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/SE00648

Certificate no.: MV0806-005LR

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 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. This method enables the user to obtain a separate *E. coli* count (blue colonies only), as well as a total coliform count (combined red and blue colony counts) using this method. The Compact Dry EC method provides an alternative method to the standard pour plate method using TBX (ISO 16649-2-2:2001) for the enumeration of *E. coli* in foods.

SCOPE

All human food products

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 16649-2-2 (2001) "Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of β-glucuronidase-positive *Escherichia coli* – Part 2: Colony count technique at 44°C using 5-bromo-4-chloro-3-indolyl β-D-glucuronide".

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= 0.116 + 1.036 x
Poultry products	Cooked chicken	y= 0.081 + 1.011 x
Fish and seafood products	Frozen fish	y= -0.047 + 1.018 x
Fruit and vegetable based products	Lettuce	y= 0.071 + 1.053 x
Dairy products	Milk powder	y= 0.029 + 0.966 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:
$y = 0.059 + 0.995\ x$
$R^2 = 0.973$
y = log (N alternative method)
x = log (N reference method)

Conclusion: *for the linearity and relative accuracy:* The results of the method comparison study clearly showed the Compact Dry EC method to be equivalent to the reference method ISO 16649-2-2 (2001) for 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 ISO 16140. The inclusivity results revealed all 31 *E. coli* strains grew and produced typical colonies on the Compact Dry EC medium and the TBX medium (ISO 16649-2:2001), although 5 *E. coli* strains (CRA 1905, 1917 , 1871, 1880 and 1882) yielded weak growth with TBX incubated at 44°C. Subsequent investigations showed that growth of these strains on TBX medium was good when this medium was incubated at 37°C, indicating that these strains were being adversely affected by the elevated incubation temperature. Whether this was just medium or brand related was not investigated. One *E. coli* strain (CRA 1882) grew poorly on TBX medium and β-glucuronidase activity was weak or poorly visible. This was highlighted by colonies that appeared pale blue or green in colour and colonies or which developed as cream-coloured (atypical) colonies. The results from the 21 strains of non-target organisms used to determine the exclusivity of the EC method (Table 12), showed that the majority (18 cultures) failed to grow or produced atypical colonies on the Compact Dry EC medium and in TBX medium. Two strains of *Shigella* did yield typical

colonies by both methods which is not surprising, because strains of *Shigella* have β-glucuronidase activity which would give rise to typical colonies with chromogenic media developed to show this activity.

PRACTICABILITY (Alternative Method only)

The Compact Dry EC method provides a convenient alternative to the conventional culture method for the enumeration *Escherichia coli* 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, but also other coliform bacteria which appear as red colonies.

Unlike the reference method which incorporated a 4h resuscitation step followed by incubation at 44°C, the Compact Dry EC method is incubated for 24h at 37°C only.

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 appearance of the colonies and ease of interpretation. One laboratory commented that *E. coli* colonies on the Compact Dry EC medium were very distinct and easy to count. Another laboratory commented that colonies appeared more magenta rather than blue in colour. A third laboratory noted that in contrast to plates with fewer colonies, the colonies on some plates inoculated with the highest concentration of sample these colonies were less well-defined. These observations were not reported by every laboratory 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 forth 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		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Low (10^2)	18	0.199	0.488	0.182	0.514	-0.042
Medium (10^3)	18	0.293	0.472	0.258	0.486	-0.035
High (10^4)	18	0.332	0.950	0.227	0.565	0.099

The data provided by 4 laboratories was omitted from the statistical analysis. Two laboratories failed to test their samples on the agreed date. One of these laboratories also failed to follow the reference method correctly. A further 2 laboratories failed to test their samples for *E. coli* by the reference method.

CONCLUSION

The results from the method comparison study and inter-laboratory study revealed that there was no substantial differences between the Compact Dry EC *E. coli* method and the reference method ISO 16649-2 (2001) for the enumeration of *E. coli* (plate count method).

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