NMKL - NordVal International
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NordVal International Certificate

Issued for:	Compact Dry X-SA Method for the Enumeration of *Staphylococcus aureus* in Foods
NordVal No:	042
First approval date:	1 June 2011
Renewal date:	1 June 2023
Valid until:	1 June 2025

Compact Dry X-SA

Manufactured and supplied by:

Shimadzu Diagnostics Corporation,
20th Floor Ueno Frontier Tower,
3-24-6 Ueno, Taito-ku, Tokyo,
110-8736 JAPAN

fulfils the requirements of the NordVal validation protocol. The reference method was 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.

NordVal International has reviewed the method and the validation studies conducted by Campden BRI, UK. The studies have been conducted according to ISO 16140-2:2016. The results document no statistical difference in the performances between Compact Dry X-SA and the ISO 6888-1: 1999.

Date: 01 June 2023

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Compact Dry X-SA method contains a ready-to-use dry chromogenic medium, and selective agents for the detection and enumeration of *Staphylococcus aureus,* which form blue colonies. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry X-SA plate. The incubation conditions tested in the study were 37 ± 1°C for 24 ± 2h.

FIELD OF APPLICATION

The method has been tested on the detection and enumeration of *Staphylococcus aureus* in foods.

HISTORY

Original studies for this certificate were conducted according NordVal protocol from 2010. Selectivity results are from this study. A renewal study was carried out in 2017 according to ISO 16140-2:2016 to test for relative trueness and accuracy profile. Further, a new selectivity was also carried out. The design of the Inter-laboratory study (ILS) is the same for the 2003 and 2016 versions of ISO16140, and therefore the existing ILS data were reanalysed using the new statistical approach outlined in ISO16140-2:2016.

RESULTS OF THE COMPARISON STUDIES

Selectivity; inclusivity and exclusivity

Inclusivity: All the 51 strains of *Staphylococcus aureus* yielded typical (blue) colonies on Compact Dry X-SA. For the reference method two strains were not detected (*S. aureus* 1236 and 1241).

Exclusivity: 31 strains of non-target organisms were tested. None of the strains appeared typical (blue colonies) on the Compact Dry X-SA. The reference method detected two strains as positive (S.delphini NCIMB 13206 and on S. hyicus CRA 254).

Precision and compliance between Compact Dry X-SA and the reference method

During this study it was necessary to artificially contaminate the majority of samples. The foods tested were cooked sliced ham, frozen prawns, raw cow's milk, fresh cream pastry products and chilled fresh pasta. At least five levels of contamination were used for each food matrix. Each sample was tested by both the reference method and the alternative method.

Relative trueness

The relative trueness is illustrated by the use of a Bland-Altman plot, i.e. the difference (bias) between paired samples analysed with the reference method and the alternative method respectively, plotted against the mean values obtained by the reference method. In the plot, Upper and Lower limits are included as the bias ± 2 times the standard deviation of the bias. The Bland-Altman Plot in Figure 1, illustrates the difference obtained in the enumeration of total Staphylococcus aureus in foods by the alternative and the reference method, respectively.

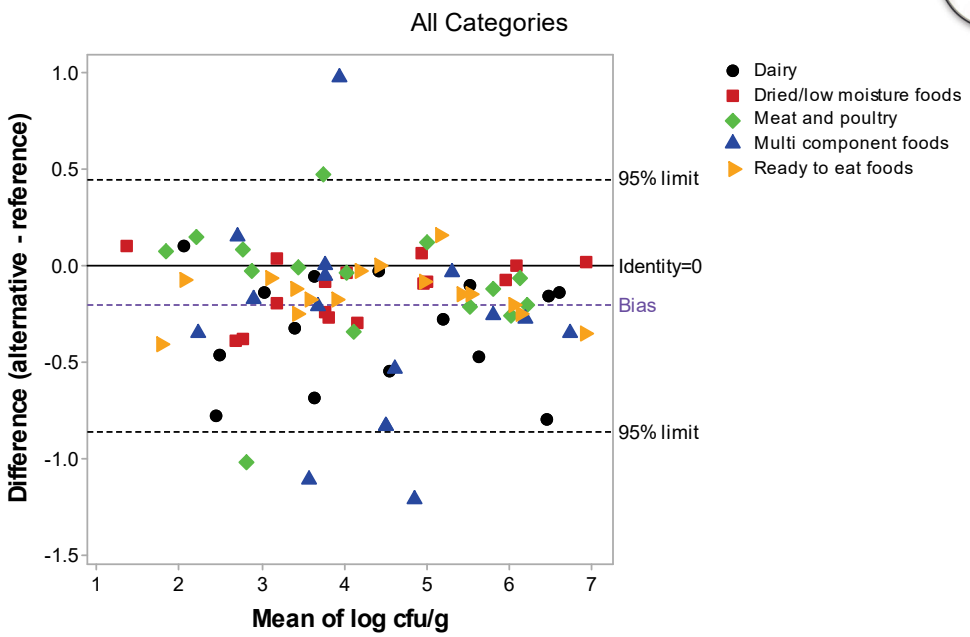

Figure 1: Bland-Altman plot for all categories

Conclusion of the comparison study:

The results of the Bland-Altman Plot provide a visual observation on the amount of bias and extreme results. It is expected that not more than one in 20 data values will lie outside the 95% Confidence Limits (CLs). The Figure 1 shows that there is a bias, as more results are obtained below the Identity =0.

For 'All Categories' there are five in 77 values which lie outside the CLs. This is a little more than the expectation of less than one in 20. For this circumstance or more extreme (≥ 5/77) a binomial distribution with mean = 1/20 gives a probability of 0.341, substantially greater than the commonly accepted critical significance level of 0.05.There were no identifiable trends in the five data outside the CLs and they covered 4 different food categories, 2 different inoculated strains and naturally contaminated samples.

Accuracy profiles:

The accuracy profile study is a comparative study between the results obtained by the reference and the results of the alternative method. This study is conducted using artificially contaminated samples. One type per category is tested for this.

Food matrices

For each of 5 food categories, one type of food was tested using 6 samples per type. Of the 6 samples, there were 2 at a low level, 2 at a medium level and 2 at a high level of contamination. For each of the 6 samples per category, 5 replicate test portions were tested. Non –inoculated samples (5) of each product type were also tested. Each sample was bulk inoculated and separate replicate test portions examined.

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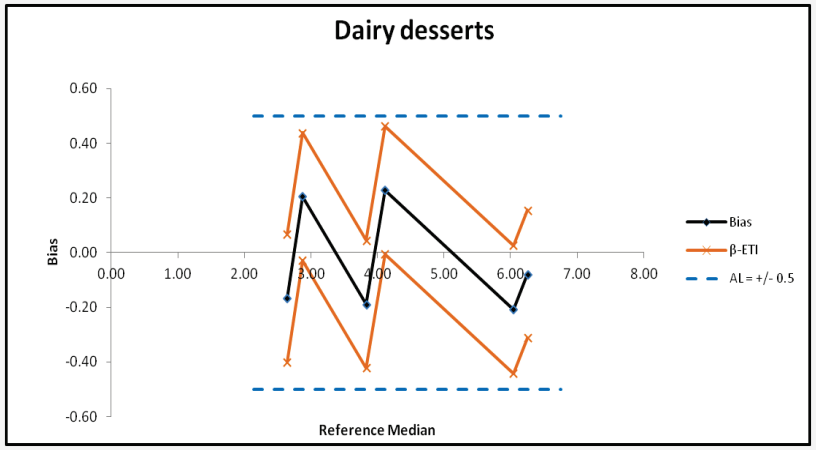
The observed profiles are within the 0.5 log AL or the recalculated AL limit calculated according to ISO16140-2:2015 section 6.1.3.3.

All the accuracy profiles, shown in figure 2, fulfil the performance criteria and the alternative method is accepted as being equivalent to the reference method.

Figure 2a: Dairy products

(Food) Category	Dairy
(Food) Type	Dairy desserts

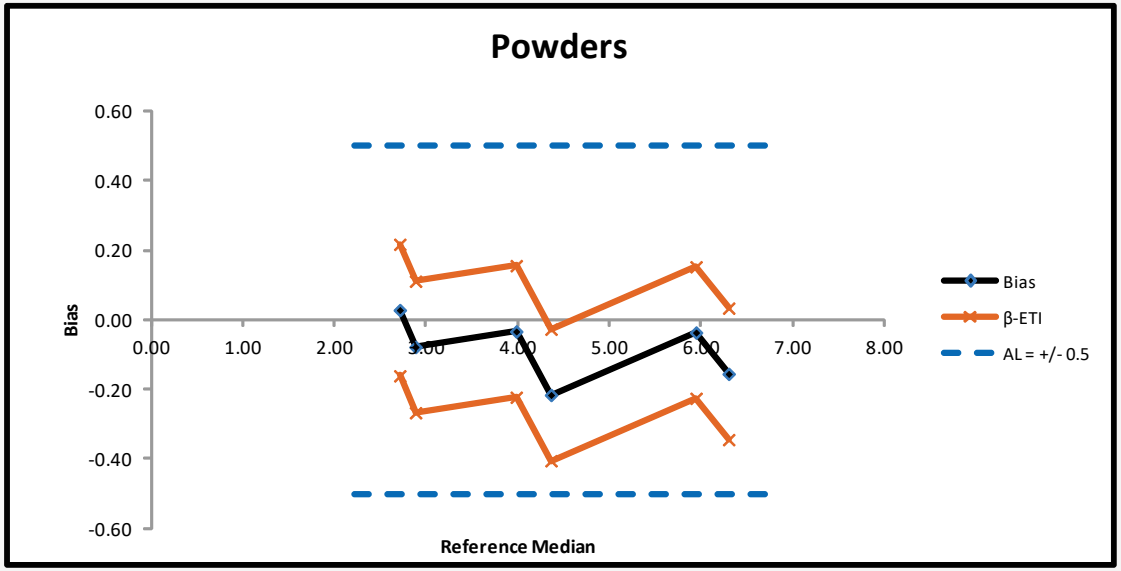


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
36	2.64	-0.166	-0.400	0.068	YES	YES
8	2.88	0.204	-0.030	0.438	YES	YES
17	3.83	-0.189	-0.423	0.045	YES	YES
16	4.11	0.228	-0.005	0.462	YES	YES
29	6.04	-0.209	-0.443	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 2b: Dried/rehydrated & low moisture products

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

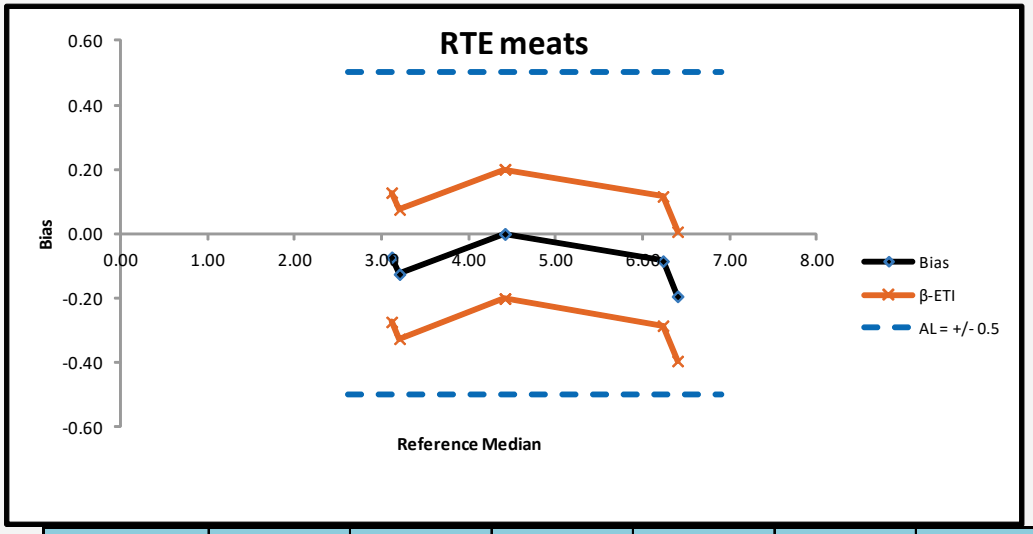


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
5	2.71	0.029	-0.161	0.218	YES	YES
13	2.88	-0.077	-0.267	0.112	YES	YES
9	3.98	-0.033	-0.222	0.156	YES	YES
26	4.36	-0.216	-0.405	-0.026	YES	YES
38	5.94	-0.036	-0.225	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 2c: Meat and poultry

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

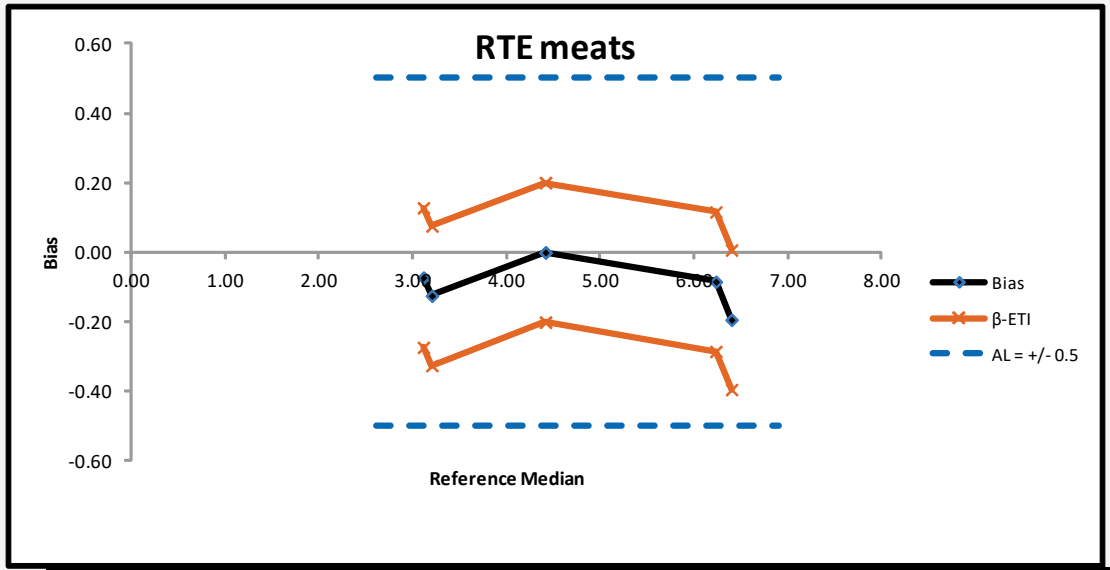


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
34	3.11	-0.073	-0.273	0.128	YES	YES
31	3.20	-0.125	-0.326	0.076	YES	YES
32	4.41	0.000	-0.201	0.201	YES	YES
25	4.41	0.000	-0.201	0.201	YES	YES
4	6.23	-0.084	-0.285	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 2d: Ready to eat foods

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

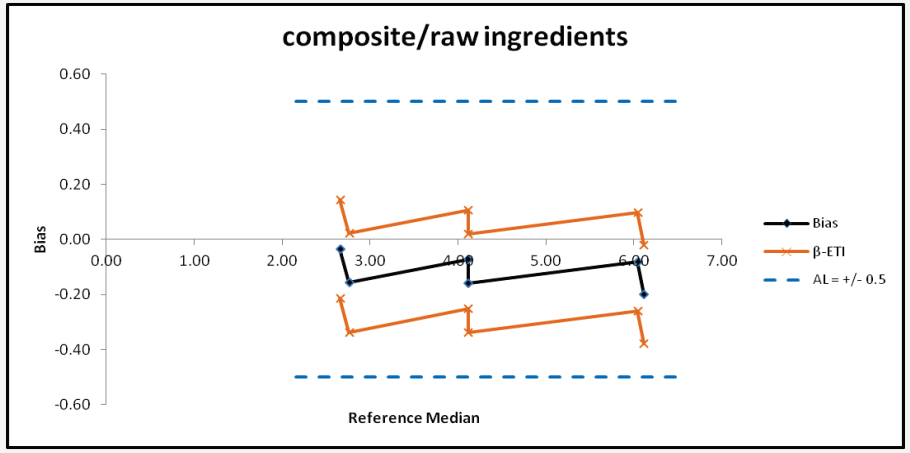


Sample Name	Reference central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
34	3.11	-0.073	-0.273	0.128	YES	YES
31	3.20	-0.125	-0.326	0.076	YES	YES
32	4.41	0.000	-0.201	0.201	YES	YES
25	4.41	0.000	-0.201	0.201	YES	YES
4	6.23	-0.084	-0.285	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 2e: 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-ETI compared to final AL Acceptable
11	2.66	-0.036	-0.215	0.143	YES	YES
6	2.76	-0.158	-0.337	0.021	YES	YES
2	4.11	-0.073	-0.252	0.106	YES	YES
7	4.11	-0.160	-0.339	0.019	YES	YES
12	6.04	-0.082	-0.261	0.097	YES	YES
24	6.11	-0.200	-0.379	-0.021	YES	YES

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

RESULTS OF THE COLLABORATIVE STUDY

Ten collaborative laboratories (as well as the Expert Laboratory) participated in the collaborative study. A single strain of *S.aureus* isolated from milk powder was incubated. Raw whole milk was used as matrix. Two milk samples remained uninoculated. For six samples, appropriated dilutions of the *S.aureus* culture were used to individually inoculated 2 x 20 ml milk samples at the lower (100 cfu/ml) middle (1000 cfu/ml) and higher (10000 cfu/ml) contamination levels. The results obtained are given in table 1.

Table 1. 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

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.

	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**			
Relative Upper TI limit (beta = 80%)	**0.034**	**-0.018**	**0.308**			
Lower Acceptability Limit	**-1.11**	**-1.11**	**-1.11**			
Upper Acceptability Limit	**1.11**	**1.11**	**1.11**			

TRUE
FALSE

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

New acceptability limits may be based on reference method pooled variance	
Pooled repro standard dev of reference	0.337

CONCLUSION

According to the comparison and the collaborative study no statistical differences were found between the Compact Dry X-SA method and the reference method (ISO 6888-1:1999) for the enumeration of *Staphylococcus aureus*.

The observed profiles are within the 0.5 log AL or the recalculated AL limit calculated according to NordVal International Protocol /ISO16140-2:2016, and hence the alternative method is accepted as being equivalent to the reference method.