**NMKL - NordVal International**
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# NordVal International Certificate

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|---|---|
| Issued for: | **Easy Plate SA for the enumeration of *Staphylococcus aureus* in a broad range of foods** |
| NordVal No: | 062 |
| First approval date: | 01 September 2023 |
| Valid until: | 01 September 2025 |

## Easy Plate SA

**Manufactured by:**

Kikkoman Biochemifa Company
2-1-1, Nishi-shinbashi, Minato-ku,
Tokyo 105-0003, Japan

NordVal International has reviewed the method validation documentation. The validation was conducted by Campden BRI, UK according to ISO 16140-2. The reference method was ISO 6888-1:2021: 2021 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 concludes that it has been satisfactorily demonstrated that the data and interpretations comply with the EN ISO 16140-2 requirements and demonstrate comparable performance of the alternative method Easy Plate SA to the ISO reference method for the enumeration of *Staphylococcus aureus* in a broad range of foods.

The production of Easy Plate SA is certified according to ISO 9001.

Date: 01 September 2023

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Executive Director

## PRINCIPLE OF THE METHOD

Easy Plate SA is a microbiological culture device made up of a waterproof sheet, a readymade dry medium on the sheet and a transparent cover over the medium. The Easy Plate SA method is intended to indicate the level of *Staphylococcus aureus* in food and beverage products. After incubation (at 37 ± 1°C for 24 ± 2h), *S. aureus* appears as blue colonies on the growth medium contained in the Easy Plate SA plate. Easy Plate SA is selective for *S. aureus* therefore other coagulase positive Staphylococci are not included in the scope of this validation.

## FIELD OF APPLICATION

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

## HISTORY

The method was approved by NordVal International and the certificate issued X of August 2023.

## METHOD COMPARISON STUDY

### Selectivity; inclusivity and exclusivity

Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains. Exclusivity is the lack of interference from a relevant range of non-target strains of the alternative method.

#### Inclusivity

Fifty strains of *S. aureus* were analysed in the study. The analysis was carried out once with the alternative method, the reference method and a non-selective method. All 50 strains of *S. aureus* gave typical colonies on the alternate and reference method. Three isolates gave typical colonies on the reference and alternate media however did not give a positive reaction with the coagulase test. The level enumerated on the reference method and alternative method were similar with no negative or positive bias shown.

#### Exclusivity

Thirty strains of non-target organisms were analysed. The analysis was carried out once with the alternative method, the reference method, and a non-selective method. 29 out of the 30 isolates tested gave the expected results on the alternative method and 22 by the reference method.

In conclusion, the alternative method, Easy Plate SA, gave comparable performance to the reference method and is therefore selective and specific to *S. aureus*. Data from the study showed that the Easy Plate SA was more selective for *S. aureus* than the reference method.

### Relative trueness

The trueness study is a comparative study between the results obtained by the reference method and the results of the alternative method. **Table 1** shows the categories, types and items tested and the ISO standards for the sample preparation used. Five samples for each three items of each five categories (equals 15 samples each category) were tested by both the reference method and the alternative method in the relative trueness study.

**Table 1. Categories, types and items tested and the ISO standards used for the sample preparation.**

| Category | Types | Items | ISO |
|---|---|---|---|
| Milk and dairy products (combined category raw and heat processed Milk and dairy products) | Raw milk and dairy products | Raw milk, raw milk cheese | 6887-5 |
| | Pasteurised milk and milk based products | Processed cheese, milk based drinks, creams, ice cream, pasteurised skim milk (non-fat milk) | 6887-5 |
| | Dry milk products | Milk powders and powder for milk based desserts | 6887-5 |
| Fishery products<br>Combined category: raw, RTE, RTRH, RTC | Raw fish (unprocessed) | Raw salmon filet, tuna, bonito | 6887-3 |
| | RTE/RTC/RTRH fish and seafoods | Smoked salmon, frozen seafoods, semi-dried fish | 6887-3 |
| | Crustaceans | Shrimp, crab | 6887-3 |
| Produce and fruits (combined category fresh and processed) | Cut ready-to-eat vegetables/leafy greens and sprouts | Bagged pre-cut lettuce shredded carrot, radish sprouts, alfalfa | 6887-4 |
| | Fresh fruit/Cut RTE fruit and vegetable products | Cut fruits, freshly squeezed juice, smoothies | 6887-4 |
| | Heat treated fruit and vegetables | Past smoothies/juice, blanched frozen vegetables | 6887-4 |
| Multi-component foods or meal components | Composite foods with substantial raw ingredients | Chilled pasta salad, egg and cress sandwich | 6887-1, 6887-4 |
| | RTRH/RTE foods (chilled, frozen) | Cooked chilled pasta, frozen fries, rice products, quiche | 6887-1, 6887-4 |
| | Mayonnaise based deli-salads | Vegetable salad, egg mayonnaise | 6887-1, 6887-4 |
| Meat and poultry products (RTE/RTRH) | cooked meat and poultry products | Cooked cured hams, pate, cooked poultry, | 6887-2 |
| | Fermented or dried products | Salami, chicken sausage | 6887-2 |
| | Raw cured products | Dry cured hams, smoked turkey products | 6887-2 |

The difference (bias) between paired samples analysed with the alternative method and the reference method, and the standard deviation thereof, were calculated. The results are provided in Table 2 and illustrated by a Bland-Altman plot. The difference is 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.

**Table 2. Summary of the calculated values per category**

| Category | N | Bias | SD |
|---|---|---|---|
| Milk and dairy products | 15 | 0.17 | 0.12 |
| Fishery products | 15 | 0.07 | 0.10 |
| Produce and fruit | 15 | 0.17 | 0.19 |
| Raw and RTC Meat and poultry | 15 | 0.11 | 0.15 |
| Multicomponent | 15 | 0.13 | 0.20 |
| **All categories** | **75** | 0.13 | 0.16 |

Bias: Average difference between the alternative method and the reference method, SD: standard deviation of differences, N: number of samples

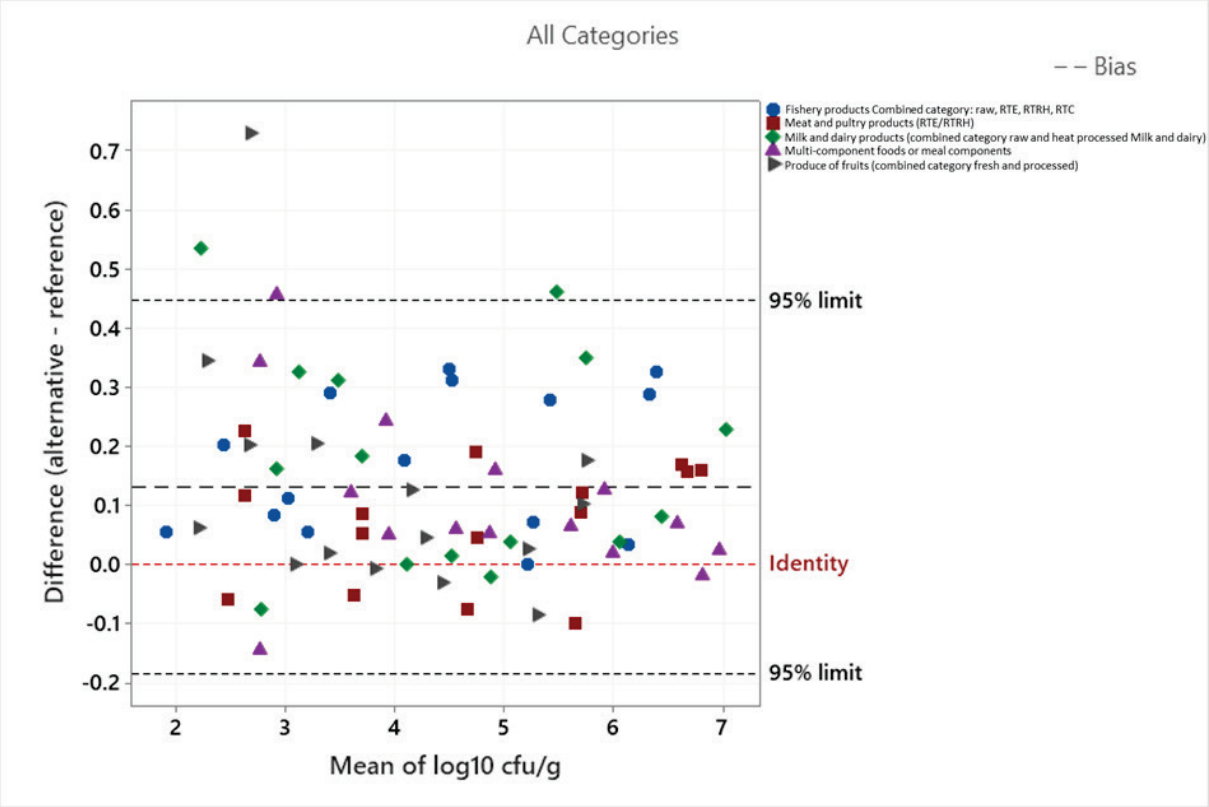



**Figure 1. Bland-Altman plot for all samples**

In conclusion, it is expected that not more than one in 20 data values will lie outside the confidence levels. For this data set there are 4 in 75 data values which lie outside the confidence levels, which is in line with expectations.

## 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.

## Samples

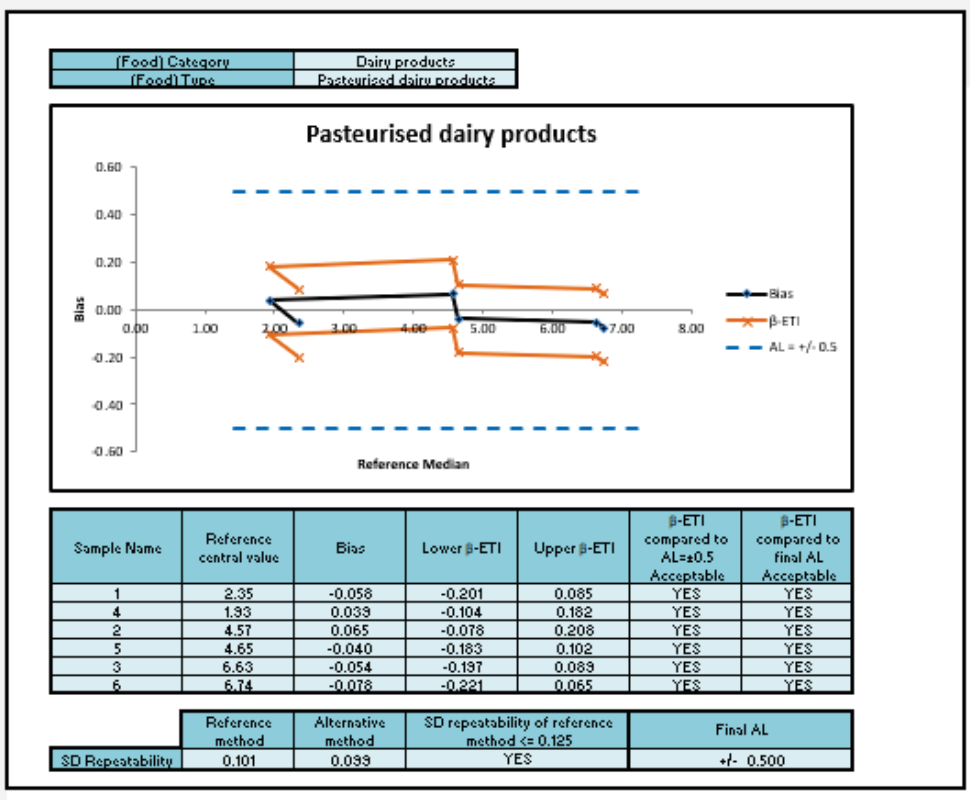
Five categories were tested with a single batch of two different food types using 6 samples per type.

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. The tested categories, types and items in the accuracy profile study are provided in **Table 3**.

**Table 3. Categories, types, items, strains and inoculation levels for Accuracy profile study.**

| Category | Types | Strain | Item | Target Level* cfu/g | Test portions |
|---|---|---|---|---|---|
| Milk and dairy products (combined category raw and heat processed Milk and dairy products) | Pasteurised dairy products | *S.aureus* CRA 1215 Isolated from cheese | Chilled custard | 100-250 | 5 |
| | | | | 25000-55000 | 5 |
| | | | | 3000000 -5500000 | 5 |
| | | | Cream cheese | 70-200 | 5 |
| | | | | 25000 -50000 | 5 |
| | | | | 3500000- 7500000 | 5 |
| Produce and fruits (combined category fresh and processed) | Fresh produce | *S.aureus* CRA1242 Outbreak isolate | Baby spinach | 100-350 | 5 |
| | | | | 10000- 30000 | 5 |
| | | | | 1500000-2500000 | 5 |
| | | | Vegetable juice | 100-200 | 5 |
| | | | | 10000-50000 | 5 |
| | | | | 1000000-2000000 | 5 |
| Meat and poultry products (RTE/RTRH) | RTE meats | *S.aureus* CRA 1217 Isolated from cooked beef | Pastrami | 150-350 | 5 |
| | | | | 15000-40000 | 5 |
| | | | | 2500000-5000000 | 5 |
| | | | Cooked sliced chicken roll | 100-350 | 5 |
| | | | | 10000-45000 | 5 |
| | | | | 2000000-8000000 | 5 |
| Fishery products Combined category: raw, RTE, RTRH, RTC | Cooked fish products e.g. prawns | *S.aureus* CRA 1208 Isolated from smoked fish | Fresh cooked prawns | 1000- 17000 | 5 |
| | | | | 14000-24000 | 5 |
| | | | | 100000-700000 | 5 |
| | | | Smoked salmon | 70-200 | 5 |
| | | | | 7000—45000 | 5 |
| | | | | 600000-1500000 | 5 |
| Multi component foods or meal components | Composite foods with raw /processed ingredients | *S.aureus* CRA 3097 Isolated from pasta | Pasta salad | 350-600 | 5 |
| | | | | 2500-25000 | 5 |
| | | | | 700000-1500000 | 5 |
| | | | Sandwich spread | 400-700 | 5 |
| | | | | 5500-15000 | 5 |
| | | | | 650000-1500000 | 5 |

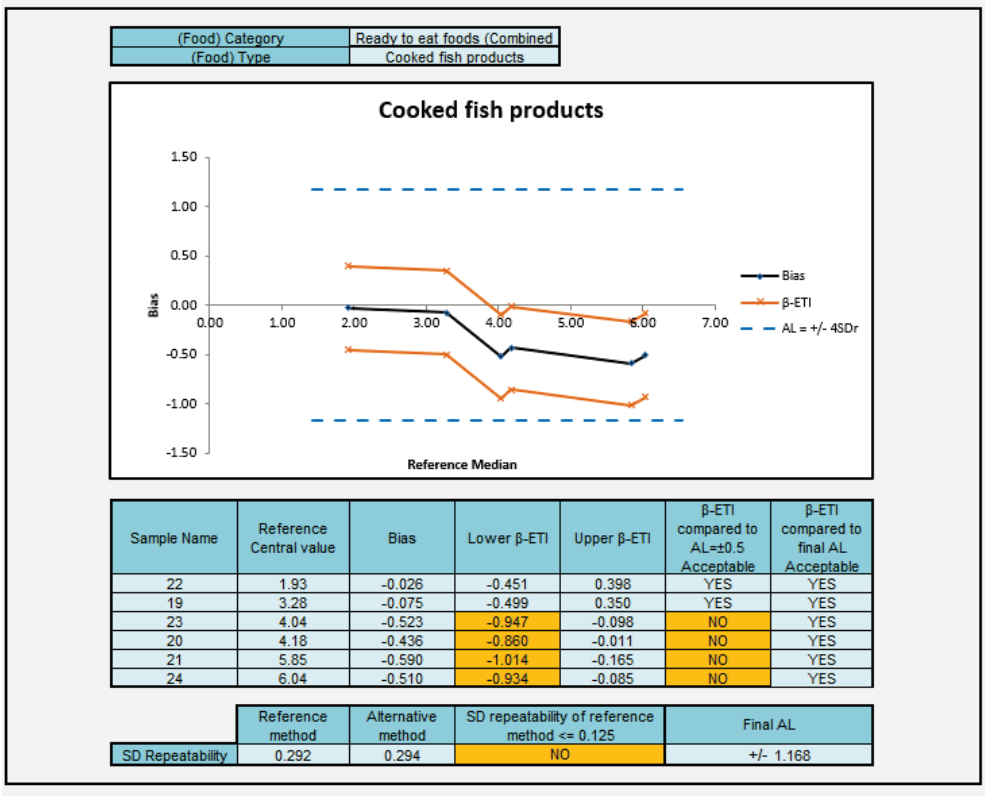
All results were tabulated, calculated and interpreted According to ISO 16140-2. Accuracy profiles were obtained for all categories as shown in **Figures 2 to 6**.




| 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 |
|---|---|---|---|---|---|---|
| 1 | 2.35 | -0.058 | -0.201 | 0.085 | YES | YES |
| 4 | 1.33 | 0.039 | -0.104 | 0.182 | YES | YES |
| 2 | 4.57 | 0.065 | -0.078 | 0.208 | YES | YES |
| 5 | 4.65 | -0.040 | -0.183 | 0.102 | YES | YES |
| 3 | 6.63 | -0.054 | -0.197 | 0.089 | YES | YES |
| 6 | 6.74 | -0.078 | -0.221 | 0.065 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.101 | 0.093 | YES | +/- 0.500 |

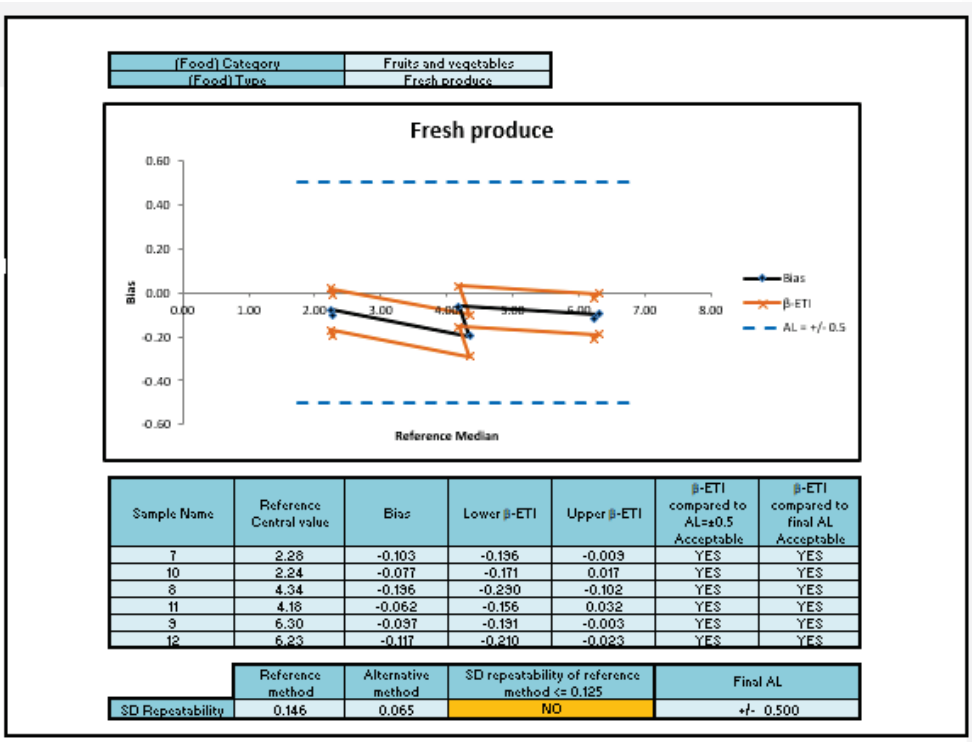
**Figure 2. Accuracy profile of dairy products (Pasteurised dairy products) for Easy Plate SA method**




| 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 |
|---|---|---|---|---|---|---|
| 22 | 1.93 | -0.026 | -0.451 | 0.398 | YES | YES |
| 19 | 3.28 | -0.075 | -0.499 | 0.350 | YES | YES |
| 23 | 4.04 | -0.523 | -0.947 | -0.098 | NO | YES |
| 20 | 4.18 | -0.436 | -0.860 | -0.011 | NO | YES |
| 21 | 5.85 | -0.590 | -1.014 | -0.165 | NO | YES |
| 24 | 6.04 | -0.510 | -0.934 | -0.085 | NO | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.292 | 0.294 | NO | +/- 1.168 |

**Figure 3. Accuracy profile of Fishery products (Cooked fish products) for Easy Plate SA method**

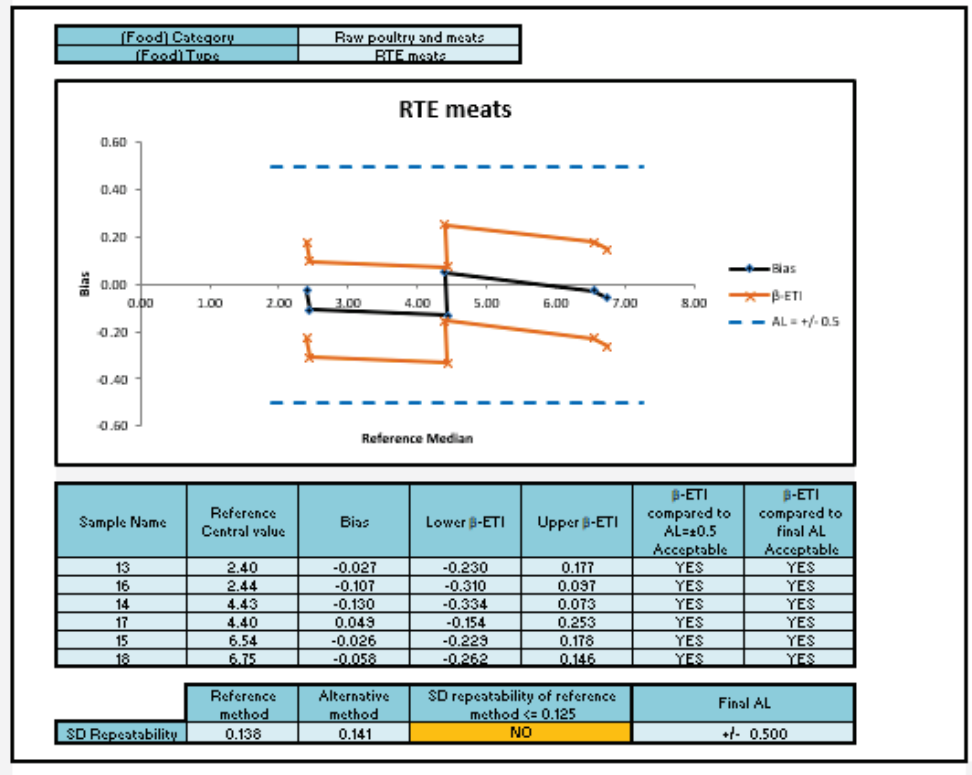



| (Food) Category | Fruits and vegetables |
|---|---|
| (Food) Type | Fresh produce |

| 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 |
|---|---|---|---|---|---|---|
| 7 | 2.28 | -0.103 | -0.136 | -0.069 | YES | YES |
| 10 | 2.24 | -0.077 | -0.171 | 0.017 | YES | YES |
| 8 | 4.34 | -0.136 | -0.230 | -0.102 | YES | YES |
| 11 | 4.18 | -0.062 | -0.156 | 0.032 | YES | YES |
| 9 | 6.30 | -0.037 | -0.131 | -0.003 | YES | YES |
| 12 | 6.23 | -0.117 | -0.210 | -0.023 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.146 | 0.065 | NO | +/- 0.500 |

**Figure 4. Accuracy profile for Fresh produce for Easy Plate SA method**



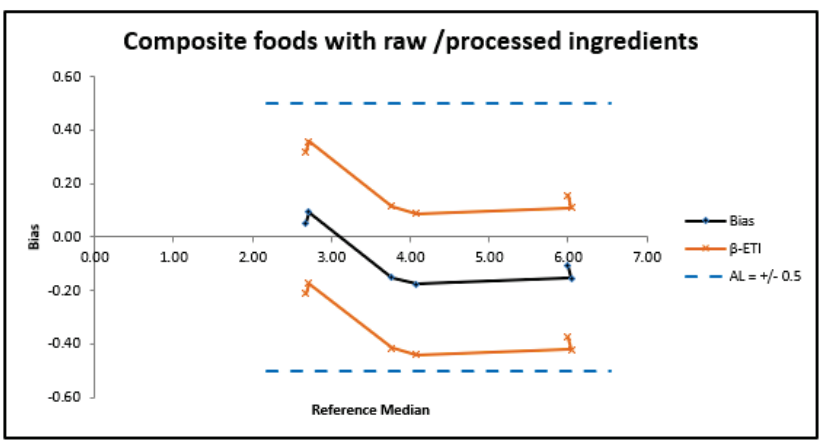

| (Food) Category | Raw poultry and meats |
|---|---|
| (Food) Type | RTE meats |

| 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 |
|---|---|---|---|---|---|---|
| 13 | 2.40 | -0.027 | -0.230 | 0.177 | YES | YES |
| 16 | 2.44 | -0.107 | -0.310 | 0.097 | YES | YES |
| 14 | 4.43 | -0.130 | -0.334 | 0.073 | YES | YES |
| 17 | 4.40 | 0.049 | -0.154 | 0.253 | YES | YES |
| 15 | 6.54 | -0.026 | -0.229 | 0.178 | YES | YES |
| 18 | 6.75 | -0.058 | -0.262 | 0.146 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.138 | 0.141 | NO | +/- 0.500 |

**Figure 5. Accuracy profile for RTE/RTRH Meat and poultry for Easy Plate SA method**

| (Food) Category | Multi component |
|---|---|
| (Food) Type | Composite foods with raw |




| 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 |
|---|---|---|---|---|---|---|
| 25 | 2.68 | 0.054 | -0.212 | 0.320 | YES | YES |
| 28 | 2.71 | 0.093 | -0.172 | 0.359 | YES | YES |
| 26 | 3.76 | -0.151 | -0.416 | 0.115 | YES | YES |
| 29 | 4.08 | -0.176 | -0.442 | 0.090 | YES | YES |
| 27 | 6.04 | -0.155 | -0.421 | 0.111 | YES | YES |
| 30 | 6.00 | -0.108 | -0.374 | 0.158 | YES | YES |

| | Reference method | Alternative method | SD repeatability of reference method <= 0.125 | Final AL |
|---|---|---|---|---|
| SD Repeatability | 0.116 | 0.184 | YES | +/- 0.500 |

**Figure 6. Accuracy profile for Multicomponent (composite foods with raw/processed ingredients) for Easy Plate SA method**

In conclusion, the observed profiles are within the 0.5 log acceptability limit, AL, or the recalculated AL limit calculated according to ISO16140-2. This fulfils the performance criteria, and the alternative method is accepted as being equivalent to the reference method.

## INTERLABORATORY STUDY

The interlaboratory study is a study performed by multiple collaborators testing identical samples at the same time, the results of which are used to estimate alternative method performance characteristics.

Eleven laboratories participated in the study. Smoked salmon inoculated with *Staphylococcus aureus* CRA 1208 (isolated from smoked fish) were used as matrix. For each collaborator, a set of samples was prepared containing 2 samples at a low level, two samples at a medium level, two samples at a high level and a single uninoculated blank sample.

All the samples were delivered on time and in appropriate conditions to 11 laboratories. One laboratory was excluded due to sample storage issues and another laboratory was omitted due to cross contamination of the samples. Thus, valid results from nine laboratories were obtained. The results are summarized in **Table 4** and illustrated in an accuracy profile in **Figure 7**.

**Table 4. Summary of the interlaboratory study**

| | Alternative method | | | Reference method | | |
|---|---|---|---|---|---|---|
| **Level (log cfu/g)** | **Low** | **Medium** | **High** | **Low** | **Medium** | **High** |
| Average | 2.27 | 5.87 | 6.88 | 2.28 | 6.08 | 7.17 |
| Repeatability standard deviation, sr | 0.118 | 0.112 | 0.081 | 0.111 | 0.108 | 0.211 |
| Reproducibility standard deviation, sR | 0.182 | 0.340 | 0.362 | 0.171 | 0.311 | 0.364 |
| Bias | -0.01 | -0.21 | -0.29 | | | |
| Lower AL | -0.97 | -0.97 | -0.97 | | | |
| Upper AL | 0.97 | 0.97 | 0.97 | | | |

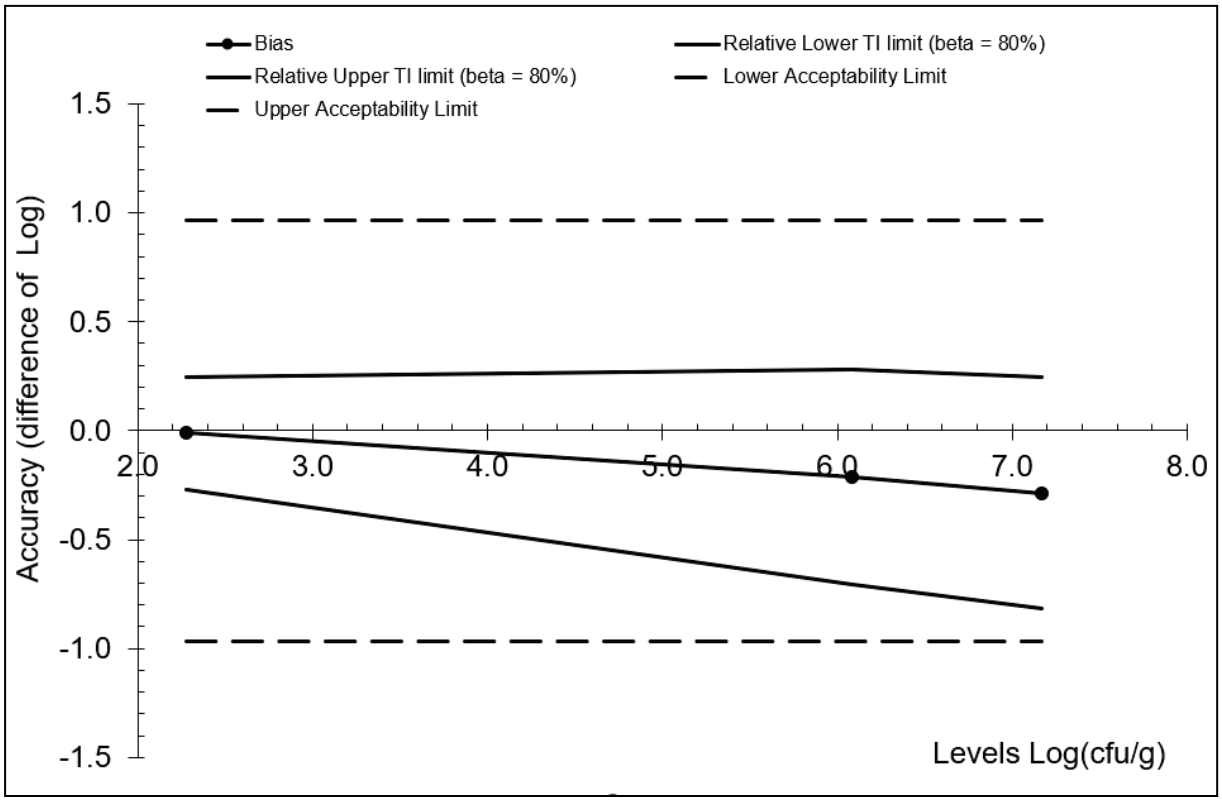



**Figure 7. Accuracy profile for Easy Plate SA.**

In conclusion, the results in the interlaboratory study falls within the acceptability limits, and hence the alternative method show satisfactory performance.

## CONCLUSION

The method comparison study and the interlaboratory study performed according to ISO 16140-2, show that the alternative method Easy Plate SA for enumeration of *Staphylococcus aureus* shows comparable performance to the reference method ISO 6888-1: 2021 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.