aureus method and the reference method with poured plate inoculation.

The Bland-Altman difference plots are presented for all categories in figure 5 for surface spreading inoculation and in figure 6 for poured plate inoculation.

As on scatter plots:

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

Figure 5: Bland-Altman difference plot for all categories – surface spreading



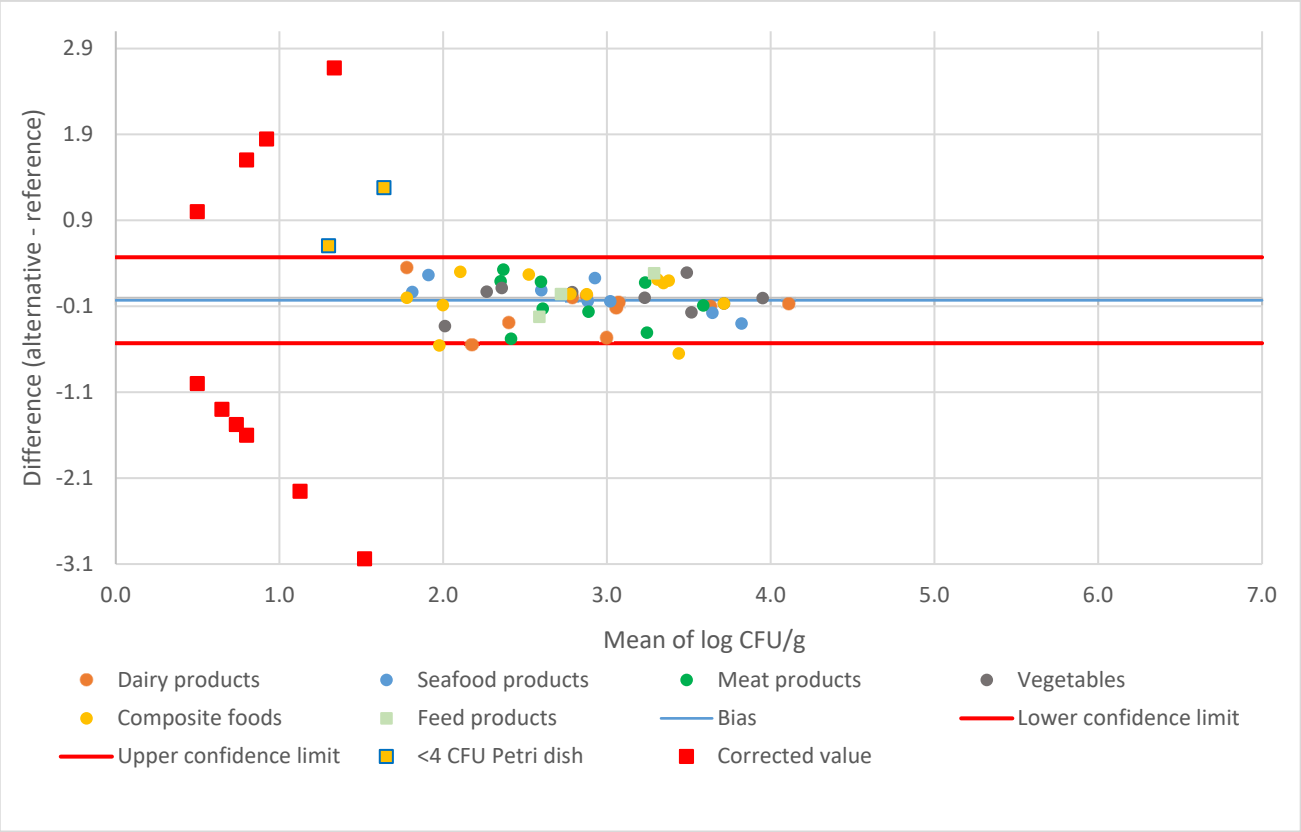
Samples for which the average difference is lower or higher than the confidence limits are listed in table 5 for surface spreading inoculation.

Table 5: values outside the confidence limits on the Bland-Altman difference plot (green cases: values <4 CFU/plate, yellow cases: values lower and higher than the quantification limits, blue: values higher than the confidence limits, red: values lower than the quantification limits) – surface spreading

Cat.	#	Matrix	RM CFU/g	AM CFU/g	RM log CFU/g	AM log CFU/g	Mean	Difference
MP	2263500	Chopped steak	320	970	2.51	2.99	2.75	**0.48**
	2263499	Plain sausage	<10	30	0.00	1.48	0.74	1.48
	2263497	Breaded cutlet	10	<10	1.00	0.00	0.50	-1.00
DP	2281366	Cheese Trebois	280	80	2.45	1.90	2.18	**-0.54**
	2263493	Raw milk goat cheese "Chabis" fat 20%	20	<10	1.30	0.00	0.65	-1.30
	2263491	Pasteurized milk cheese	10	<10	1.00	0.00	0.50	-1.00
VP	2263513	Celery remoulade	170	60	2.23	1.78	2.00	**-0.45**
CF	2263482	Viennese tuna sandwich	180	50	2.26	1.70	1.98	**-0.56**
	2263487	Tuna salad, tomates, corn, red beans	40	<10	1.60	0.00	0.80	-1.60
	2263486	Vegetarian salad	20	<10	1.30	0.00	0.65	-1.30
	2263485	Endive walnut pear salad	30	<10	1.48	0.00	0.74	-1.48
	2263480	Hachis Parmentier	70	20	1.85	1.30	1.57	-0.54
	2263665	Salad salmon tomatoes pasta potatoes	<10	70	0.00	1.85	0.92	1.85

Thirteen samples are outside the confidence limits: 9 concern corrected values or samples with less than 4 CFU/plate. Among the 4 interpretable samples, 1 is higher than the upper confidence limit and 3 are lower than the lower confidence limit.

Figure 6: Bland-Altman difference plot for all categories – Poured plate



Samples for which the average difference is lower or higher than the confidence limits are listed in table 6 for Poured plate inoculation.

Table 6: values outside the confidence limits on the Bland-Altman difference plot (green cases: values <4 CFU/ plate, yellow cases: values lower and higher than the quantification limits, blue: values higher than the confidence limits, red: values lower than the quantification limits) – Poured plate

Cat.	#	Matrix	RM CFU/g	AM CFU/g	RM log CFU/g	AM log CFU/g	Mean	Difference
MP	2263499	Plain sausage	<10	470	0.00	2.67	1.34	2.67
	2263498	Raw chicken	<10	40	0.00	1.60	0.80	1.60
	2263497	Breaded cutlet	10	40	1.00	1.60	1.30	0.60
	3363496	Brest of chicken	10	190	1.00	2.28	1.64	1.28
DP	2281366	Cheese Trebois	280	80	2.45	1.90	2.18	**-0.54**
	2263494	Raw milk cow's cheese "Mont d'or"	1 100	<10	3.04	0.00	1.52	-3.04
	2263491	Pasteurized milk cheese	10	<10	1.00	0.00	0.50	-1.00
CF	2263482	Viennese tuna sandwich	180	50	2.26	1.70	1.98	**-0.56**
	2263507	Caramel pear cake	5 800	1 300	3.76	3.11	3.44	**-0.65**
	2263487	Tuna salad, tomatoes, corn, red beans	40	<10	1.60	0.00	0.80	-1.60
	2263486	Vegetarian salad	20	<10	1.30	0.00	0.65	-1.30
	2263485	Endive walnut pear salad	30	<10	1.48	0.00	0.74	-1.48
	2263482	Viennese tuna sandwich	180	<10	2.26	0.00	1.13	-2.26
	2263481	Tartiflette croissants	<10	10	0.00	1.00	0.50	1.00
	2263480	Hachis Parmentier	70	10	1.85	1.00	1.42	-0.85
	2263665	Salad salmon tomatoes pasta potatoes	<10	70	0.00	1.85	0.92	1.85

Sixteen samples are outside the confidence limits: 13 concern corrected values or samples with less than 4 CFU/Petri dish. The 3 interpretable samples are lower than the lower confidence limit.

3.1.5. Samples with abundant background microflora

Five samples considering contaminated with background microflora, were analyzed with CHROMagar™ Staph aureus media supplemented with 2.5 mg/L of potassium tellurite with poured plate inoculation.
The results are shown in table 7.

Table 7: samples analyzed with CHROMagar™ Staph aureus + 2.5 mg/L of tellurite

Sample code	Sample name	Contamination	Dil.	Reference method ISO 6888-2			Surface spreading - Incubation 18 h at 37°C			Poured plate - Incubation 18 h at 37°C		
				CFU after 24h	CFU after 48h	Result	CFU	Conf.	Result	CFU	Conf.	Result
2247843	Pastry *	nc	-1	0	0	<10	0	/	<10	0	/	<10*
			-2	0	0		0	/		0	/	
2263509	Pastry *	ac	-1	23	27	245	47	100%	500	46	100%	455*
			-2	0	0		8	100%		4	100%	
2281362	Pearl pasta surimi and prawns *	nc	-1	0	0	<10	0	/	<10	0	/	<10*
			-2	0	0		0	/		0	/	
2263665	Salad salmon tomatoes pasta potatoes *	nc	-1	0	0	<10	7	100%	70	7	100%	70*
			-2	0	0		0	/		0	/	
2281309	Pastry *	nc	-1	7	11	110	8	100%	80	9	100%	90*
			-2	0	0		1	100%		0	/	

For four samples (2247843, 2263509, 2281362 and 2281309), the addition of potassium tellurite in the CHROMagar™ Staph aureus media gave similar results between Baird-Parker + RPF and CHROMagar™ Staph aureus.
For one sample (2263665), the recovery of *Staphylococcus aureus* is better with CHROMagar™ Staph aureus supplemented than Baird-Parker + RPF. For this sample, the background microflora was very abundant on the Baird-Parker + RPF medium, which did not allow enumeration of *Staphylococcus aureus*.

3.1.6. Conclusion

The relative trueness study of the CHROMagar™ Staph aureus method is satisfactory.

3.2. Accuracy profile study

3.2.1. Protocols

Three matrix-strain couples were tested by both methods.
For this study, only the poured plate protocol was tested but two modalities of preparation were tested.

The first modality is the use of the CHROMagar™ Staph aureus media extemporaneously after its preparation. The second modality is the use of the CHROMagar™ Staph aureus media after its melting in a water bath at 100°C for 40 minutes previously pre-poured in bottle and kept at 2-8°C. Two batches of a matrix, representative of each category, were inoculated with a strain of *Staphylococcus aureus* at three levels (low, medium and high). For each sample, 5 replicates,

represented by 5 different test portions, were tested by each method. This represents a total of 30 analyses per method.

The matrix-strain couples are presented in table 8.

Table 8: matrix-strain pairs for the accuracy profile study

Category	Matrix	Strain	Code	Origin	Contamination level CFU/mL
Meat products	Ground beef	*Staphylococcus aureus*	LBZ517	Meat	100 5000 100 000
Dairy products	Raw milk cheese	*Staphylococcus aureus*	EXX511	Raw milk cheese	
Composite foods	Vegetables mixed with mayonnaise (macédoine)	*Staphylococcus aureus*	CRKP93	Mixed salad	

3.2.2. Results

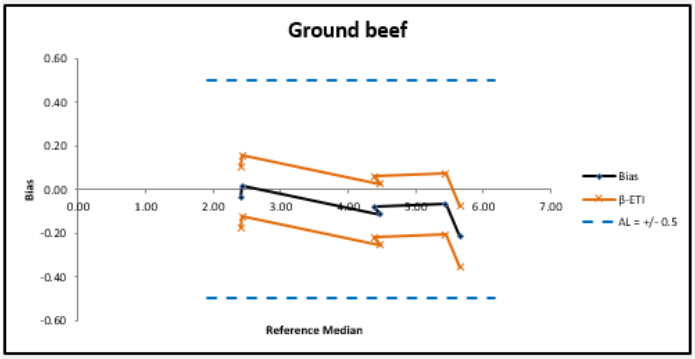
Raw data are provided in Appendix F.

The statistical data and the accuracy profiles are shown in figures 7 and 8.
Statistical calculations were done according to the Excel spreadsheet named AP_calculation_tool MCS 16140-2_clause 6-1-3-3 ver_31-07-2018.xlsx available at http://standards.iso.org/iso/16140.

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

Figure 7: Accuracy profiles per category without melting

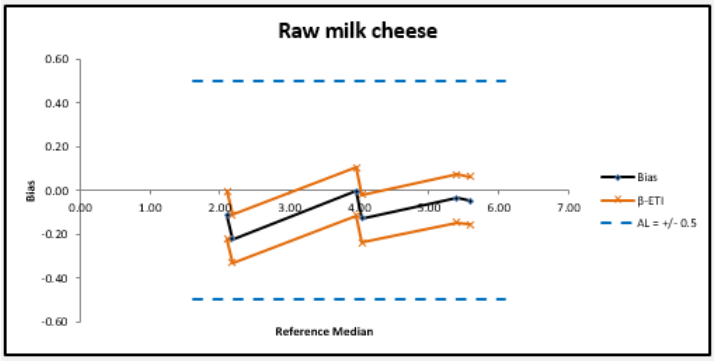
(Food) Category	Meat products
(Food) Type	Ground beef



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R1 to R5	2.41	-0.035	-0.175	0.105	YES	YES
R6 to R10	2.43	0.016	-0.124	0.156	YES	YES
R11 to R15	4.48	-0.115	-0.255	0.025	YES	YES
R16 to R20	4.38	-0.079	-0.219	0.061	YES	YES
R21 to R25	5.45	-0.067	-0.207	0.073	YES	YES
R26 to R30	5.66	-0.216	-0.356	-0.076	YES	YES

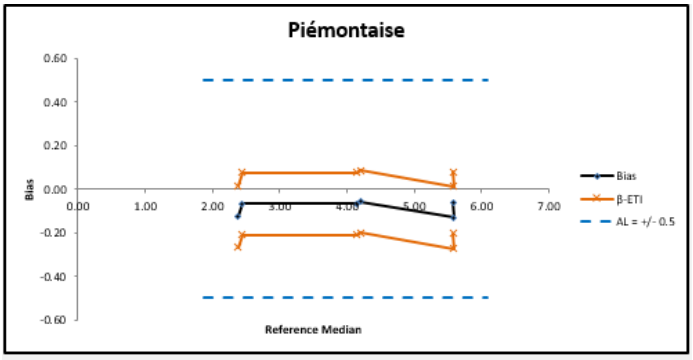
	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.123	0.097	YES	+/- 0.500

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



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R31 to R35	2.11	-0.114	-0.224	-0.004	YES	YES
R36 to R40	2.18	-0.222	-0.332	-0.112	YES	YES
R41 to R45	3.96	-0.005	-0.115	0.105	YES	YES
R46 to R50	4.04	-0.128	-0.237	-0.018	YES	YES
R51 to R55	5.40	-0.036	-0.146	0.074	YES	YES
R56 to R60	5.59	-0.047	-0.157	0.063	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.100	0.076	YES	+/- 0.500

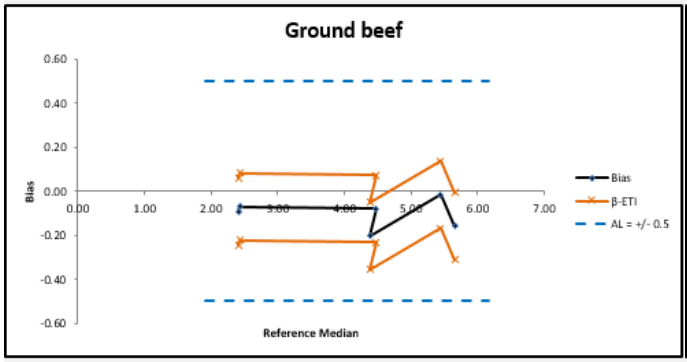


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R61 to R65	2.38	-0.125	-0.268	0.018	YES	YES
R66 to R70	2.45	-0.067	-0.210	0.076	YES	YES
R71 to R75	4.15	-0.067	-0.210	0.076	YES	YES
R76 to R80	4.20	-0.058	-0.201	0.085	YES	YES
R81 to R85	5.59	-0.129	-0.272	0.014	YES	YES
R86 to R90	5.58	-0.061	-0.204	0.082	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.101	0.099	YES	+/- 0.500

Figure 8: Accuracy profiles per category with melting

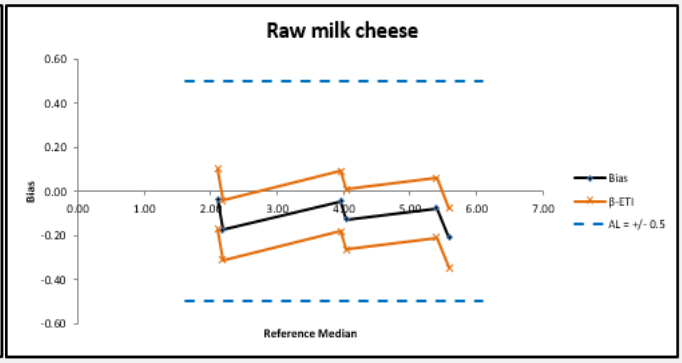
(Food) Category	Meat products
(Food) Type	Ground beef



Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R1 to R5	2.41	-0.093	-0.246	0.060	YES	YES
R6 to R10	2.43	-0.070	-0.223	0.083	YES	YES
R11 to R15	4.48	-0.079	-0.232	0.074	YES	YES
R16 to R20	4.38	-0.204	-0.357	-0.051	YES	YES
R21 to R25	5.45	-0.016	-0.169	0.137	YES	YES
R26 to R30	5.66	-0.158	-0.311	-0.005	YES	YES

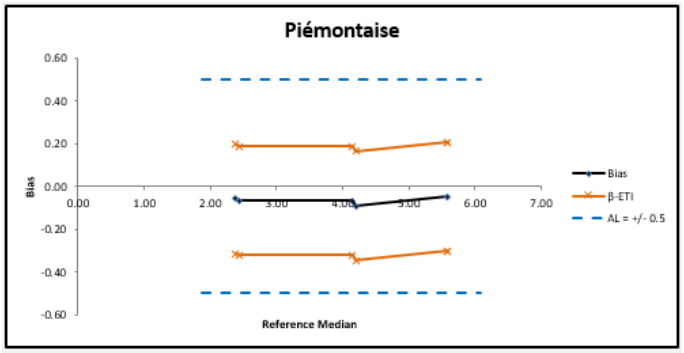
	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.123	0.106	YES	+/- 0.500

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



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R31 to R35	2.11	-0.035	-0.170	0.101	YES	YES
R36 to R40	2.18	-0.176	-0.312	-0.040	YES	YES
R41 to R45	3.96	-0.045	-0.181	0.090	YES	YES
R46 to R50	4.04	-0.128	-0.263	0.008	YES	YES
R51 to R55	5.40	-0.076	-0.211	0.060	YES	YES
R56 to R60	5.59	-0.211	-0.347	-0.075	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.100	0.094	YES	+/- 0.500



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
R61 to R65	2.38	-0.058	-0.314	0.198	YES	YES
R66 to R70	2.45	-0.067	-0.322	0.189	YES	YES
R71 to R75	4.15	-0.067	-0.322	0.189	YES	YES
R76 to R80	4.20	-0.090	-0.346	0.165	YES	YES
R81 to R85	5.59	-0.047	-0.303	0.209	YES	YES
R86 to R90	5.58	-0.048	-0.304	0.207	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.101	0.177	YES	+/- 0.500

The tolerance intervals fall into the acceptability limits for all categories tested and for the two modalities of preparation of the CHROMagar™ Staph aureus media.

3.2.3. Conclusion

The CHROMagar™ Staph aureus method is accepted as being equivalent to the reference method for the two modalities of preparation for the three categories tested.

3.3. Inclusivity / exclusivity studies

3.3.1. Protocols

- Inclusivity

Thirty pure strains of coagulase-positive staphylococci were inoculated at approximately 100 CFU and counted with the media CHROMagar™ Staph aureus Poured plate and surface spreading in comparison with Baird-Parker and RPF agar media.

- Exclusivity

Thirty pure strains of species close to coagulase-positive staphylococci were inoculated with a high concentration of stationary phase cells and then counted with CHROMagar™ Staph aureus media Poured plate and surface spreading in comparison with Baird-Parker and RPF agar media.

3.3.2. Results

The results for inclusiveness are provided in Table 9 and those for exclusivity in Table 10.

Table 9: results of inclusivity

#	Reference Microsept	Strain	Origin	Decimal dilution of the initial suspension	Number of CFU on TSA agar medium	ISO 6888-2 (BP+RPF)			Alternative method CHROMagar™ Staph aureus					
						Aspect of the colonies		Number of CFU	Poured plate - Incubation 18 h at 37°C			Surface spreading - Incubation 18 h at 37°C		
						24h	48h		Number of CFU	Colour of the colonies	Aspect of the colonies	Number of CFU	Colour of the colonies	Aspect of the colonies
1	DSM18597	*S. aureus ATCC 13565*	Ham, food poisoning	-1	**84**	2mm black colonies with halo	5mm black colonies with halo	**104**	**84**	purple	1mm purple colonies	**118**	purple	2mm purple colonies
2	DSM18587	*S. aureus ATCC 19095*	Human abscess	-1	**87**	1mm black colonies with halo	3mm black colonies with halo	**121**	**95**	purple	<1mm pink colonies	**133**	purple	1mm purple colonies
3	STA1.1 (50)	*S. aureus ATCC 25923*	Clinical isolate	-1	**98**	1,5mm black colonies with halo	3,5mm black colonies with halo	**158**	**111**	purple	<1mm purple colonies	**135**	purple	1,5mm purple colonies
4	STA1.2 (51)	*S. aureus ATCC 6538*	Enteric desease research	-1	**107**	1mm black colonies with halo	2mm black colonies with halo	**25**	**110**	purple	<1mm purple colonies	**49**	purple	1mm purple colonies
5	DSM683	*S. aureus ATCC 9144*	Enteric desease research	-1	**130**	<1mm black colonies with halo	2mm black colonies with halo	**167**	**126**	purple	<1mm purple colonies	**202**	purple	1mm purple colonies
6	DSM20373	*S. intermedius*	/	-1	**67**	1mm black colonies with halo	3mm black colonies with halo	**25 à 24h et 74 à 48h**	**57**	grey (very weak purple coloration)	<1mm grey colonies	**61**	grey (very weak purple coloration)	1mm grey colonies
7	DSM17421	*S. hyicus*	/	-1	**92**	1mm grey colonies without halo	4mm, black colonies without halo	**145**	**98**	pink	<0,5mm pink colonies	**123**	pink	1mm pink colonies
8	EAR487	*S. delphini*	Duck	-1	**19**	2mm colonies without halo	4mm, black colonies with weak halo	**20**	**11**	pink	<1mm pink colonies	**14**	pink	2mm pink colonies
9	DSM10244	*S. lutrae*	/	-1	**139**	1mm black colonies, weak halo	black colonies, 4mm, weak halo	**185**	**0 (no growth)**	/	/	**0 (no growth)**	/	/
10	AFHK27	*S. aureus*	Tuna salad, red beans, corn, tomatoes	-1	**172**	2mm black colonies with halo	5mm black colonies with halo	**123**	**158**	purple	<1mm purple colonies	**148**	purple	1,5mm purple colonies
11	GAK477	*S. aureus*	Burger	-1	**192**	1-1,5mm black colonies with weak halo	3mm black colonies with halo	**185**	**175**	purple	<1mm purple colonies	**150**	purple	1,5mm purple colonies
12	BDW129	*S. aureus*	Tuna and raw vegetables sandwich	-1	**98**	1,5mm black colonies with halo	7mm black colonies with halo	**54**	**101**	purple	<1mm purple colonies	**94**	purple	1mm purple colonies
13	HBL504	*S. aureus*	Lasagna	-1	**158**	2mm black colonies with halo	5mm black colonies with halo	**164**	**125**	purple	1mm purple colonies	**179**	purple	1,5mm purple colonies
14	KER837	*S. aureus*	Paella rice	-1	**116**	2mm black colonies with halo	4mm black colonies with weak halo	**132**	**114**	purple	1mm purple colonies	**154**	purple	1,5mm purple colonies
15	MDV990	*S. aureus*	Rabbit terrine with leeks	-1	**135**	2mm black colonies with weak halo	5mm black colonies with weak halo	**104**	**130**	purple	1mm purple colonies	**138**	purple	2mm purple colonies
16	BHLT86	*S. aureus*	Complete tuna sandwich	-1	**108**	1mm black colonies with halo	2mm black colonies with halo	**76**	**89**	purple	<1mm purple colonies	**101**	purple	1-1,5mm purple colonies
17	CRKP93	*S. aureus*	Mixed salad	-1	**92**	1mm black colonies with halo	2mm black colonies with halo	**105**	**89**	purple	<1mm purple colonies	**103**	purple	1-1,5mm purple colonies
18	KNWQ77	*S. aureus*	Strasbourg salad	-1	**123**	2mm black colonies with halo	3mm black colonies with halo	**121**	**110**	purple	<1mm purple colonies	**145**	purple	1-1,5mm purple colonies
19	LQVP54	*S. aureus*	Organic sausage	-1	**111**	2 mm black colonies with halo	3mm black colonies with halo	**114**	**106**	purple	<1mm purple colonies	**130**	purple	1-1,5mm purple colonies
20	BBF899	*S. aureus*	Turkey carcass	-1	**132**	1mm black colonies without halo	2mm black colonies with halo	**141**	**141**	purple	<1mm purple colonies	**>300**	purple	1mm purple colonies
21	DBP642	*S. aureus*	Small turkey meat	-1	**4**	1mm black colonies without halo	6mm black colonies with halo	**21**	**5**	purple	<1mm purple colonies	**43**	purple	1mm purple colonies
22	CFQ207	*S. aureus*	Chicken thigh	-1	**82**	1mm black colonies with halo	6mm black colonies with halo	**115**	**89**	purple	<1mm purple colonies	**132**	purple	1mm purple colonies
23	LBZ517	*S. aureus*	Meat	-1	**96**	1mm black colonies with halo	6mm black colonies with halo	**190**	**109**	purple	<1mm purple colonies	**158**	purple	1mm purple colonies
24	AVJD48	*S. aureus*	Herb sausage	-1	**91**	1mm black colonies with halo	5mm black colonies with halo	**93**	**95**	purple	<1mm purple colonies	**124**	purple	1mm purple colonies
25	BYEL73	*S. aureus*	Raw turkey wing	-1	**100**	1mm black colonies with halo	6mm black colonies with halo	**126**	**97**	purple	<1mm purple colonies	**129**	purple	<1mm purple colonies
26	ACY440	*S. aureus*	Shrimps	-1	**114**	<1mm black colonies without halo	5mm black colonies with halo	**93**	**110**	purple	<1mm purple colonies	**95**	purple	1mm purple colonies
27	BATB46	*S. aureus*	Smoked herring	-1	**105**	1mm black colonies with halo	5mm black colonies with halo	**99**	**91**	purple	<1mm purple colonies	**119**	purple	1mm purple colonies
28	EXX511	*S. aureus*	Raw milk cheese "Saint-Mathurin"	-1	**87**	<1mm black colonies with halo	5mm black colonies with halo	**110**	**104**	purple	<1mm purple colonies	**112**	purple	1mm purple colonies
29	GAU875	*S. aureus*	Tomme of ewe cheese with thermized milk	-1	**112**	1mm black colonies with halo	6mm black colonies with halo	**90**	**96**	purple	0,5mm purple colonies	**105**	purple	<1mm purple colonies
30	HCU266	*S. aureus*	Custard	-1	**90**	<1mm black colonies with halo	3-4mm black colonies with halo	**178**	**76**	purple	<1mm purple colonies	**127**	purple	<1mm purple colonies

Table 10: results of exclusivity

#	Reference Microsept	Strain	Origin	ISO 6888-2 (BP+RPF)				Alternative method CHROMagar™ Staph aureus					
								Surface spreading			Poured plate		
				Dil.	Number of CFU on TSA agar medium	Dil.	Number of CFU	Aspect of the colonies	Dil.	Number of CFU	Colour of the colonies	Dil.	Number of CFU	Colour of the colonies
1	WJM246	*Bacillus subtilis*	Curcuma	-3	3	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-2	40	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
2	CBB622	*Bacillus cereus groupe III*	Piemontaise	-4	16	-3	0 (no growth)	1mm white colonies without halo	-3	0 (no growth)	1mm pink colonies characteristic of *Bacillus*	-3	0 (no growth)	<1mm pink colonies characteristic of *Bacillus*
				-3	>200	-2	1		-2	24		-2	26	
3	YFJ492	*Carnobacterium divergens*	Salmon steak	-5	>300	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-4	>300	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
4	ARP296	*Citrobacter brakii*	Raw porc	-7	25	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	152	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
5	NWR779	*Pantoea agglomerans*	Well water	-7	74	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	>200	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
6	GCQ471	*Enterococcus faecalis*	Raw milk cheese	-7	11	-3	0 (no growth)	<1mm white colonies without halo	-3	1	1mm blue colonies	-3	0 (no growth)	<1mm blue colonies
				-6	159	-2	0 (no growth)		-2	23		-2	4	
7	GBL293	*Escherichia coli*	Unbaked chocolate cookie	-7	183	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	>300	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
8	ANW492	*Lactobacillus brevis*	Dairy product	-4	5	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-3	80	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
9	EFP530	*Lactobacillus gasseri*	Probiotic	-6	2	-3	0 (no growth)	<1mm white colonies without halo	-4	126	1mm blue colonies	-4	212	<1mm blue colonies
				-5	37	-2	3		-3	>300		-3	>300	
10	DZW418	*Leuconostoc mesenteroides*	Zucchini flan	-4	30	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-3	>200	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
11	ALB478	*Listeria monocytogenes*	Salmon tagliatelles	-7	81	-4	>300	<1mm white colonies without halo	-4	>300	1mm blue colonies	-4	>300	<1mm blue colonies
				-6	>200	-3	>300		-3	>300		-3	>300	
12	NBV721	*Micrococcus luteus*	Pond water	-5	170	-4	>300	<1mm white colonies without halo	-4	>300	1mm white colonies	-4	>300	<1mm white colonies
				-4	>300	-3	>300		-3	>300		-3	>300	
13	NEF806	*Enterococcus faecium*	Well water	-6	47	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-5	168	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
14	YEG730	*Pseudomonas aeruginosa*	Water	-7	12	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	140	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
15	MDD911	*Salmonella* Enteritidis	Cooked spinach salmon turnover	-7	110	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	>300	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
16	CXZ948	*Brochotrix thermosphacta*	Sushi	-6	0 no grow	-3	0 (no growth)	/	-4	76	1mm white colonies	-4	58	<1mm blue colonies
				-5	10	-2	0 (no growth)		-3	193		-3	201	
17	ADLA78	*Micrococcus sp*	Vegetal extract	-7	47	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-6	>200	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
18	FND144	*Brevibacillus parabrevis*	Cheese	-2	4	-2	0 (no growth)	/	-2	0 (no growth)	/	-2	0 (no growth)	/
				-1	59	-1	0 (no growth)		-1	0 (no growth)		-1	0 (no growth)	
19	EDG277	*Bifidobacterium lactis*	Probiotic	-9	5	-3	0 (no growth)	/	-3	0 (no growth)	/	-3	0 (no growth)	/
				-8	44	-2	0 (no growth)		-2	0 (no growth)		-2	0 (no growth)	
20	DSP656	*Lactococcus lactis*	Water	-8	17	-3	0 (no growth)	/	-3	0 (no growth)	1,5mm white colonies	-3	0 (no growth)	/
				-7	81	-2	0 (no growth)		-2	5		-2	0 (no growth)	
21	UMP052	*Corynebacterium frankenforstense*	Raw chicken	-8	6	-5	>300	<1mm white colonies without halo	-5	>300	<1mm white colonies	-5	>300	<1mm white colonies
				-7	78	-4	>300		-4	>300		-4	>300	
22	UJC759	*Staphylococcus caprae*	Goat milk	-6	218	-8	4	<1mm white colonies without halo	-8	5	1mm white colonies	-8	23	<1mm white colonies
				-5	>300	-7	55		-7	52		-7	163	
23	WRM124	*Staphylococcus carnosus*	Dry sausage	-7	13	-6	0 (no growth)	/	-6	45	1mm white colonies	-6	48	<1mm white colonies
				-6	123	-5	0 (no growth)	/	-5	110		-5	158	
24	VNY469	*Staphylococcus epidermidis*	Human skin	-8	>300	-8	90	1mm white colonies without halo	-8	0 (no growth)	/	-8	0 (no growth)	/
				-7	>300	-7	>300		-7	0 (no growth)		-7	0 (no growth)	
25	DSM30515	*Staphylococcus hominis*	Human skin	-5	22	-5	0 (no growth)	/	-5	0 (no growth)	/	-5	0 (no growth)	/
				-4	>200	-4	0 (no growth)		-4	0 (no growth)		-4	0 (no growth)	
26	AAY895	*Staphylococcus saprophyticus*	Lasagna	-7	1	-7	1	<1mm white colonies without halo	-7	2	<1mm white colonies	-7	4	<1mm white colonies
				-6	48	-6	57		-6	53		-6	62	
27	NDN153	*Staphylococcus equorum*	Fountain water	-5	8	-4	0 (no growth)	<1mm white colonies without halo	-4	0 (no growth)	<1mm white colonies	-4	1	<1mm white colonies
				-4	62	-3	26		-3	45		-3	44	
28	TYY149	*Staphylococcus warneri*	Beef	-8	>300	-8	>300	1mm white colonies without halo	-8	>300	<1mm white colonies	-8	>300	<1mm white colonies
				-7	>300	-7	>300		-7	>300		-7	>300	
29	VGH553	*Staphylococcus xylosus*	Raw milk cheese	-8	>300	-8	2	1mm white colonies without halo	-8	51	<1mm white colonies	-8	41	<1mm white colonies
				-7	>300	-7	55		-7			-7	163	
30	CCN391	*Staphylococcus sciuri*	Hake	-7	6	-6	0 (no growth)	/	-6	0 (no growth)	/	-6	0 (no growth)	/
				-6	83	-5	0 (no growth)		-5	0 (no growth)		-5	0 (no growth)	

3.3.3. Interpretation

- Inclusivity

For twenty-seven strains tested, a growth was observed on CHROMagar™ Staph aureus media with presence of characteristic colonies.

For the *Staphylococcus intermedius* strain, a growth was observed but with presence of non-characteristic colonies on CHROMagar™ Staph aureus media (grey with weak purple coloration colonies).

For the *Staphylococcus hyicus* strain (DSM17421), a growth was observed on CHROMagar™ Staph aureus media with presence of pink colonies. *Staphylococcus hyicus* species is coagulase variable and this enzymatic activity can be revealed when using porcine plasma (Pickering and al. 2021).
Since DSM17421 strain gave negative results on Baird-Parker + RPF medium and in coagulase assay, it was not considered in the specificity calculation.
For the *Staphylococcus lutrae* strain, no growth was observed on the CHROMagar™ Staph aureus media, unlike the Baird-Parker + RPF medium. No other strain of *Staphylococcus lutrae* could be retested.
Two *Staphylococcus aureus* strains (KER837 and MDV990) grew black colonies with weak halo on Baird-Parker + RPF plates after 48 hours incubation at 34-38°C, whereas those strains grew characteristic colonies on CHROMagar™ Staph aureus after 18 hours incubation at 37°C.
Two coagulase positive staphylococci, *Staphylococcus delphini* and *Staphylococcus lutrae* showed weak halos surrounding the colonies on reference plates.

- Exclusivity

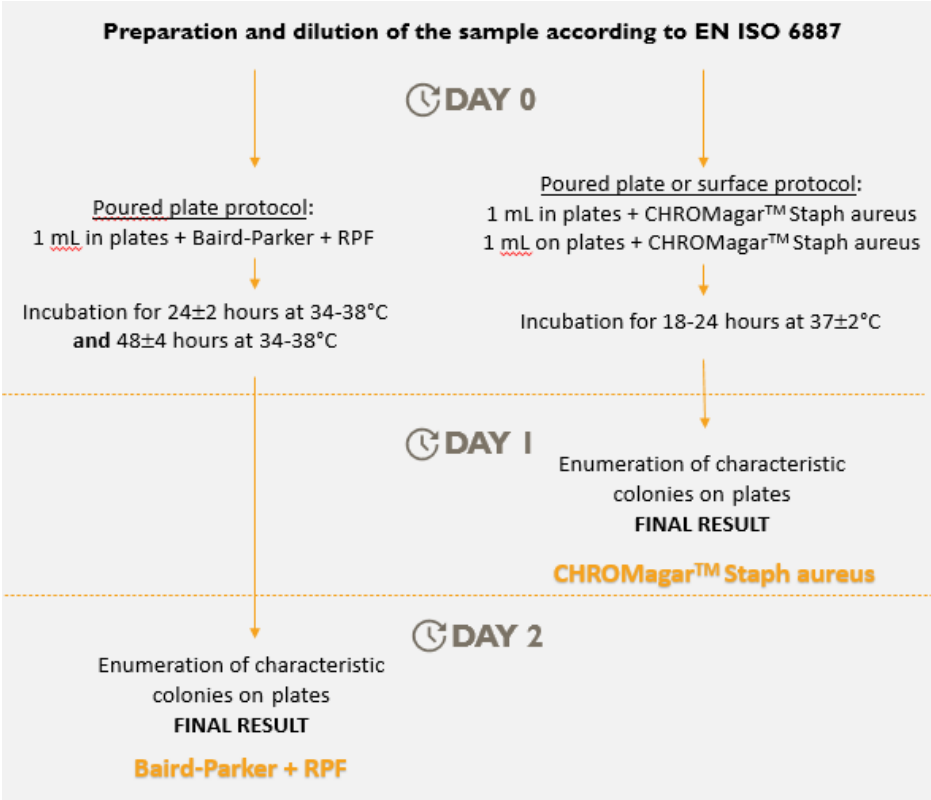
For all the non-target strains tested, no cross-reaction was observed.

3.3.4. Conclusion

The inclusivity and the exclusivity of the CHROMagar™ Staph aureus medium are satisfactory.

3.4. General conclusion for the methods comparison study

The CHROMagar™ Staph aureus medium offers 2 inoculation methods (poured plate and surface spreading) both allowing a result in 24 hours maximum against 48 hours for the Baird-Parker + RPF medium.



The relative accuracy study shows a good correlation between the CHROMagar™ Staph aureus medium and the Baird-Parker + RPF used in the reference method for all the food categories tested. The bias observed between the CHROMagar™ Staph aureus medium and the Baird-Parker + RPF medium is +0.03 log for surface spreading and -0.03 log for poured plate inoculation.

The accuracy profile study illustrates that the performances of the alternative method are comparable to those of the reference method. For the 3 categories tested in poured plate modality with or without fusion of the CHROMagar™ Staph aureus medium, the profiles clearly show that all the results obtained were within the acceptability limits defined at ± 0.5 log.

The selectivity of the CHROMagar™ Staph aureus medium is 100%. The specificity of the CHROMagar™ Staph aureus medium is 100% for *Staphylococcus aureus* strains and 93.1% for coagulase-positive *Staphylococcus* strains.

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Deputy manager of R&D department
Le Lion d'Angers, January 2nd, 2023

APPENDICES