Method Comparison Study Report for the ISO 16140-2:2016 validation of Compact Dry X-SA, for the detection of coagulase positive staphylococci (*Staphylococcus aureus*) in a broad range of foods

MicroVal study number: 2008LR14

Method/Kit name: Compact Dry XSA

Report version:MCS ILS Summary report created 03/09/2019

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Foreword

This report is prepared in accordance with ISO 16140-2:2016 and MicroVal Technical Committee interpretation of ISO 16140-2 v.1.0

Company: Nissui Pharmaceutical Co. Ltd

Expert Laboratory: Campden BRI

Method/Kit name: Compact Dry XSA

Validation standard: ISO 16140-2:2016 Microbiology of the food chain —Method validation —Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

Reference methods: ISO 6888-1:1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium

Scope of validation: A broad range of foods based on categories

1. Dairy products
2. Dried/low moisture foods
3. Meat and Poultry
4. Ready to eat foods
5. Multi component foods

Certification organisation: Lloyd's Register

List of abbreviations

- AL Acceptability Limit
- AP Accuracy Profile
- Art. Cont. Artificial contamination
- CFU Colony Forming Units
- CL confidence limit (usually 95%)
- EL Expert Laboratory
- $\bar{D}$ Average difference
- g Gram
- h Hour
- ILS Interlaboratory Study
- Inc/Ex Inclusivity and Exclusivity
- LOQ Level of Quantification
- MCS Method Comparison Study
- min minute
- ml Millilitre
- MR (MicroVal) Method Reviewer
- MVTC MicroVal Technical Committee
- EL Expert Laboratory
- n number of samples
- na not applicable
- neg negative (target not detected)
- NG no growth
- nt not tested
- RT Relative Trueness
- SD standard deviation of differences
- 10^{-1} dilution 10-fold dilution of original food
- 10^{-2} dilution 100-fold dilution of original food
- PSD Peptone salt diluent

Contents

1 Introduction

In this project a MicroVal validation study, based on ISO 16140-2:2016, of alternative method(s) for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus*) in five different food categories was carried out by Campden BRI as the MicroVal Expert Laboratory.

The alternative method used was:

- Enumeration of *Staphylococcus aureus* on Compact Dry XSA, incubated at 37°C±1°C for 24 ± 2h

The reference method used was:

- ISO 6888-1 :1989 Microbiology of food and animal feeding stuffs- Horizontal method for of coagulase-positive staphylococci (Staphylococcus aureus and other species) - Part 1: Technique using Baird-Parker agar medium

Categories included :

- Dairy products
- Dried/low moisture foods
- Meat and Poultry
- Ready to eat foods
- Multi component foods

Criteria evaluated during the study have been:

- Relative trueness study;
- Accuracy profiles;
- Limits of quantification (LOQ);
- Inclusivity and exclusivity
- Interlaboratory Study

The final conclusion on the Method Comparison Study and ILS is summarised below:

The alternative method Compact Dry XSA shows comparable performance to the reference methods (ISO 6888-1:1989) for the enumeration of coagulase-positive staphylococci in a broad range of foods.

2 Method protocols

The Method Comparison Study was carried out using 10g gram portions of sample material.

According to ISO 16140-2 the reference method and alternative methods were performed with the same sample. The study was therefore a paired study design.

2.1 Reference method

See the flow diagram in Annex A.

Sample preparations used in the reference method were done according to ISO 6887-series parts 1, 2, 3, 4 and 5. Plating was done according to ISO 7218:2007+A1:2013 section 10.2.2 which says at least one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution may also be used to improve reliability. If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results. Depending on the sample being tested and the expected contamination level, single or multiple dilutions were used with single or duplicate plates if considered necessary to improve the reliability of the calculated result and ensure at least two relevant plates were available for use in calculations.

2.2 Alternative method

See the flow diagram of the alternative method in Annex A.

Compact Dry XSA plates are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the centre of the self-diffusible medium.
The Compact Dry X-SA method contains chromogenic medium and selective agents for the detection and enumeration of *Staphylococcus aureus* which form blue colonies after 24+/-2h at 37±1ºC.

Samples of product containing the target organism were diluted 1 in 10 with an appropriate diluent according to ISO 6887 and homogenised in a stomacher.

Appropriate serial dilutions were made, and all relevant dilutions were analysed using the reference method and alternative method.

3 Method comparison study

3.1 Relative trueness study

The trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. This study was conducted using naturally or artificially contaminated samples. Different categories, types and items were tested for this.

A total of 5 categories were included in this validation study. A minimum of 15 items for each category were tested by both the reference method and the alternative method in the relative trueness study, with a minimum of 15 interpretable results per category.

Each category was made up of 3 types, with at least 5 items representative for each type.

3.1.1 Number of samples

The categories, the types and the number of samples analyzed are presented in Table 1.

Table 1 – Categories, types and number of samples analyzed

Categories	Types	Number samples analysed	Number of interpretable results
Dairy products	Dairy desserts e.g. chilled custard, trifle, cream, ice cream, custard slice	5	5
	Pasteurised / raw milk products, yogurt, milk drinks	5	5
	Cheese e.g. soft cheese, hard cheese, raw milk cheese	5	5
Dried/ low moisture products	Chilled RTC batters and pasta e.g. filled tortellini, ravioli	5	5
	Infant formula and cereals e.g. probiotic infant cereals, rusks, infant milk	6	6
	Dehydrated powders e.g. soups, gravy, milk powders	5	5
Meat and poultry	Poultry: cooked sliced chicken, cooked chicken fillets, cooked BBQ chicken chunks	5	5
	Cooked and fermented meat e.g. salami, pepperoni, chorizo, ham	5	5
	Raw meats: mince, sausages, chicken breast fillet	5	5
Ready to eat foods	Ready to eat/reheat chilled/frozen foods e.g. quiche, pizza, cottage pie	5	5
	Cooked/cured fish products e.g. prawns, smoked salmon, seafood terrine, salmon Pate	5	5
	Cut ready to eat fresh produce e.g. fruit mixes, bagged leafy vegetables, carrot batons	6	6

Categories	Types	Number samples analysed	Number of interpretable results
Multi component foods	Composite foods with substantial raw ingredients e.g. sandwiches, pasta salads,	5	5
	Mayonnaise based raw and processed salads e.g. coleslaw, sandwich spreads	5	5
	Composite processed meals e.g. .lasagne, fish pie, spaghetti bolognese	5	5
TOTAL		77	77

78 samples were analysed, leading to 78 interpretable results

3.1.2 Test sample preparation

It is preferable to have naturally contaimated samples where possible, however, it is also necessary to artificially inoculate some samples where naturally contamianted samples cannot be sourced. Artificial contamination was carried out by spiking or seeding protocols. Samples were inoculated and held either frozen for 1 week, chilled for 2 days or ambient for 2 weeks, or cultures were exposed to pH2 for 60 min or heated at 55°C for 5min.

Injury efficiency was evaluated by enumerating the pure culture on selective and non-selective agars.

The observed injury measurements varied from 0.31 to more than 0.57 log cfu/g difference between non-selective and selective plates
65 samples were artificially contaminated; 10 contaminated naturally.

A further 42 samples were screened for natural contamination- all were negative.

3.1.3 Protocols applied during the validation study

A single protocol was applied for the study.

Reference method plates were incubated at 37±1ºC for a total of 48±4h . Compact Dry XSA plates were incubated at 24+/-2h at 37±1ºC.

In all cases the minimum incubation times were used.

3.1.4 Test results

The samples were analysed by the reference and the alternative methods in order to have at least 15 interpretable results per category, and at least 5 interpretable results per tested type by the two methods.

3.1.5 Calculation and interpretation of relative trueness study

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

Figures 1 to 5 shows the data plotted per category and Figure 6 summarises all the data.

Figure 1 : Dairy products

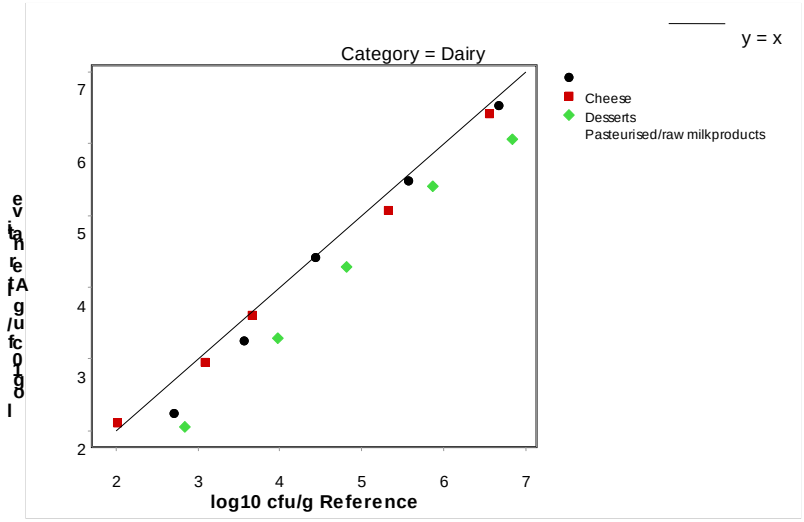


Figure 2: Dried/Low Moisture Foods

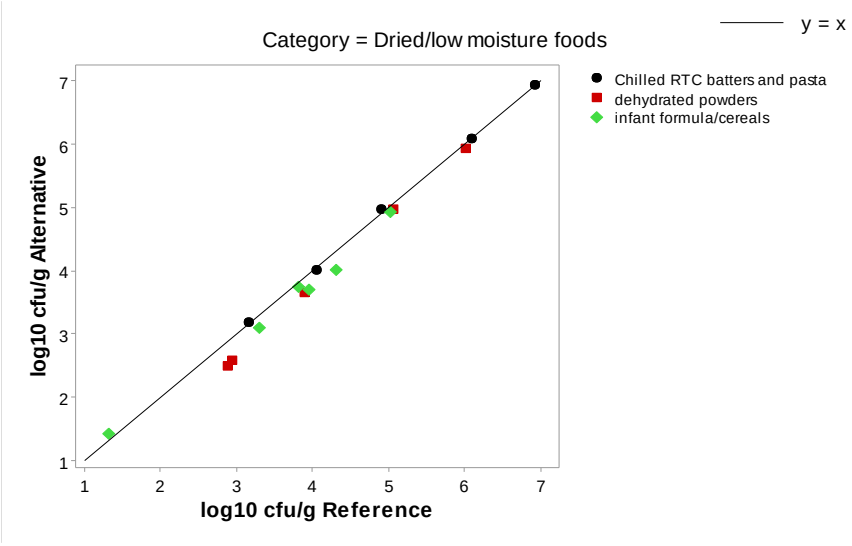


Figure 3: Meat and poultry

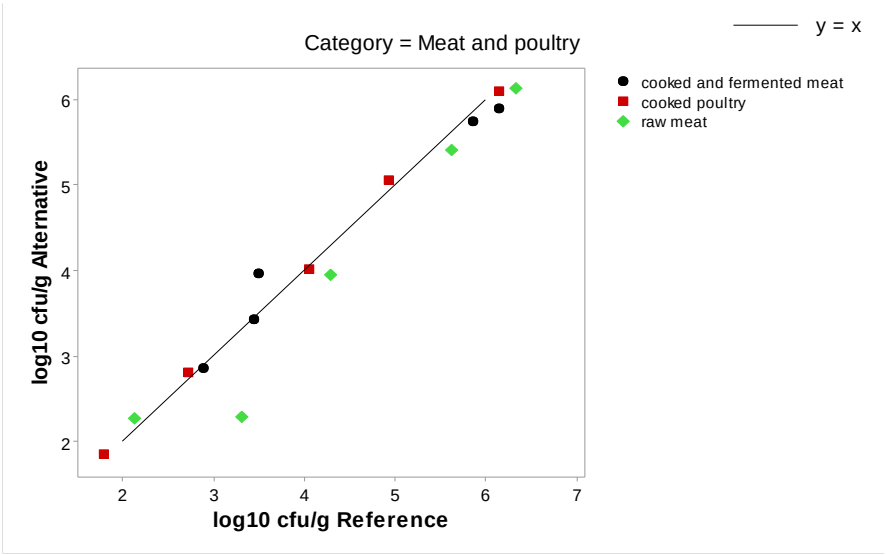


Figure 4: Multi-component Foods

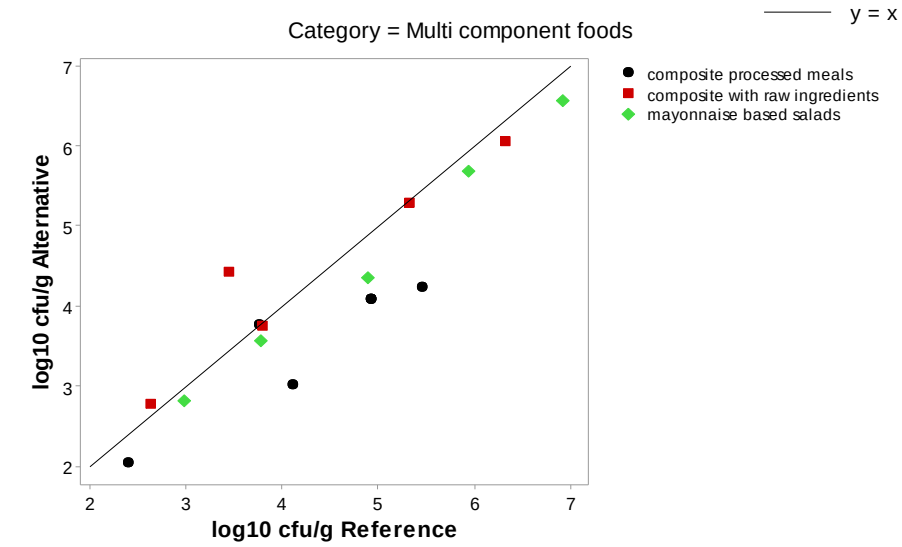


Figure 5: Ready to eat Foods

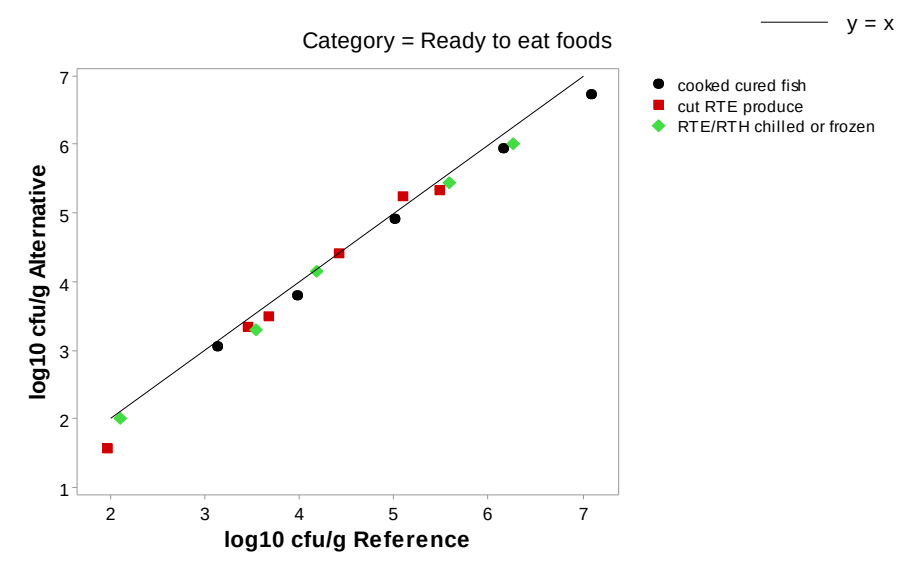
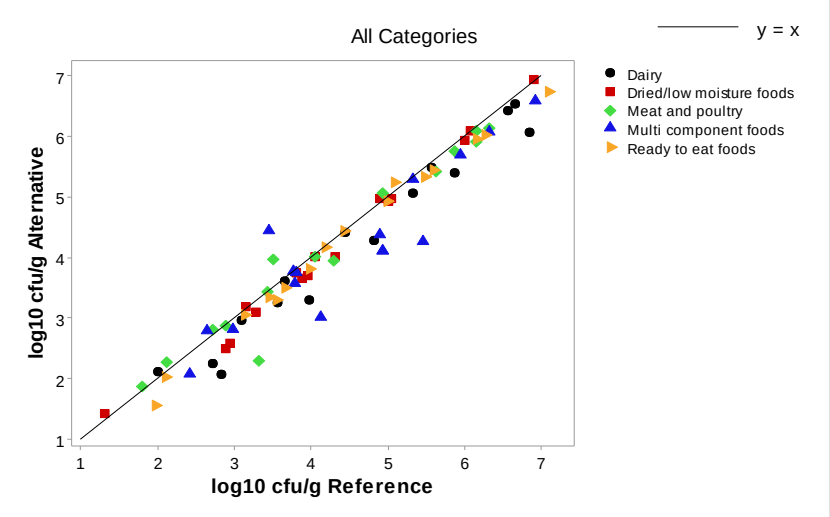


Figure 6: All categories plot



According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results.

According to ISO 16140-2:2016 6.1.2.3 the results of the scatter plot are interpreted based on a visual observation on the amount of bias and extreme results. The data appears acceptable on the whole but there is some evidence of a negative bias for the alternate method for multicomponent foods, particularly processed composite meals and for dairy products, in particular pasteurized /raw milk products. This can be seen from the individual product figures (1 and 4) and from the all categories figure (6). These products were spiked with heat treated. Cells stressed in this way may under-recover on the alternative method compared to the reference method.

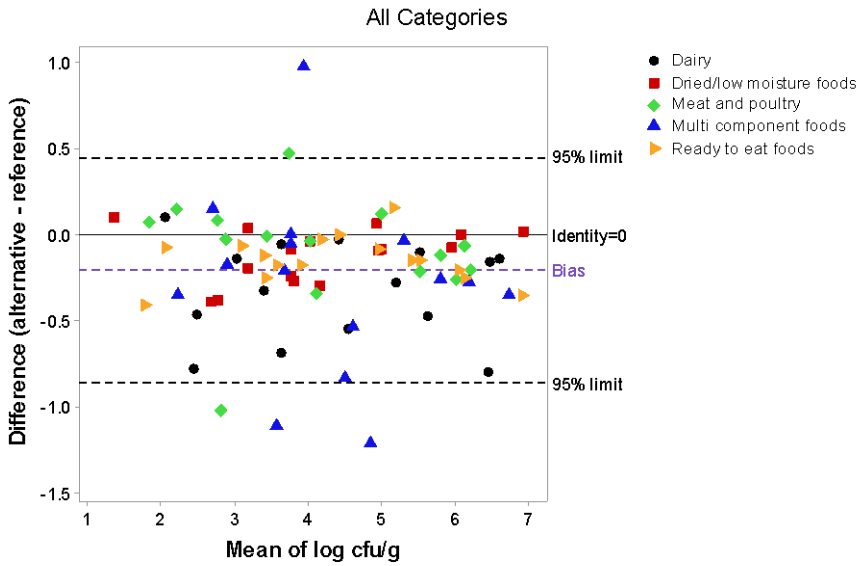
A summary of the calculated values per category is provided in Table 2. The Bland-Altman difference plot for all the samples is given Figure 7

Table 2 - Summary of the calculated values per category

Category.	n	$\overline{D}$	s_D	95% Lower limit	95% Upper limit
Dairy	15	-0.329	0.286	-0.962	0.305
Dried/low moisture	16	-0.125	0.156	-0.467	0.218
Meat and poultry	15	-0.097	0.323	-0.813	0.619
Multi component	15	-0.291	0.527	-1.459	0.877
Ready to eat foods	16	-0.149	0.137	-0.449	0.151
All Categories	77	-0.196	0.321	-0.839	0.446

$\overline{D}$: Average difference SD: standard deviation of differences n: number of samples

Figure 7 – Bland-Altman difference plot for all the samples



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 3.

Table 3 - Data which are outside of the accepted limits -

Food Category	Food type	Sample code	Food item	Strain	Spiking/seeding protocol	Difference log cfu/g (alternative – reference)
Meat and poultry	Cooked and fermented meats	1C	Salami	3097	Ambient/2 weeks	0.486
Meat and poultry	Raw meat	28B	Pork loin steak	Natural	none	-1.022
Multi-component foods	RTE meals	10B	Tuna pasta bake	1238	55°C/5mim heating	-1.217
Multi-component foods	RTE meals	35	Fish pie	1238	55°C/5mim heating	-1.114
Multi-component foods	Product with raw ingredients	41B	sweet chilli chicken noodle salad	natural	none	0.968

Comments

It is expected that not more than one in 20 data values will lie outside the CLs. Any disagreements with the expectation should be recorded.

For this data set there are 5 in 77 data values which lie outside the CLs (All categories plot). There were no identifiable trends in these data, and they covered 4 different food categories, 2 different inoculated strains and naturally contaminated samples

3.1.6 Conclusion (RT study)

The relative trueness of the Alternative method for *S.aureus* (coagulase-positive staphylococci) is satisfied.

3.2 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. This study is conducted using artificially contaminated samples, using one type per category.

3.2.1 Categories, sample types and strains

In this study five food categories were tested with a single batch of two different food types using 6 samples per type. Two samples were contaminated at a low level, 2 at intermediate level, 2 at a high level. For each sample, 5 replicates (5 different test portions) were tested. A total of 30 samples were analysed per food type. Each sample was bulk inoculated and five replicate test portions examined from the bulk sample.

The tested categories, types and items are provided in Table 4.

Table 4 - Categories, types, items, strains and inoculation levels for accuracy profile study

Category	Types	Strain	Item	Level	Test portions
Dairy products	Dairy desserts	*S.aureus* CRA 1215 from cheese	Chilled custard	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Raw milk cheese	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Dried/rehydrated & low moisture products	Powders	*S.aureus* CRA 2095 from milk powder	RTC pasta	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Infant cereal	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Meat and poultry	RTE meats	*S.aureus* CRA 1217 from cooked beef	Pastrami	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Cooked sliced chicken roll	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Ready to eat foods	Cooked fish products e.g. prawns	*S.aureus* CRA 1208 from smoked fish	Fresh cooked prawns	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
			Smoked salmon	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5
Multi component foods	Composite foods with raw	*S.aureus* CRA 3097 from pasta	Pasta salad	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5

Category	Types	Strain	Item	Level	Test portions
	/processed ingredients		Sandwich spread	Zero	5
				Low:500cf/g	5
				Medium : 10000cfu/g	5
				High : 1000000cfu/g	5

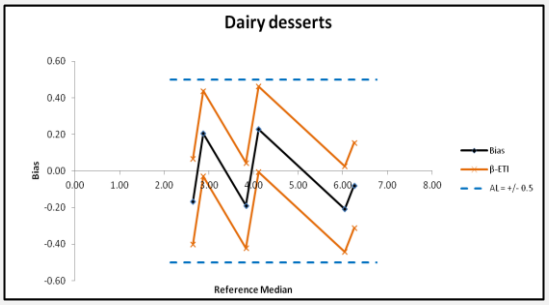
Total number of samples tested= 150

3.2.2 Calculations and interpretation of accuracy profile study
The statistical results and the accuracy profiles are provided in Figures 8 to 12.

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

Figure 8 Accuracy profile for Category: Dairy products (type desserts)

(Food) Category	Dairy
(Food) Type	Dairy desserts

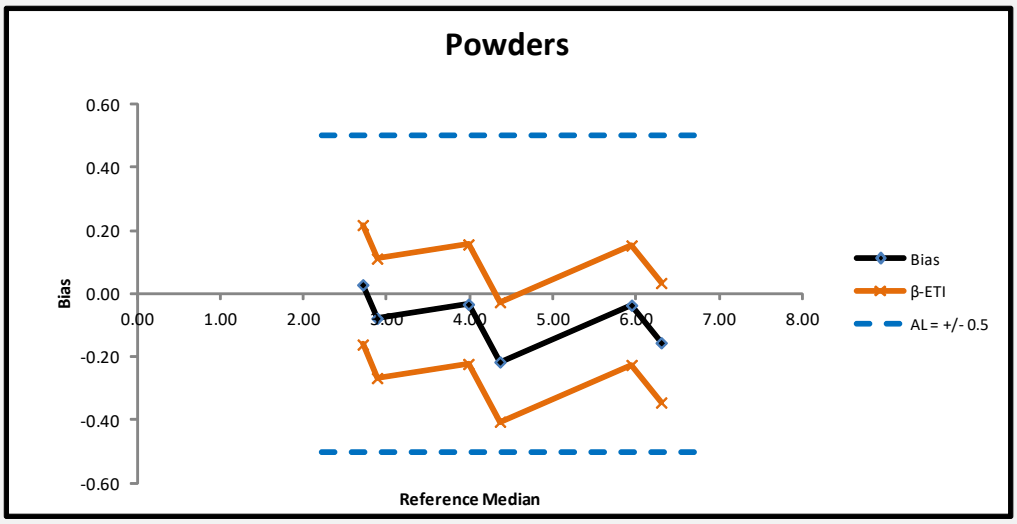


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
36	2.64	-0.166	-0.400	0.068	YES	YES
8	2.88	0.204	-0.030	0.438	YES	YES
17	3.83	-0.189	-0.423	0.045	YES	YES
16	4.11	0.228	-0.005	0.462	YES	YES
29	6.04	-0.209	-0.443	0.025	YES	YES
30	6.26	-0.079	-0.313	0.155	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.311	0.162	NO	+/- 0.500

Figure 9: Dried/rehydrated & low moisture products

(Food) Category	dried/dehydrated foods
(Food) Type	Powders

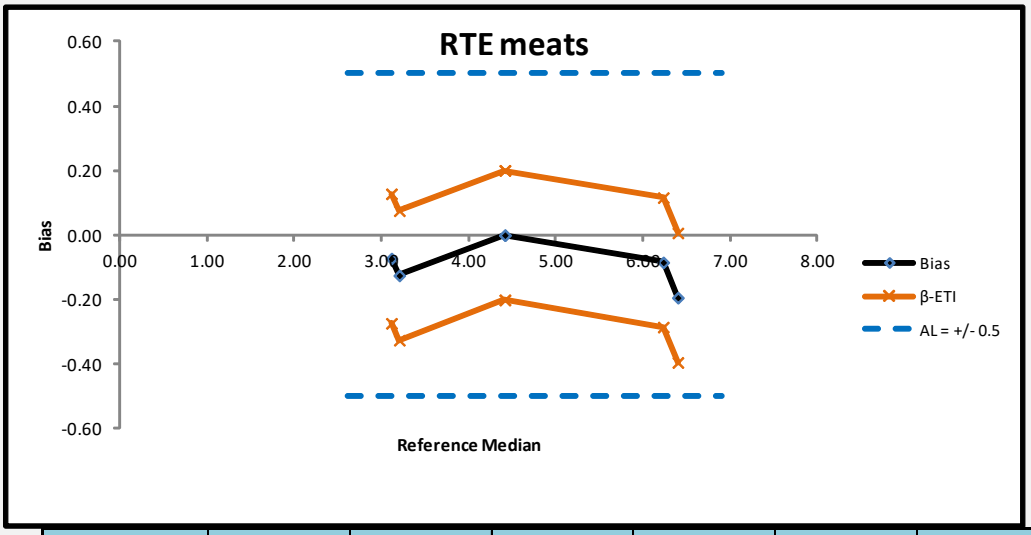


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
5	2.71	0.029	-0.161	0.218	YES	YES
13	2.88	-0.077	-0.267	0.112	YES	YES
9	3.98	-0.033	-0.222	0.156	YES	YES
26	4.36	-0.216	-0.405	-0.026	YES	YES
38	5.94	-0.036	-0.225	0.153	YES	YES
39	6.30	-0.155	-0.344	0.034	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.116	0.131	YES	+/- 0.500

Figure 10: Meat and poultry

(Food) Category	Meat and poultry
(Food) Type	RTE meats

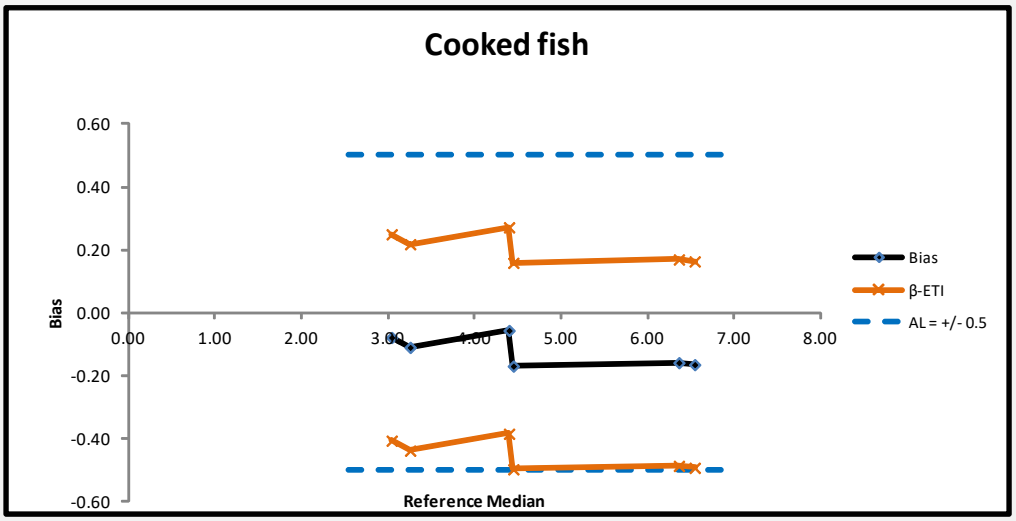


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
34	3.11	-0.073	-0.273	0.128	YES	YES
31	3.20	-0.125	-0.326	0.076	YES	YES
32	4.41	0.000	-0.201	0.201	YES	YES
25	4.41	0.000	-0.201	0.201	YES	YES
4	6.23	-0.084	-0.285	0.116	YES	YES
35	6.40	-0.194	-0.394	0.007	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.325	0.139	NO	+/- 0.500

Figure 11: Ready to eat foods

(Food) Category	Ready to eat foods
(Food) Type	Cooked fish

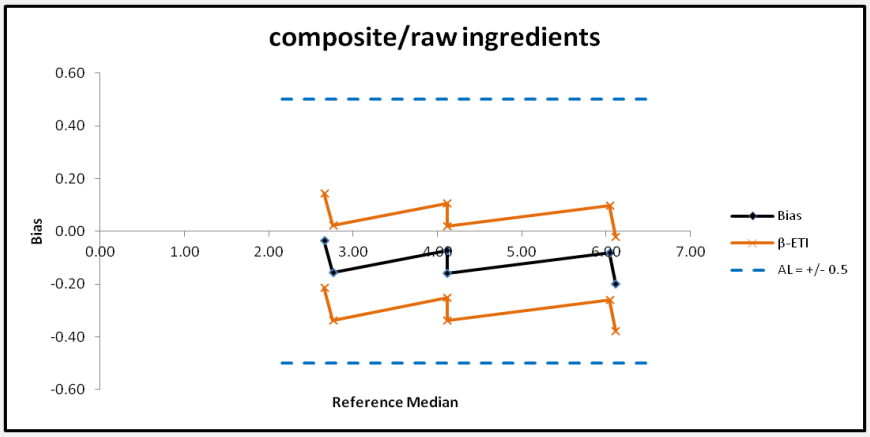


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
18	3.04	-0.078	-0.405	0.250	YES	YES
27	3.26	-0.109	-0.437	0.219	YES	YES
33	4.40	-0.056	-0.383	0.272	YES	YES
14	4.45	-0.168	-0.496	0.159	YES	YES
28	6.36	-0.158	-0.485	0.170	YES	YES
10	6.54	-0.164	-0.492	0.164	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.116	0.227	YES	+/- 0.500

Figure 12: Multi component foods

(Food) Category	Multi component
(Food) Type	composite/raw ingredients



Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β comp fin Acc
11	2.66	-0.036	-0.215	0.143	YES	Y
6	2.76	-0.158	-0.337	0.021	YES	Y
2	4.11	-0.073	-0.252	0.106	YES	Y
7	4.11	-0.160	-0.339	0.019	YES	Y
12	6.04	-0.082	-0.261	0.097	YES	Y
24	6.11	-0.200	-0.379	-0.021	YES	Y

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.084	0.124	YES	+/- 0.500

According to ISO 16140, if any of the upper or lower limits for the six samples exceeds the 0.5log Acceptability Limits (ALs) and the standard deviation, *S*ref > 0,125, then an additional evaluation procedure is followed:

New ALs are calculated as a function of the standard deviation: AL *s* = 4_ sref. If for all *i* in the accuracy profile *Ui* ≤ AL*s* and *Li* _ −AL*s* , the alternative method is accepted as being equivalent to the reference method for the given combination category and type.

For some of the food categories the additional AL calculation was required. This was for the dairy products and RTE meat products, however, the re-calculated AL's were still ±0.5log.

3.3 Inclusivity / exclusivity

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

3.3.1Protocol

After being grown according to appropriate conditions, decimal dilutions were made, and the 53 target strains and 31 non-target strains were enumerated by the alternative method, the reference method and a non selective agar (TSA).

3.3.2 Results

Of the 53 inclusivity strains tested, 51 strains were detected using both methods and 2 strains gave typical colonies on both media but did not confirm using the coagulase test.

Of the 31 exclusivity strains tested, none were detected by the alternate method and 2 were detected by the reference method these were S.delphini NCIMB 13206 and on S. hyicus CRA 254. The identity of these strains was re-checked using the MALDI and was confirmed as *S.delphini* and *S. hyicus..*

3.4 Limit of quantification (LOQ)

The limit of Quantification (LOQ) is only required for instrumental measurements. It was not done in this study

3.5 Conclusion (MCS)

Overall, the conclusions for the Method Comparison are:

- The Compact Dry X-SA for enumeration of *S.aureus* in foods method shows satisfying trueness
- The Compact Dry X-SA for enumeration of *S.aureus* in foods method shows satisfactory and accuracy profile.
- The Compact Dry X-SA for enumeration of *S.aureus* in foods method was shown to be specific and selective.

4 Interlaboratory 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 Calculation and interpretation of data

The data from the collaborative trial were calculated and interpreted according to section 6.2.3 of ISO 16140-2:2016 using the freely available Excel® spreadsheet (http://standards.iso.org/iso/16140). Version 14-03-2016 was used for these calculations.

The results obtained by the collaborators are shown in Tables 5

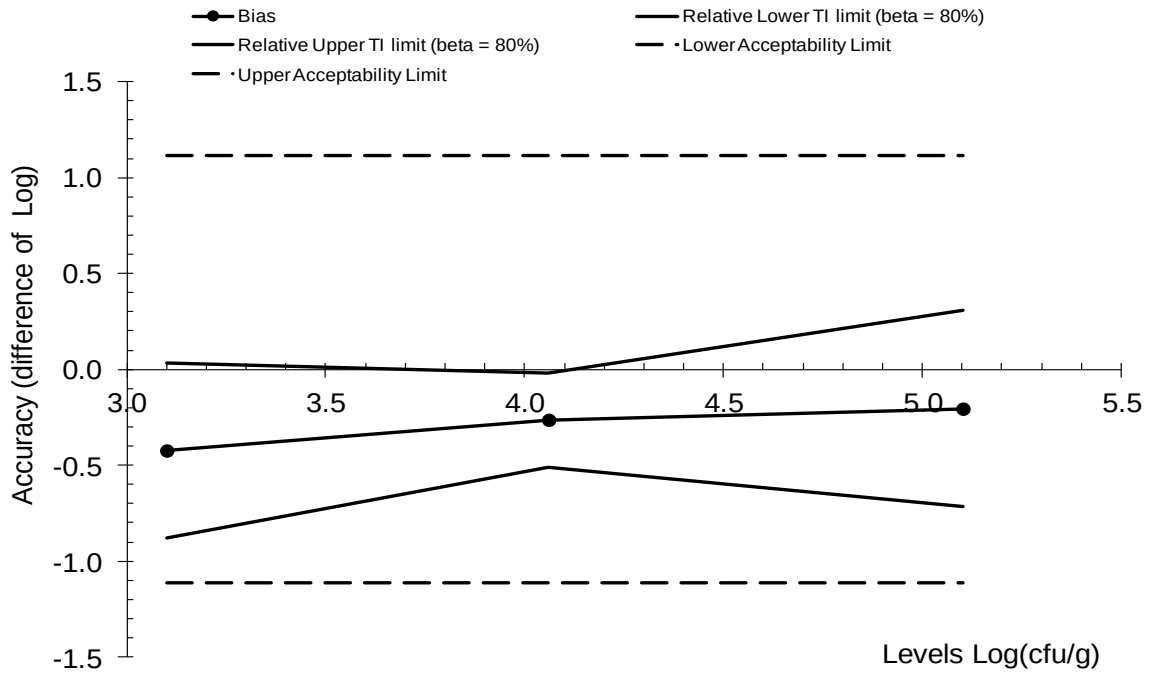
The accuracy profile plot is shown in Figures 12 and the statistical analysis of the data is shown in Tables 6.

Table 5: Summary of the results of the interlaboratory study per analyte level

		Reference method x_{ijk}		**Alternative method k_{ijk}**	
Collaborators (i)	**Level (k)**				
1	Blank	<10		<10	
2	Blank	<10		<10	
3	Blank	<10		<10	
4	Blank	<10		<10	
5	Blank	<10		<10	
6	Blank	<10		<10	
7	Blank	<10		<10	
8	Blank	<10		<10	
9	Blank	<10		<10	
10	Blank	<10		<10	
		Duplicate 1	Duplicate 2	Duplicate 1	Duplicate 2
1	Low	3.411	3.310	2.630	2.850
2	Low	3.080	2.970	2.850	2.730
3	Low	2.750	3.240	2.580	2.710
4	Low	3.310	3.300	3.160	2.950
5	Low	3.030	3.150	2.439	2.700
6	Low	3.140	3.330	2.630	3.130
7	Low	3.340	3.240	2.950	3.000
8	Low	3.050	3.150	2.710	2.500
9	Low	2.820	2.600	2.079	1.881
10	Low	2.960	2.820	2.590	2.470
1	Medium	4.560	4.180	3.870	3.790
2	Medium	3.960	4.020	3.840	3.770
3	Medium	3.700	3.630	3.580	3.610

		Reference method x_{ijk}		Alternative method k_{ijk}	
Collaborators (i)	**Level (k)**				
4	Medium	4.230	4.180	4.160	4.160
5	Medium	3.970	4.000	3.700	3.810
6	Medium	4.060	4.040	3.730	3.820
7	Medium	4.270	4.460	3.910	3.970
8	Medium	4.120	4.100	3.720	3.670
9	Medium	3.810	4.160	3.460	3.830
10	Medium	3.850	3.910	3.731	3.820
1	High	5.520	5.510	5.000	5.030
2	High	5.120	5.190	4.990	4.940
3	High	4.840	4.790	4.630	4.710
4	High	5.510	5.350	5.640	5.680
5	High	5.020	5.060	4.860	4.850
6	High	5.310	5.260	4.920	4.850
7	High	5.530	5.750	5.040	5.030
8	High	5.120	5.190	4.570	4.840
9	High	4.180	5.130	4.060	4.510
10	High	3.660	5.000	4.890	4.900

Figure 12. Accuracy profile of Compact Dry XSA from the ILS



The statistical analysis of the existing ILS data is shown in Table 6 below. It can be seen that the repeatability standard deviation (S_r) was better for the alternate method than the reference method ranging from 0.096 to 0.165 for XSA and 0.126 to 0.373 for the reference method.

The between-labs standard deviation (S_L) was similar for the alternative method (0.145 to 0.336) and the reference method (0.178 to 0.309) and the reproducibility standard deviation (S_R) was better for the alternative method (0.174 to 0.358) than the reference method (0.228 to 0.485).

According to the ISO 16140-2:2016 standard, if any of the values of the β-ETI fall outside of the ±0.5log AL then a further calculation is done to calculate the pooled average S_R of the reference method. This was done and gave an S_R value of 0.337. This value was used to recalculate the new AL as a function of the standard deviation (AL_s) using the formula 3.3 x $S_{R,ref}$ which gives new AL_s values of +1.11 and -1.11. These are plotted in Figure 4 and it can be seen that no values lie outside of these AL_s values and therefore the alternative method is accepted as being equivalent to the reference method.

It can be seen from Figure 12 and Table 6 that there is a slight bias in the data with the alternate method giving slightly lower average values than the reference method for the low, medium and high categories (-2.05 to -0.423). This was previously reported in the original ILS where the average bias across the three levels was - 0.283 which is similar to the average bias in the new calculation of -0.297.

It was previously accepted that whilst there was evidence of a small underlying bias between the two methods with the ISO method giving slightly higher plate count results than the Compact Dry X-SA, however this was considered to have no major microbiological implications considering the magnitude of the bias and the different formats of the test methods.

The alternative method is therefore accepted as being equivalent to the reference method in the Inter laboratory study although the data shows that there is the potential for the alternative method to give a lower count that the reference method.

Table 9. Statistical analysis of the ILS data according to the ISO spreadsheet

Accuracy profile	0.5						
Study Name	XSA ILS analysis						
Date	Campden BRI						
Coordinator	22/12/2016						
Tolerance probability (beta)	80%	80%	80%				
Acceptability limit in log (lambda)	1.11	1.11	1.11				
				TRUE			
	Alternative method				**Reference method**		
Levels	**Low**	**Medium**	**High**		**Low**	**Medium**	**High**
Target value	**3.100**	**4.061**	**5.102**				
Number of participants (K)	10	10	10		10	10	10
Average for alternative method	2.677	3.798	4.897		3.100	4.061	5.102
Repeatability standard deviation (sr)	0.165	0.096	0.121		0.142	0.126	0.373
Between-labs standard deviation (sL)	0.277	0.145	0.336		0.178	0.196	0.309
Reproducibility standard deviation (sR)	0.323	0.174	0.358		0.228	0.233	0.485
Corrected number of dof	11.659	12.162	10.090		13.149	12.044	15.678
Coverage factor	1.417	1.412	1.435				
Interpolated Student t	1.359	1.355	1.371				
Tolerance interval standard deviation	0.3364	0.1810	0.3740				
Lower TI limit	2.220	3.552	4.384				
Upper TI limit	3.134	4.043	5.410				
Bias	**-0.423**	**-0.263**	**-0.205**				
Relative Lower TI limit (beta = 80%)	**-0.880**	**-0.508**	**-0.718**	TRUE			
Relative Upper TI limit (beta = 80%)	**0.034**	**-0.018**	**0.308**	FALSE			
Lower Acceptability Limit	**-1.11**	**-1.11**	**-1.11**				
Upper Acceptability Limit	**1.11**	**1.11**	**1.11**				
New acceptability limits may be based on reference method pooled variance							
Pooled repro standard dev of reference	0.337						

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.

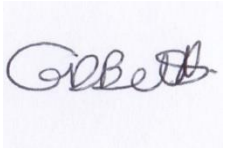
Select ALL blue lines to draw the accuracy profile as illustrated in the worksheet "Graph Profile"

5 Overall conclusions of the validation study

- The alternative method Compact Dry XSA for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory results for relative trueness;
- The alternative Compact Dry XSA for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory results for accuracy profile;
- The alternative Compact Dry XSA for enumeration of *S.aureus* (coagulase-positive staphylococci is selective and specific.
- The alternative Compact Dry XSA for enumeration of *S.aureus* (coagulase-positive staphylococci shows satisfactory performance in the ILS

The alternative Compact Dry XSA for enumeration of *S.aureus* (coagulase-positive staphylococci) comparable performance to the reference method ISO 6888-1for enumeration of coagulase-positive staphylococci in a broad range of foods

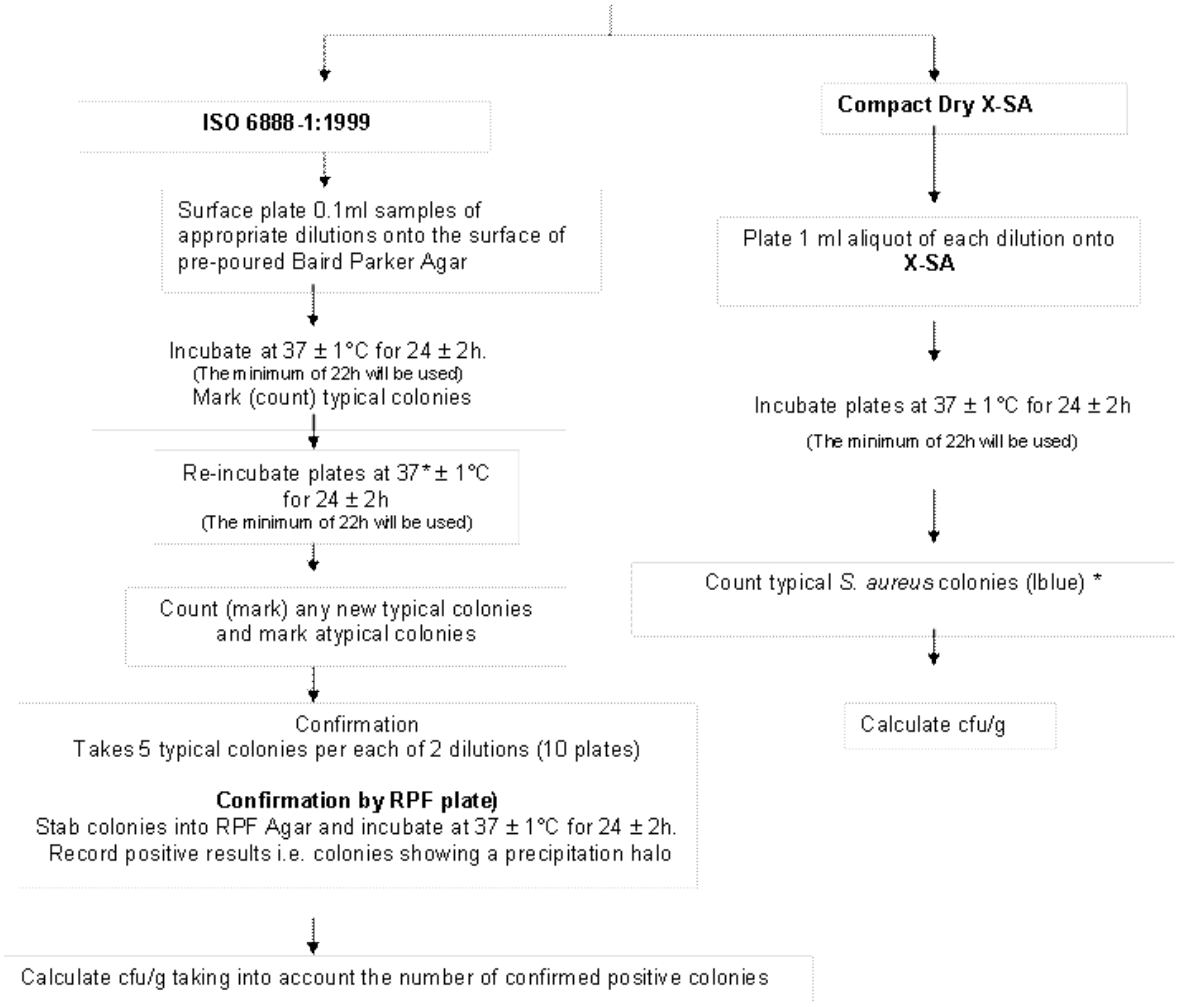
Date : 03/03/2019

Signature: 

Annexes A: Flow diagram of the reference and alternative method. B: Test kit insert

ANNEX A: Flow diagram of the alternative method and reference methods

Food sample (10g) + appropriate diluents (90ml) dilution (according to ISO 6887)
Homogenise and dilute further as required



*no confirmation is required for X-SA according to the manufacturers' instructions
If there are any naturally contaminated samples found then 5 colonies per sample will be confirmed using RPF agar. Confirmation will not be done on artificially inoculated samples.

ANNEX B Kit insert

Compact Dry X-SA medium for Staphylococcus aureus

40 plates/Platten/plaques/placas/lastre/placas	ID-No. 1 002 960
240 plates/Platten/plaques/placas/lastre/placas	ID-No. 1 002 961
500 plates/Platten/plaques/placas/lastre/placas	ID-No. 1 402 961
1400 plates/Platten/plaques/placas/lastre/placas	ID-No. 1 502 990

English

Compact Dry X-SA is a ready to use, chromogenic plate for detection of Staphylococcus aureus

Sample pretreatment

Viable count in water or liquid foodstuff
Drop 1 ml of specimen (dilute if necessary) on the middle of the Compact Dry plate.

Viable count in solid foodstuff

Add buffer solution to the sample and homogenize by stomacher®. Drop 1 ml of specimen (dilute if necessary) in the middle of the dry sheet of the Compact Dry plate.

Viable count in swab test specimen

Use the swab to wipe the surface, put into the device with wiping solution. Drop 1 ml of wiping solution (dilute if necessary) in the middle of the Compact Dry plate. It is recommended to use "Swab for Compact Dry" offered by HyServe Id-No. 1 002 952/3 / 1 402 954 (40/240/600 pieces).

Test instructions
1. Open the cap and drop 1 ml of specimen in the middle of the Compact Dry plate.
2. Specimen diffuses automatically and evenly into the sheet and transforms the dried sheet into a gel within seconds.
3. Put the cap again on the plate and write the information needed on the memorandum section.
4. Turn over the capped plate and put in the incubator.
5. After incubation count the number of blue colonies White paper placed under the plate helps to count the colonies.

Incubation time	**24 hours ± 2**
Incubation temperature	**35 ± 2 °C**
AOAC approved	**35 ± 2 °C**
NordVal/MicroVal approved	**37 ± 1 °C**

Interpretation of the results
Blue Colonies are *Staphylococcus aureus*.

Storage and shelf life
Keep at room temperature (+ 5 to +30 °C). Total shelf life 21 months after manufacturing.

Notes
- High cell concentrations on plates will cause the entire growth area to become colored/bluish. In this case dilute the sample.
- After use please follow the current disposal regulations.
- The growth area is 20 cm². The back of the plate has a grid carved of 1 cm x 1 cm to make the colony counting easier. In case of any difficulties to count colonies due to large number of colonies grown, total viable count can be obtained by multiplying 20 by an average number of colonies per grid counted from several grids.
- Compact Dry plates are produced at an ISO 9001 certified site.

- **AOAC Approval No. 081001**
- **MicroVal approval No. 2008-LR14**
- **NordVal Certificate No. 042, ISO 6888-1:1999**

Deutsch

Compact Dry X-SA ist eine gebrauchsfertige, chromogene Platte zum Nachweis von Staphylococcus aureus

Probenvorbereitung

Lebendkeimzahl in Wasser oder flüssigen Lebensmitteln
1 ml der Probe (evtl. verdünnen) in der Mitte der Compact Dry Platte aufbringen.

Lebendkeimzahl in festen Lebensmitteln

Zugabe von Pufferlösung und Homogenisierung der Lebensmittelprobe im Stomacher® ist erforderlich. 1 ml der Probe (evtl. verdünnen) in der Mitte der Compact Dry Platte aufbringen.

Lebendkeimzahl aus Tupfer-Proben

Mit dem sterilen, feuchten Wattetupfer kann z.B. die Oberfläche gewischt werden. Der Tupfer wird zurück in die Aufnahmeflüssigkeit überführt. Nach Schütteln wird die gesamte Lösung (1 ml) in der Mitte der Compact Dry Platte aufgebracht. Es wird empfohlen den Swab für Compact Dry von HyServe, Id-No. 1 002 952/3 / 1 402 954 (40/240/600 Stück) zu verwenden.

Testanweisung
1. Öffnen Sie den Deckel und tröpfeln Sie 1 ml Probenmaterial auf die Mitte der Compact Dry Platte.
2. Das Probenmaterial breitet sich automatisch und gleichmäßig auf dem Film aus und rehydriert diesen zu einem Gel.
3. Platte mit Deckel erneut verschließen und beschriftbare Fläche zur Kennzeichnung verwenden.
4. Geschlossene Platte umdrehen und in einen Brutschrank legen.
5. Nach Inkubation die Anzahl der blauen Kolonien zählen. Ein weißes Papier als Unterlage erleichtert den Zählvorgang.

Inkubationszeit	**24 Stunden ± 2**
Inkubationstemperatur	**35 ± 2 °C**
AOAC approved	**35 ± 2 °C**
NordVal/MicroVal approved	**37 ± 1 °C**

Interpretation des Ergebnisses
Blaue Kolonien weisen auf *S. aureus* hin.

Lagerung und Haltbarkeit
Bei Raumtemperatur aufbewahren (5 bis +30 °C). Haltbarkeit bis zu 21 Monate nach Herstellung.

Anmerkungen
- Hohe Wachstumskonzentrationen auf den Platten verursachen eine bläuliche Färbung des gesamten Kulturbereichs. In diesem Fall muss das Probenmaterial verdünnt werden.
- Nach Gebrauch entsprechend den geltenden Abfallbestimmungen entsorgen.
- Die Plattenfläche umfasst 20 cm². Auf der Plattenrückseite ist zur Erleichterung der Koloniezählung ein 1 cm x 1 cm großes Raster eingraviert. Sollte es auf Grund hoher Koloniedichte Probleme beim Auszählen einer ganze Platte geben, kann man einzelne Quadrate auszählen und den Mittelwert der Kolonien aus verschiedenen Feldern mit 20 multiplizieren.
- Compact Dry-Platten werden in einem ISO 9001 zertifizierten Betrieb gefertigt.

- **AOAC Approval No. 081001**
- **MicroVal approval No. 2008-LR14**
- **NordVal Certificate No. 042, ISO 6888-1:1999**

Français

Compact Dry X-SA est une plaque chromogène prête à l'utilisation pour détecter le nombre total de Staphylococcus aureus

Traitement préliminaire de l'échantillon

Nombre de germes revivifiables dans l'eau ou dans des aliments liquides
Appliquer 1 ml de l'échantillon (le diluer si nécessaire) au centre de la plaque Compact Dry.

Nombre de germes revivifiables dans des aliments solides
Il est nécessaire d'ajouter une solution tampon à l'échantillon et de l'homogénéiser par Stomacher®. Appliquer 1 ml de l'échantillon (le diluer si nécessaire) au centre de la plaque Compact Dry.

Nombre de germes revivifiables dans des échantillons prélevés
Utiliser le tampon pour essuyer la surface, le placer dans l'unité avec la solution d'essuyage. Appliquer 1 ml de la solution d'essuyage (le diluer si nécessaire) au centre de la plaque Compact Dry. Il est recommandé d'utiliser le tampon 'Swab for Compact Dry' distribué par la société HyServe N° ID 1 002 952/3 / 1 402 954 (40/240/600 pièces).

Instructions pour le test
1. Ouvrir le couvercle et appliquer 1 ml de l'échantillon sur la plaque Compact Dry.
2. L'échantillon se répand automatiquement et uniformément sur la feuille et en l'espace de quelques secondes, il transforme la feuille sèche en un gel.
3. Refermer le couvercle de la plaque et inscrire les informations nécessaires dans la partie correspondante.
4. Retourner la plaque fermée et la placer dans l'incubateur.
5. Après le temps d'incubation, compter le nombre de colonies bleues. Les colonies peuvent être comptées plus simplement en plaçant du papier blanc sous la plaque..

Temps d'incubation	**24 heures ± 2**
Température d'incubation	**35 ± 2 °C**
AOAC approved	**35 ± 2 °C**
NordVal/MicroVal approved	**37 ± 1 °C**

Interprétation des résultats
Toutes les colonies de S. aureus se colorent en bleu.

Stockage et durée de conservation
Stockage à température ambiante (+ 5 à +30 °C). Durée totale de conservation 21 mois après fabrication.

Remarques
- Des concentrations élevées sur les plaques entraînent une coloration de toute la surface. Dans un tel cas, il faut diluer l'échantillon.
- Après l'utilisation, éliminer les plaques en respectant les réglements correspondants en vigueur.
- La surface de la plaque est de 20 cm². Une grille de 1 cm x 1 cm est taillée dans le dos de la plaque afin de faciliter le calcul des colonies. S'il est toutefois difficile de compter le nombre de colonies, suite à un grand nombre de colonies, il est possible de déterminer le nombre total de germes revivifiables dans certains carrés de la grille et d'en multiplier par 20 la valeur moyenne obtenue.
- Les plaques Compact Dry sont fabriquées dans une usine certifiée conforme à ISO 9001.

- **AOAC Approval No. 081001**
- **MicroVal approval No. 2008-LR14**
- **NordVal Certificate No. 042, ISS 6888-1:1999**

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