

NordVal International Certificate

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| Issued for: | Compact Dry YM RAPID |
| NordVal No: | 050 |
| First approval date: | 15 June 2020 |
| Renewal date: | 08 June 2022 |
| Valid until: | 15 June 2024 |

Compact Dry YM RAPID

Manufactured and supplied by:

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

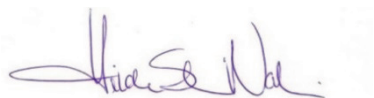
fulfils the requirements of ISO 16140-2:2016 and of the NordVal Validation Protocol No. 1. The reference method was ISO 21527-1:2008: Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds – colony count technique – Part 1: Products with a water activity (a_w) greater than 0.95.

NordVal International has reviewed the method and the validation studies conducted by Campden BRI, UK. The results document that the Compact Dry YM RAPID method provides equivalent results to the reference method.

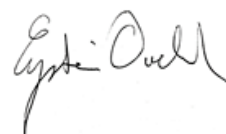
The production of Compact Dry YM RAPID fulfils the requirements given in ISO 9001 and ISO 13485.

Date: 08 June 2022

Yours sincerely,

A handwritten signature in purple ink, appearing to read 'Hilde Skår Norli'.

Hilde Skår Norli
Chair of NordVal International

A handwritten signature in purple ink, appearing to read 'Eystein Oveland'.

Eystein Oveland
NMKL Executive Director

PRINCIPLE OF THE METHOD

Compact Dry are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent. It is rehydrated by inoculating 1 ml of an appropriate diluted sample into the centre of the self-diffusible medium. The Compact Dry YM RAPID method contains chromogenic medium and selective agents for the detection and enumeration of yeasts and moulds. Yeasts form blue colonies and moulds form “cottony colonies” with characteristic colours. The incubation conditions are $25 \pm 1^\circ\text{C}$ for 3 days.

FIELD OF APPLICATION

The method has been tested for enumeration of viable yeasts and moulds in a broad range of foods with an a_w of >0.95 . Like the reference method, this method is not intended for mould spores or for heat resistant mould species.

RESULTS OF THE COMPARISON STUDIES

The validations were carried out at Campden BRI in 2017. The food categories and types tested are listed in Table 1.

Table 1. Categories and types tested

| Category | Type |
|---------------------------|---|
| Confectionary-bakery-eggs | Cheese, e.g. grated cheese, soft cheese, blue cheese |
| | Yogurts with fruit |
| | Fermented milk drinks |
| Dairy | Bakery products with custard |
| | Egg products without additives, e.g. chilled quiches |
| | Par-baked egg products |
| Fruits and vegetables | Fresh fruit salad and fruit purees |
| | Chilled fruit juices |
| | Fermented vegetables e.g. sauerkraut, olives |
| Multi-component foods | Ready-to-eat meat and poultry, e.g. turkey fillet, pate |
| | Cooked and cured fish products, e.g. roll herring, seafood terrine |
| | Cured meats, e.g. salami, ham |
| RTE foods | Composite foods with raw ingredients, e.g. sandwiches, pasta salads |
| | Mayonnaise-based chilled salads |
| | Ambient stable acidified foods, e.g. ketchup |

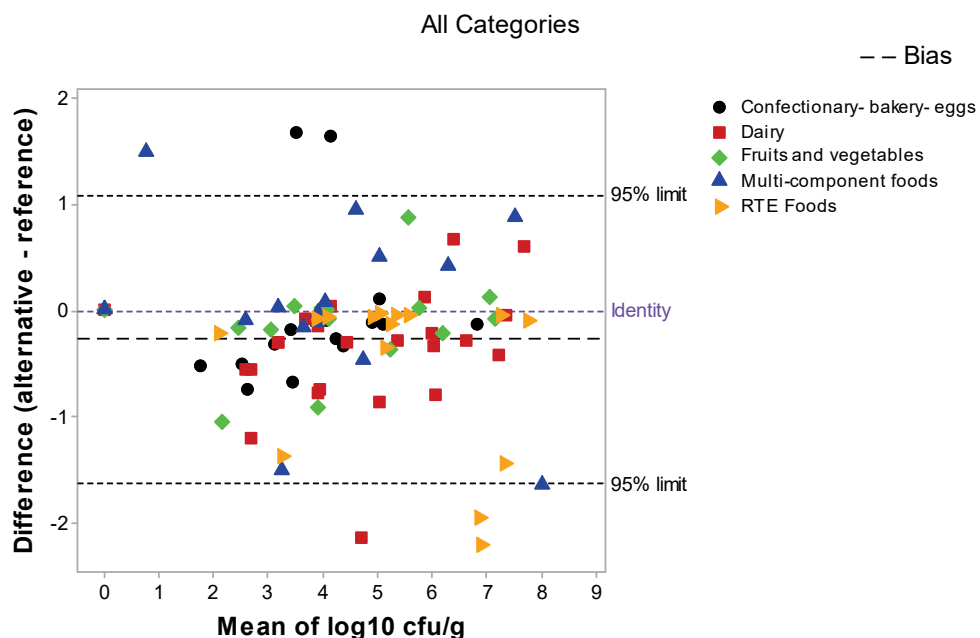
Relative trueness

Five categories were tested, dairy products; confectionary, bakery and eggs; fruits and vegetables; ready-to-eat (RTE) foods and multi-component foods. In total, 85 samples were analysed using both methods, leading to 80 interpretable results. All of the samples tested were naturally contaminated. The water activity of representative food types within each category was measured to ensure $a_w > 0.95$. The average difference D , the standard deviation of difference S_D and the limits of agreement were calculated per category and for all categories (Table 2). The results of the relative trueness study are presented in a Bland-Altman Plot, Figure 1.

Table 2. Summary of calculated differences ($\log_{10}CFU/g$)

| Category | n | D | S_D | 95% Lower limit | 95% Upper limit |
|---------------------------|----|--------|-------|-----------------|-----------------|
| Confectionary-bakery-eggs | 15 | -0.045 | 0.278 | -1.66 | 1.57 |
| Dairy | 23 | -0.037 | 0.578 | -1.62 | 0.83 |
| Fruits and vegetables | 14 | -0.150 | 0.457 | -1.17 | 0.87 |
| Multi-component foods | 13 | -0.102 | 0.777 | -1.86 | 1.66 |
| RTE foods | 15 | -0.549 | 0.780 | -2.28 | 1.18 |
| All categories | 80 | -0.268 | 0.676 | -1.62 | 1.07 |

Figure 1: Bland-Altman plot for all categories after 3 days of incubation



The Bland-Altman plot shows the differences in enumeration obtained by the two methods, the bias. Further, the 95% confidence levels are included. It is expected that not more than one in 20 data values will lie outside the 95% Confidence Limits (CLs).

For 'All categories' there are 7 of 80 data values which lie outside the CLs. This is outside the expectation of 1 in 20.

There were no identifiable trends in these outliers that covered 4 different food categories and showed no particular trends for food type. It is concluded that these samples are individual cases where there is disagreement with no identifiable explanation. These differences are not unexpected as this data is for a total count of naturally present yeast and moulds, which may vary considerably between samples.

Accuracy profiles

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. One type per category is tested.

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.

The statistical results and the accuracy profiles are provided in Figure 2a to e.

Figure 2a: Dairy products with *Saccharomyces cerevisiae*

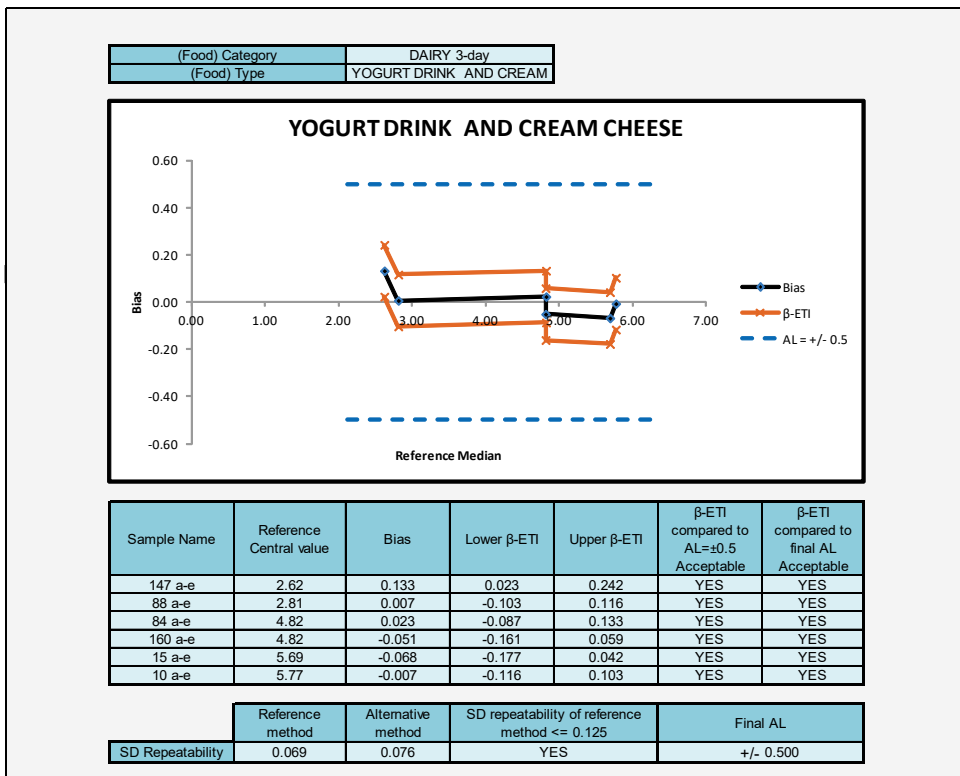


Figure 2b: Fruits and vegetables inoculated with *Debaryomyces hansenii*

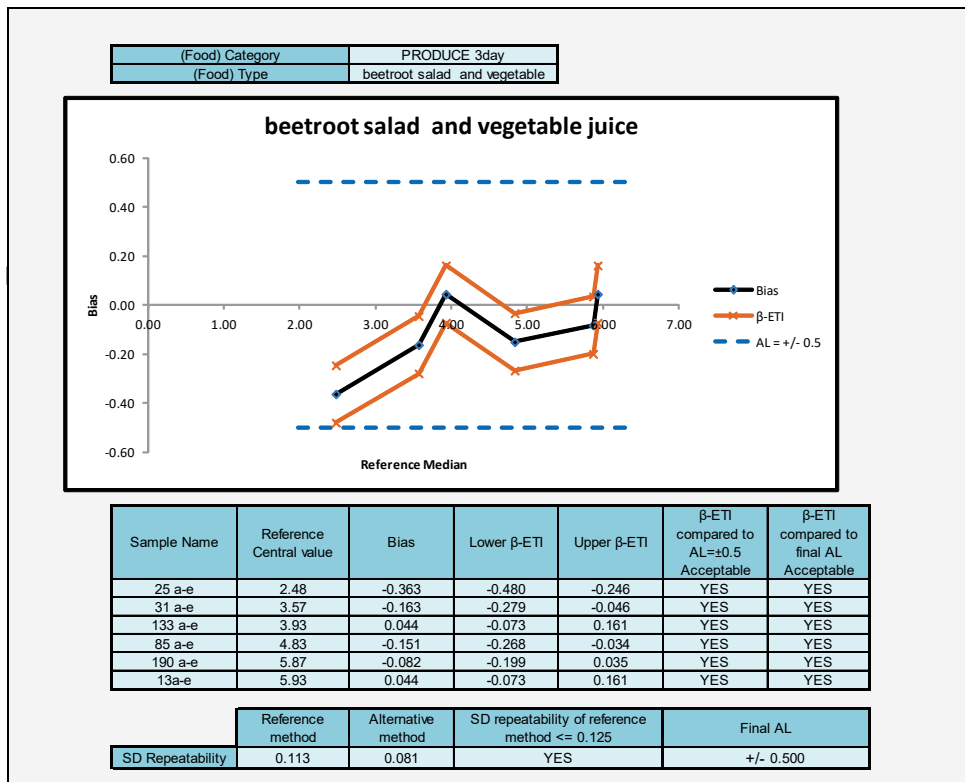


Figure 2c: Bakery products inoculated with *Aspergillus niger*

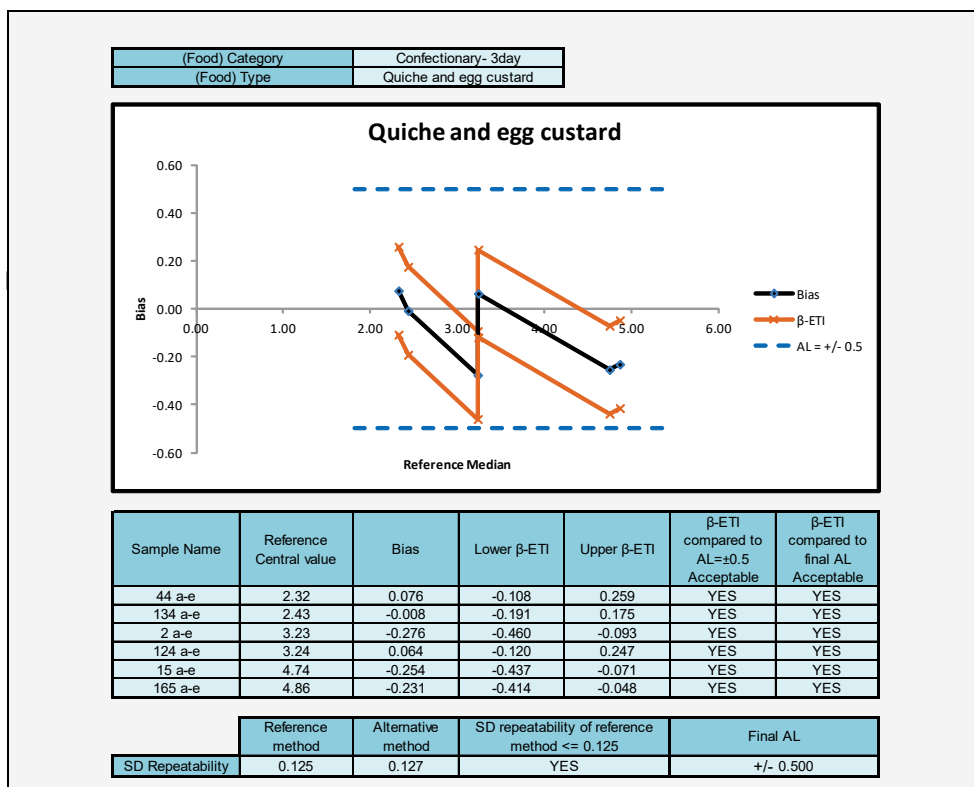


Figure 2d: Multi-component foods inoculated with *Geotrichum candidum*

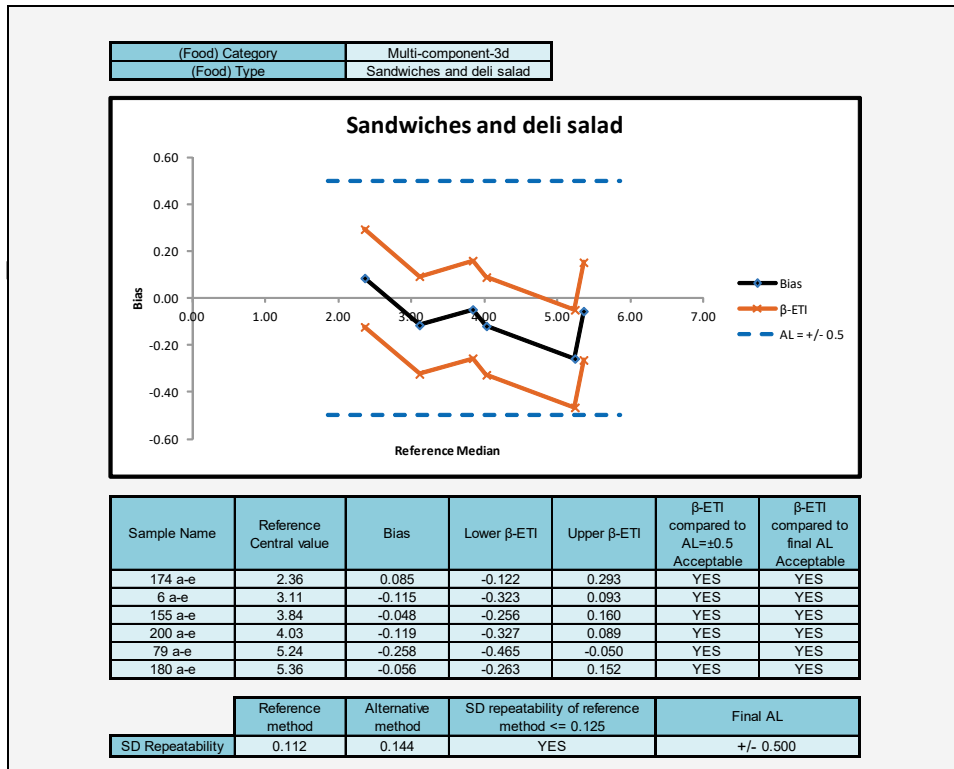
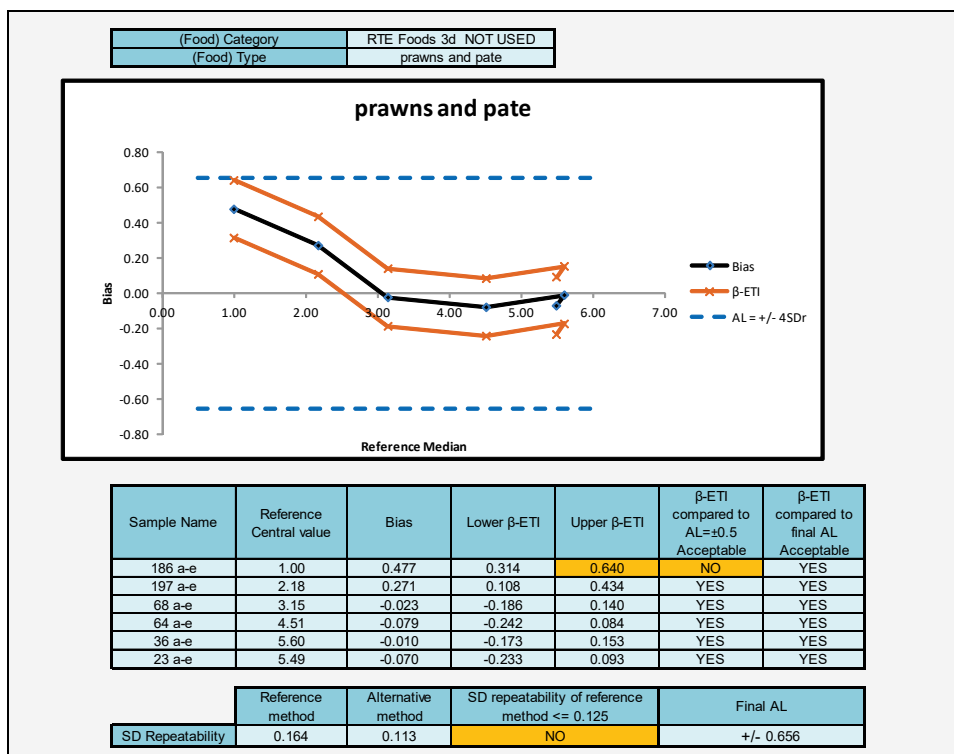


Figure 2e: RTE foods inoculated with *P. chrysogenum*



The comparison of the reference method and alternative method was within the Acceptability Limit (AL) of 0.5 log cfu. For the 5th category, the RTE foods, the AL was exceeded for the lowest level in prawns.

If any of the upper or lower values exceeded the limits for any category and the standard deviation of the reference method was $>0.125 \log \text{ cfu}$, new acceptability limits were calculated as a function of the standard deviation. After re-calculation of the limits the RTE food met the re-calculated limits.

All the accuracy profiles fulfil the performance criteria, and the alternative method is accepted as being equivalent to the reference method using a 3-day incubation period.

RESULTS OF THE COLLABORATIVE STUDY

In this study, there were 7 participating organisations, each with two collaborators, giving a total of 14 data sets. In total 4 different countries were represented by the organisations.

The matrix for this study was chilled salmon pâté. The samples were inoculated with a cocktail of one strain of yeast, *S. cerevisiae* CRA 15968, and one strain of mould, *P. chrysogenum* DSM 848, mixed in equal concentration.

For each of the 14 collaborators 7 x 10 g samples of salmon pâté were weighed into sterile stomach bags. Appropriate dilutions of the yeast and mould cocktail were used to individually inoculate 10g samples at the low ($\sim 10^2 \text{ cfu/ml}$), middle ($\sim 10^4 \text{ cfu/ml}$) and high ($\sim 10^6 \text{ cfu/ml}$) contamination levels. Each laboratory received two samples at each contamination level and one sample of negative control.

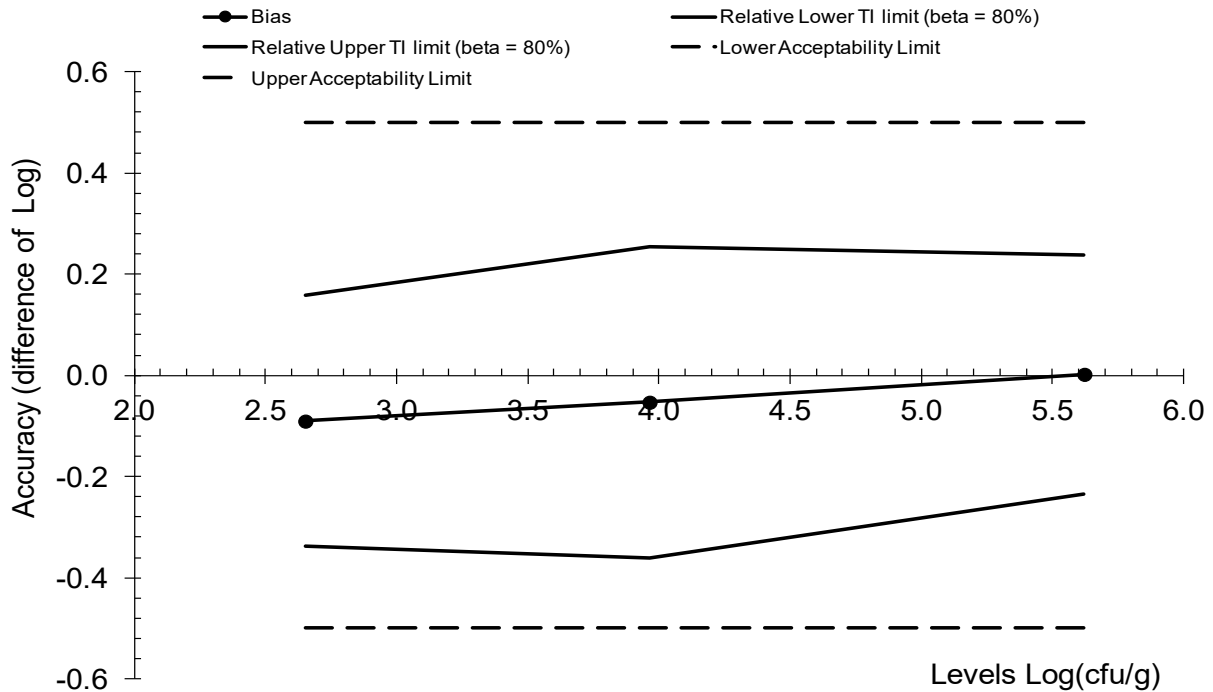
The results are shown in Table 3.

Table 3. Results of the ILS, the levels are given in cfu/g

| Method | Alternative | Reference | Alternative | Reference | Alternative | Reference |
|---------------------------|-------------|-----------|-------------|-----------|-------------|-----------|
| Levels | Low | | Medium | | High | |
| No of participants | 14 | 14 | 14 | 14 | 14 | 14 |
| Mean | 2.56 | 2.65 | 3.91 | 3.96 | 5.62 | 5.62 |
| Repeatability, sr | 0.13 | 0.11 | 0.11 | 0.13 | 0.095 | 0.077 |
| Between Lab, sL | 0.13 | 0.12 | 0.20 | 0.14 | 0.14 | 0.11 |
| Reproducibility, sR | 0.18 | 0.16 | 0.22 | 0.19 | 0.17 | 0.13 |
| Bias (alt- ref) | -0.09 | | -0.05 | | - | |
| Lower tolerance limit | -0.34 | | -0.36 | | -0.23 | |
| Higher tolerance limit | 0.16 | | 0.25 | | 0.24 | |
| Acceptability limit \pm | 0.5 | | 0.5 | | 0.5 | |

The alternative method and the reference method provide equivalent results, the bias is close to zero and the standard deviations for repeatability, between lab and reproducibility, are within the same magnitude. This is illustrated in the accuracy profile given in Figure 3.

Figure 3: Accuracy profile of the alternative method in the inter-laboratory study



The bias is close to zero and all the values of the Tolerance Interval (TI) fall within AL (± 0.5 log units). The results of the ILS are satisfactory.

CONCLUSION

Based on the results of the method comparison study and the inter-laboratory study, NordVal International concludes that the alternative method Compact Dry YM RAPID provides equivalent results to the reference method for the enumeration of yeasts and moulds in a broad range of foods.