LRQA
Business Assurance

CERTIFICATE OF APPROVAL

MICROVAL

THIS IS TO CERTIFY THAT THE FOLLOWING METHOD

COMPACT DRY YM

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

Supplied by:
HyServe GmbH & Co.
Hechenrainer Str. 24
82449 Uffing
GERMANY

has been approved by Lloyd's Register Quality Assurance Limited in accordance with The MicroVal Rules and Certification Scheme. The validation has been performed in accordance with:

EN ISO 16140:2003

as demonstrated by the report: MICROVAL EN ISO 16140 VALIDATION OF THE HYSERVE COMPACT DRY YM METHOD FOR THE ENUMERATION OF YEASTS AND MOULDS, MB/REP/111835/5

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Expiry date: 25 August 2015

ISSUED BY: Lloyd's Register Nederland B.V.
Rotterdam, The Netherlands

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PRINCIPLE OF THE METHOD

Compact Dry (Nissui Pharmaceutical Co. Ltd., supplied by HyServe Gmbh & Co. KG) 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. Compact Dry YM is a ready-to-use plating system for the enumeration of yeasts and moulds. The Compact Dry YM plate contains chromogenic medium and selective agents for the detection and enumeration of yeasts, which appear as blue colonies and moulds which form "cottony colonies". This method is an alternative to the standard method, enabling determination yeast and mould counts in foods after 3-7 days incubation.

SCOPE

All human food products

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 21527-1:2008. Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and moulds. Part 1: Colony count technique in products with water activity greater than 0.95.

LINEARITY and RELATIVE ACCURACY

Comparison of performances of the alternative method and the reference method.

LINEARITY STUDY

The tests were performed in 2008 and 2009 on five food categories (meat products, fruit and vegetable products, dairy products, bakery products and other products (mayonnaise). All samples were naturally contaminated, with the exception of mayonnaise samples from the other products category which were artificially contaminated.

Five levels of contamination were used for each food matrix, covering a minimum, a central and a maximum level, plus two intermediary levels. The samples were analysed by each of the two methods, with contamination levels ranging from <10 to 10^7 CFU/g.

Table of results: Comparison of Compact Dry (CD) YM with the ISO 21527-1 method

Food category	Food product	Regression line CD YM 3d	Regression line CD YM 7d
Meat products	Cooked turkey	y = -0.857 + 1.123 x	y = -0.749 + 1.11 x
Fruit and vegetable products	Fresh tomatoes	y = -0.511 + 0.979 x	y = -0.459 + 1.01 x
Dairy products	Cheese	y = 0.035 + 0.967 x	y = -0.333 + 1.03 x
Bakery products	Bread	y = -0.056 + 0.912 x	y = -0.152 + 0.980 x
Other products	Mayonnaise	y = -0.660 + 1.098 x	y = -0.637 + 1.10 x

ACCURACY STUDY:

The tests were performed in 2008 and 2009 on samples from five food categories, of which 105 were tested for the presence of naturally occurring yeasts and moulds (naturally contaminated) and 20 mayonnaise samples from the other products category which were artificially contaminated. The contamination levels were:

Food category	Contamination range (in log CFU/g)
Meat products	1 to 6.53
Fruit and vegetable products	1 to 4.98
Dairy products	1 to 7.28
Bakery products	1 to 6.75
Other products	1 to 4.75

The equation of the regression line between the alternative method and the reference method, for all categories combined, is as follows:

	Compact Dry YM 3d	Compact Dry YM 7d
Regression line	y = -0.478 +1.02 x	y = -0.503 + 1.05 x
Correlation Coefficient (r)	0.977	0.987

y = log (N alternative method)
x = log (N reference method)

Repeatability for the two methods and the bias between the two methods were determined according to the calculation method used for the collaborative study (ISO16140 6.3.5 and 6.3.6).

The limits of repeatability (in log) obtained for the alternative method and the reference method are as follows:

Alternative method (3d): $r = 2.8 \times S_r(y) = 2.8 \times 0.304 = 0.851$ where y is alternative method
Alternative method (7d): $r = 2.8 \times S_r(y) = 2.8 \times 0.316 = 0.885$ where y is alternative method
Reference method: $r = 2.8 \times S_r(x) = 2.8 \times 0.333 = 0.932$ where x is reference method

Bias (in logs) between the alternative method and the reference method is as follows
the bias depends on the concentration value ranging from
Alternative method (3d): -0.47 at 0.5 CFU/ml to -0.29 at 8 CFU/ml
Alternative method (7d): -0.48 at 0.5 CFU/ml to -0.10 at 8 CFU/ml

Conclusion: *for the linearity and relative accuracy:*
The results of the methods comparison study showed the Compact Dry YM method to be equivalent to the reference method (ISO 21527-1:2008) for the enumeration of yeasts and moulds in a range of foods.

Selectivity (INCLUSIVITY/EXCLUSIVITY)
Both methods were challenged with 2-3 $\log_{10}$ (100 times limit of detection) CFU/ml of each culture twice as required by EN ISO16140. The inclusivity of the Compact Dry YM method was determined with a total of 30 strains comprising 15 moulds and 15 yeasts. The exclusivity of the Compact Dry YM method was established with 20 strains of non-target organism.

The Compact Dry YM method supported the growth of 23 of the fungal isolates (8 moulds and 15 yeasts) at 3 days and 28 of these isolates (13 moulds and 15 yeasts) at 7 days. In comparison, the DRBCA medium used for the reference method allowed the growth of 24 of the isolates (10 moulds and 14 yeasts) after 2 days and 27 after 5 days (12 moulds and 15 yeasts).
The two mould strains which failed to grow on both Compact Dry YM and DRBCA was a strain of *Monascus bisporus* and a strain of *Chrysosporium farinicola*. This observation confirms the xerophilic nature of these mould species which do not normally grow on highwater activity media. Furthermore, these are also slow growing moulds, typically requiring 2 weeks to develop into visible colonies. Therefore the results obtained can be regarded as correct for these two strains and it confirms that both media are not suitable for the isolation of xerophilic moulds.

None of the 20 non-target organisms grew on Compact Dry YM plates after 3 days, and after 7 days only one strain of *Pseudomonas fluorescens* was able to grow on this medium but the colonies were considered atypical.

PRACTICABILITY (Alternative Method only)

The Compact Dry YM method provides a convenient alternative to the conventional culture method for the enumeration of yeasts and moulds in foods. The ready-to-use format means that there is no prior preparation required except for dilution of the sample and inoculation of plates which stack easily and require less space than conventional Petri dishes. Enumeration of yeasts and moulds is achieved after 3-7 days incubation.

INTERLABORATORY STUDY

The inter-laboratory study was conducted in November 2010 with 9 collaborative laboratories from 6 different countries. Samples of orange juice were inoculated with defined levels of yeast and mould to obtain a lower, middle and upper level. Duplicate samples of each contamination level and duplicate un-inoculated controls were send to the laboratories as blind-coded samples to be tested by both methods.

Results from the analysis for Compact Dry YM at 3 days:

Contamination level	Number of samples	Reference method		Alternative method (3d)		
		Repeatability sd	Reproducibility sd	Repeatability sd	Reproducibility sd	Bias
Lower (10^3)	16	0.0635	0.1146	0.1650	0.1847	-0.0300
Middle (10^4)	16	0.1270	0.1885	0.1016	0.2015	-0.0225
Higher (10^5)	16	0.0762	0.2177	0.0762	0.2912	-0.0850

Results from the analysis for Compact Dry YM at 7 days:

Contamination level	Number of samples	Reference method		Alternative method (7d)		
		Repeatability sd	Reproducibility sd	Repeatability sd	Reproducibility sd	Bias
Lower (10^3)	18	0.0649	0.1153	0.1168	0.1662	-0.0300
Middle (10^4)	18	0.1686	0.2345	0.1168	0.2360	-0.0200
Higher (10^5)	18	0.1038	0.2239	0.1168	0.1746	-0.0550

CONCLUSION

The results from the methods comparison study and inter-laboratory study showed that the Compact Dry YM method is not substantially different from the reference method (ISO 21527-1:2008) for the enumeration of yeasts and moulds in a range of foods.

Please send any queries concerning the performance of the validated method to Lloyd's Register Quality Assurance.