NordVal International / NMKL
c/o Danish Technical University
Kemitorvet B201
DK-2800 Kgs Lyngby, Denmark
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NordVal International Certificate

Issued for:	HyServe Compact Dry YM RAPID
NordVal No:	050
Approval date:	15 June 2018
Valid until:	15 June 2020

HyServe Compact Dry YM RAPID

Manufactured by:
Nissui Pharmaceutical Co.Ltd,
3-23-9 Ueno,
Taito-ku, Tokyo, 110-8736
Japan

Supplied by:
HyServe GmbH & Co. KG,
Hechenrainerstr 24,
82449 Uffing,
Germany

fulfils the requirements of ISO 16140-2:2016 and the NordVal validation protocol 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 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 provides equivalent results to the reference method.

Date: 15 June 2018

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
NMKL Secretary General

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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 ± 1°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 aw of >0.95. Like the reference method, it 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

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Relative trueness

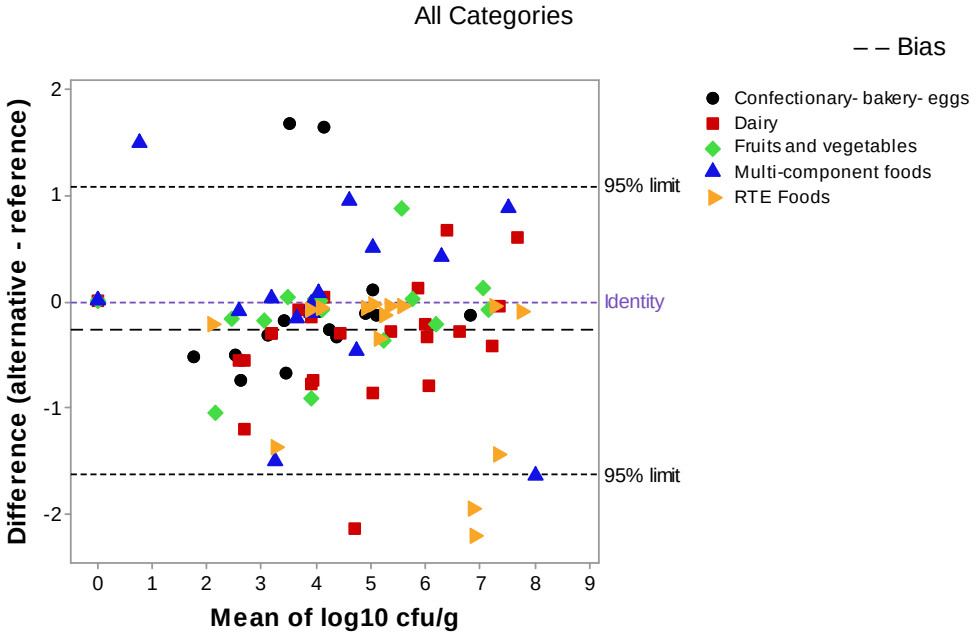
Five categories were tested, dairy products; confectionary, bakery and eggs; fruits and vegetables; ready-to-eat (RTE) foods and multi-component foods. In total, 85 samples were analysed using both method, leading to 80 interpretable results. All of the samples tested were naturally contaminated. The water activity of representative food types within each category were measured to ensure they were aw >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 incubation



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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 slightly outside the expectation of 1 in 20. There were no identifiable trends in these outliers which 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.

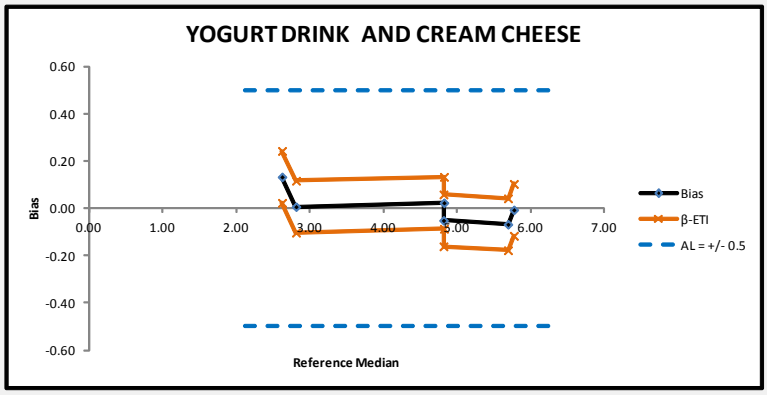
Accuracy profiles

The accuracy profile study is a comparative study between the results obtained by the reference 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 Figure 2a to e.

Figure 2a: Dairy products with S. cerevisiae

(Food) Category	DAIRY 3-day
(Food) Type	YOGURT DRINK AND CREAM



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
147 a-e	2.62	0.133	0.023	0.242	YES	YES
88 a-e	2.81	0.007	-0.103	0.116	YES	YES
84 a-e	4.82	0.023	-0.087	0.133	YES	YES
160 a-e	4.82	-0.051	-0.161	0.059	YES	YES
15 a-e	5.69	-0.068	-0.177	0.042	YES	YES
10 a-e	5.77	-0.007	-0.116	0.103	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.069	0.076	YES	+/- 0.500

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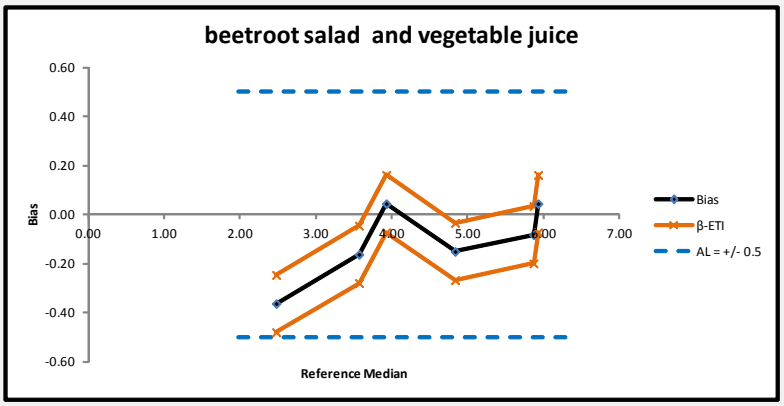
Figure 2b: Fruits and vegetables inoculated with D. hansenii

(Food) Category	PRODUCE 3day
(Food) Type	beetroot salad and vegetable

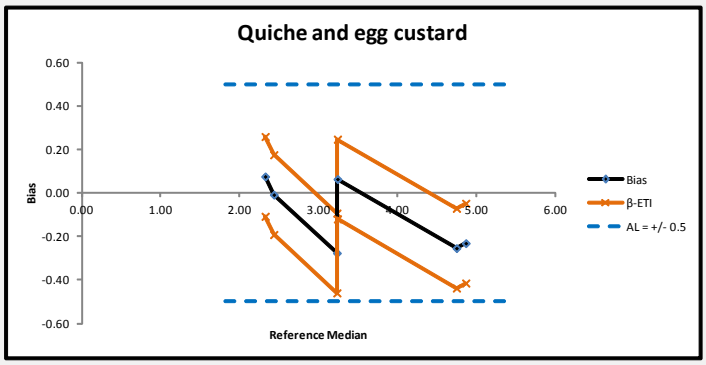


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
25 a-e	2.48	-0.363	-0.480	-0.246	YES	YES
31 a-e	3.57	-0.163	-0.279	-0.046	YES	YES
133 a-e	3.93	0.044	-0.073	0.161	YES	YES
85 a-e	4.83	-0.151	-0.268	-0.034	YES	YES
190 a-e	5.87	-0.082	-0.199	0.035	YES	YES
13a-e	5.93	0.044	-0.073	0.161	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.113	0.081	YES	+/- 0.500

Figure2c: Bakery products inoculated with A. niger

(Food) Category	Confectionary- 3day
(Food) Type	Quiche and egg custard



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
44 a-e	2.32	0.076	-0.108	0.259	YES	YES
134 a-e	2.43	-0.008	-0.191	0.175	YES	YES
2 a-e	3.23	-0.276	-0.460	-0.093	YES	YES
124 a-e	3.24	0.064	-0.120	0.247	YES	YES
15 a-e	4.74	-0.254	-0.437	-0.071	YES	YES
165 a-e	4.86	-0.231	-0.414	-0.048	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.125	0.127	YES	+/- 0.500

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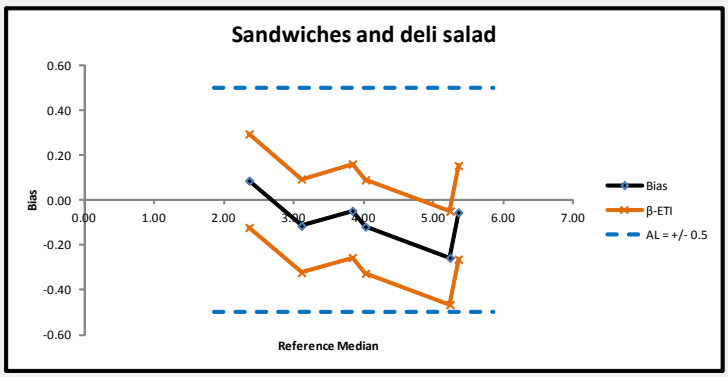
Figure 2d: Multi-component foods inoculated with G. candidum

(Food) Category	Multi-component-3d
(Food) Type	Sandwiches and deli salad

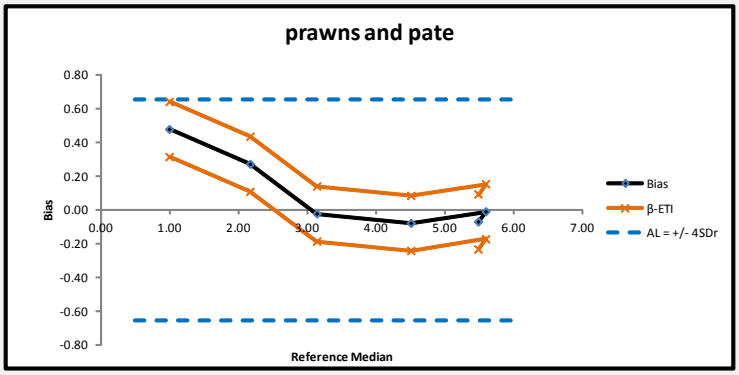


Sample Name	Reference Central value	Bias	Lower β-ETI	Upper β-ETI	β-ETI compared to AL=±0.5 Acceptable	β-ETI compared to final AL Acceptable
174 a-e	2.36	0.085	-0.122	0.293	YES	YES
6 a-e	3.11	-0.115	-0.323	0.093	YES	YES
155 a-e	3.84	-0.048	-0.256	0.160	YES	YES
200 a-e	4.03	-0.119	-0.327	0.089	YES	YES
79 a-e	5.24	-0.258	-0.465	-0.050	YES	YES
180 a-e	5.36	-0.056	-0.263	0.152	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.112	0.144	YES	+/- 0.500

Figure 2e: RTE foods inoculated with P. chrysogeum

(Food) Category	RTE Foods 3d NOT USED
(Food) Type	prawns and pate



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
186 a-e	1.00	0.477	0.314	0.640	NO	YES
197 a-e	2.18	0.271	0.108	0.434	YES	YES
68 a-e	3.15	-0.023	-0.186	0.140	YES	YES
64 a-e	4.51	-0.079	-0.242	0.084	YES	YES
36 a-e	5.60	-0.010	-0.173	0.153	YES	YES
23 a-e	5.49	-0.070	-0.233	0.093	YES	YES

	Reference method	Alternative method	SD repeatability of reference method <= 0.125	Final AL
SD Repeatability	0.164	0.113	NO	+/- 0.656

The comparison of the reference method and alternative method was within the Acceptability limit of 0.5 log cfu. For the 5th category, the RTE foods, the AL was exceeded for the lowest level of 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 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. cerevisae* CRA 15968, and one strain of mould, *P. chrysogenum* DSM 848, mixed in equal concentration.

For each of the 14 collaborators 7 x 10g 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 (~10^2 cfu/ml), middle (~10^4cfu/ml) and high (~10^6cfu/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 ±	0.5		0.5		0.5	

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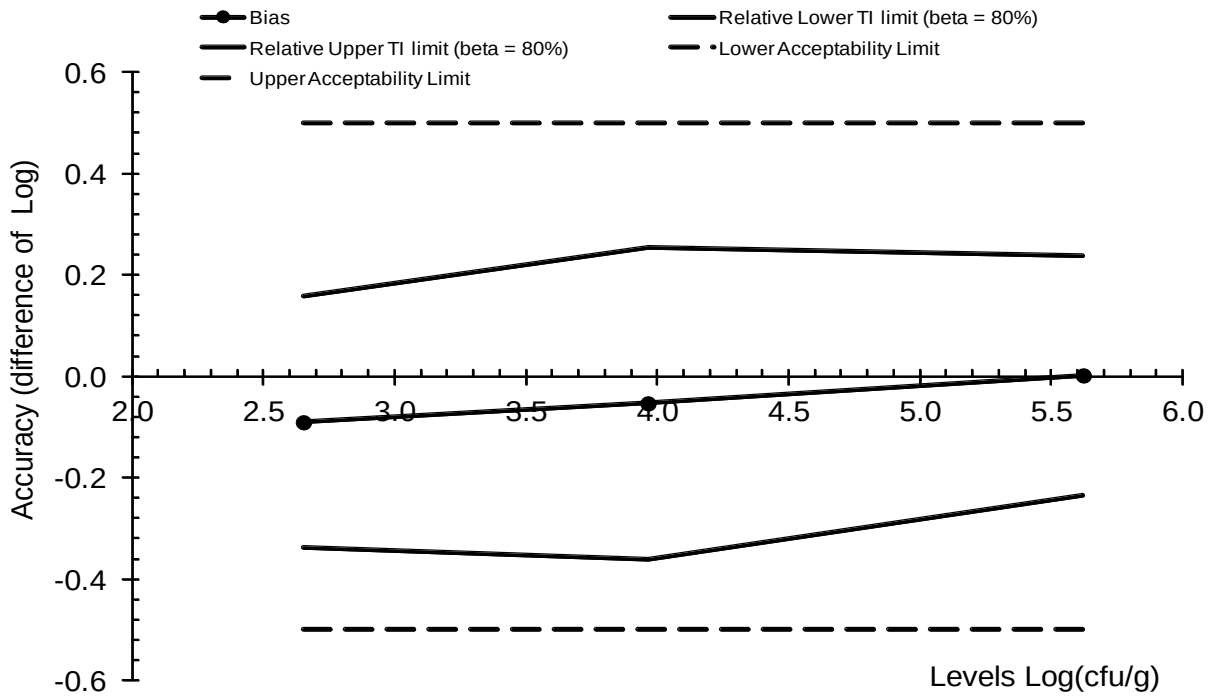
The alternative method and the reference method provide equivalent results, the bias is close to zero and the standard deviations for repeatability, between lab and reproducibility are within the same magnitude. This is illustrated in the accuracy profile given in Figure 3.

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 the Acceptability Limits AL (±0.5log units). The results of the ILS are satisfactory.

CONCLUSION

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