



CERTIFICATE OF COMPLIANCE

MICROVAL



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

COMPACT DRY EC

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/SE00645

Certificate no.: MV0806-004LR

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 EC (total coliforms) method contains chromogenic medium and selective agents for the detection and enumeration of *Escherichia coli* which form blue colonies and other coliform bacteria, which form red colonies. Adding both the blue and red colony counts together gives a total coliform count using this method. This method is an alternative method to the standard pour plate method, enabling determination of total coliform counts in foods after 24-48h incubation.

SCOPE

All human food products

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 4832 (2006) "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms – Colony count technique"

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 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.

Table of results:

Food category	Food product/strain pair	Regression line
Meat products	Raw ground beef	$y = 0.363 + 0.910 x$
Poultry products	Cooked chicken	$y = 0.054 + 1.002 x$
Fish and seafood products	Frozen fish	$y = -0.693 + 1.156 x$
Fruit and vegetable based products	Lettuce	$y = -0.844 + 1.215 x$
Dairy products	Milk powder	$y = 0.177 + 0.922 x$



ACCURACY STUDY:

The tests were performed in 2007 on five food products/strains. 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 contamination 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.

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:

$$y = -0.172 + 1.027 x$$

$$R^2 = 0.959$$

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

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

Conclusion: *for the linearity and relative accuracy:* The results of the method comparison study clearly showed the Compact Dry CF method to be equivalent to the reference method ISO 4832 (2006) 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 33 coliform strains produced typical colonies in VRBA (ISO 4832) and Compact Dry EC medium. The results from the 20 strains of non-target organisms used to determine the exclusivity of the EC method showed that 9 strains did not grow on the EC medium or in VRBA. One strain of *Yersinia enterocolitica* did not grow on the Compact Dry EC medium but did grow in VRBA. Seven strains were atypical with both Compact Dry EC medium and VRBA. The number of exclusivity strains yielding typical colonies by the Compact Dry EC method and VRBA was 4 and 7, respectively.

PRACTICABILITY (Alternative Method only)

The Compact Dry EC (total coliforms) method provides a convenient alternative to the conventional culture method for the enumeration coliform bacteria in foods.

The Compact Dry EC method employs a selective chromogenic medium which gives the added benefit of enabling recognition of presumptive *Escherichia coli* colonies which appear as blue



colonies. Other coliform bacteria appear as red colonies and by adding both the blue and red colony counts together give a total coliform count using this method.

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 from the laboratories participating in the inter-laboratory study were positive. Specific comments received by the Expert Laboratory related to the colour of colonies on the Compact Dry EC medium. One laboratory commented that in contrast to the blue colonies (*E. coli*) the red colonies (other coliforms) were less distinct and not as clear. Another laboratory noted that some the colour of some purple colonies growing on the EC medium was diffuse. Spreading colonies were also noted by two laboratories although this did not affect their ability to obtain counts. These observations were not reported by every laboratory and were not noted by the Expert Laboratory

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	0.202	0.437	0.212	0.375	-0.005
Medium (10^3)	22	0.246	0.293	0.165	0.526	-0.051
High (10^4)	22	0.210	0.773	0.214	0.424	0.116

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 EC method and the reference method ISO 4832 (2006) for the enumeration of coliforms (plate count method) in a range of foods.

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