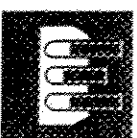




CERTIFICATE OF COMPLIANCE

MICROVAL



HEREBY DECLARES THAT THE CERTIFICATION ASSESSMENT BY
LLOYD'S REGISTER QUALITY ASSURANCE
HAS DEMONSTRATED THAT THE PRODUCT

COMPACT DRY ETB

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

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

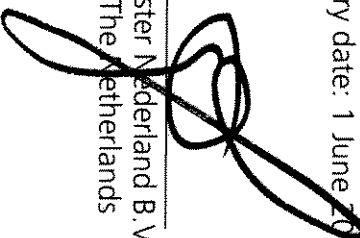
COMPLIES WITH

The MicroVal Rules and Certification Scheme version 5
The validation has been performed in accordance with EN ISO 16140: 2003

As demonstrated by Report number MB/REP/101096/SE0800653

Certificate no.: MV0806-002LR

Validation date: 2 June 2008
Surveillance date: 2 June 2008
Expiry date: 1 June 2012

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



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. The Compact Dry ETB (Total Enterobacteriaceae) method contains glucose and selective agents for the detection and enumeration of members of the Enterobacteriaceae. This method is an alternative method to the standard pour plate method, enabling determination of total Enterobacteriaceae counts in foods after 24-48h incubation.

SCOPE

All human food products

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 21528-2 (2004) Microbiology of food and animal feeding stuffs: Horizontal method for the detection and enumeration of Enterobacteriaceae – colony count method – part 2: colony count method.

LINEARITY and RELATIVE ACCURACY

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

LINEARITY STUDY

The tests were performed in 2007 on five food categories. In total 35 samples were naturally contaminated, 15 contained organisms at levels below the limit of detection of the test (<10 CFU/g), and 75 samples were artificially contaminated. The principle food product categories tested were meat products, poultry products, fish and seafood products, dairy products and fruit and vegetable based products.

The samples were analyzed in duplicate with each of the two methods, at the five naturally contaminated levels within the ranges: 10 to 100, 100 to 1000, 1000 to 10,000, 10,000 to 100,000, 100,000 to 1000,000 and 1000,000 to 10,000,000 and artificially contaminated levels: 100 to 1000, 1000 to 10,000 and 10,000 to 100,000 CFU/g.

Table of results:

Food category	Food product/strain pair	Regression line
Meat products	Raw ground beef	$y = -0.229 + 1.01 x$
	Cooked chicken	$y = -0.087 + 0.985 x$
Poultry products	Frozen fish	$y = -0.628 + 1.105 x$
	Lettuce	$y = 0.127 + 0.983 x$
Fish and seafood products		
Fruit and vegetable based products		
Dairy products	Milk powder	$y = 0.090 + 0.975 x$



ACCURACY STUDY:

The tests were performed in 2007 on five food categories, of which 110 were naturally contaminated, of which 5 contained organisms at levels below the limit of detection of the test (<10 CFU/g), and 15 were artificially contaminated, belonging to the following principle food product categories: meat products, poultry products, fish and seafood products, dairy products and fruit and vegetable based products.

The samples were analyzed in duplicate with each of the two methods, at the five naturally contamination levels within the ranges: 10 to 100, 100 to 1000, 1000 to 10,000, 10,000 to 100,000, 100,000 to 1,000,000 and 1,000,000 to 10,000,000 and artificially contaminated levels: 100 to 1000, 1000 to 10,000 and 10,000 to 100,000 CFU/g.

Food category	Contamination range (in log CFU/g)
Meat products	3.4 to 7.9
Poultry products	LOD (<1) to 6.2
Fish and seafood products	2.9 to 7.0
Fruit and vegetable based products	2.5 to 7.5
Dairy products	LOD (<1) to 5.7

LOD (limit of detection)

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

$$y = -0.157 + 1.01 x$$

$$R^2 = 0.97$$

$$y = \log (\text{N alternative method})$$

$$x = \log (\text{N reference method})$$

Conclusion: *for the linearity and relative accuracy:* The results of the method comparison study clearly showed the Compact Dry ETB method to be equivalent to the reference method ISO 21528-2 (2004) for a range of foods.

SELECTIVITY (INCLUSIVITY/EXCLUSIVITY)

Both methods were challenged with 2-3 log₁₀ (100 times limit of detection) CFU/ml of each culture twice as required by EN ISO 16140. The inclusivity results revealed all 32 strains belonging to the family Enterobacteriaceae produced typical colonies in VRBGA (ISO 21528-2) and also appeared as typical colonies on the Compact Dry ETB plates. The results from the 23 strains of non-target organisms used to determine the exclusivity of the ETB method showed that 21 strains did not grow on the ETB medium and 20 strains did not grow in VRBGA. The two strains that did grow in VRBGA included a strain of *Aeromonas hydrophila* (strain 4111) which appeared typical in this medium, a strain of *Vibrio parahaemolyticus* (strain 15737) which grew but was atypical in appearance on the ETB medium and which produced typical colonies in VRBGA, although growth was poor. One strain of *Pasteurella bettyae* yielded typical colonies by both methods whereas tests with other *Pasteurella* strains, including an additional *P. bettyae* strain showed inhibition of these bacteria by both media. *Pasteurella* spp belong to the family Pasteurellaceae and not the Enterobacteriaceae, and both members of these families are capable of fermenting glucose, and although their optimum growth temperature is 37°C most are fastidious in their growth requirements. However, unlike members of the Enterobacteriaceae *Pasteurella* spp. are oxidase-positive.



PRACTICABILITY (Alternative Method only)

The Compact Dry ETB (total Enterobacteriaceae) method provides a convenient alternative to the conventional culture method for the enumeration members of the Enterobacteriaceae in foods.

The Compact Dry ETB method employs a selective medium containing glucose which enables recognition of presumptive Enterobacteriaceae colonies which appear as coloured colonies, typically red/purple. Plates can be read at 24h and unlike the reference method the manufacturers do not stipulate the need for additional confirmation tests.

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.

Overall the comments on the Compact Dry ETB method from the laboratories participating in the inter-laboratory study were positive. Specific comments received by the Expert Laboratory related to the size and appearance of colonies. One laboratory commented that colonies were not particularly clear or easy to count and another laboratory commented that the small red/purple colonies were hard to count. These observations were not reported by other laboratories and were not noted by the Expert Laboratory. The accuracy of the final results obtained with the alternative method did not appear to be adversely affected.

INTERLABORATORY STUDY

The inter-laboratory study was conducted in November 2007 with 13 laboratories. Samples of pasteurised milk were artificially contaminated with defined numbers of *E. coli* and *E. aerogenes* to provide samples with the following contamination levels; low (10^2 cfu/ml), medium (10^3 CFU/ml) and high (10^4 CFU/ml). Uninoculated samples were used to provide the fourth contamination level (0 CFU/ml). Each laboratory received duplicate blind-coded samples for each contamination level which were tested by both methods.

Obtained results

Contamination level	Number of samples taken into account	Reference method		Alternative method		Bias
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	
Low (10^2)	22	0.113	0.321	0.351	0.550	-0.225
Medium (10^3)	22	0.307	0.365	0.272	0.537	-0.145
High (10^4)	22	0.193	0.474	0.256	0.344	-0.083

The data provided by 2 laboratories was omitted from the statistical analysis because samples were not analysed on the agreed date

CONCLUSION

The results from the method comparison study and inter-laboratory study revealed that there was no substantial differences between the Compact Dry ETB method and the reference method ISO 21528-2 (2004) for the enumeration of Enterobacteriaceae (plate count method).

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