Lloyd's Register

CERTIFICATE OF COMPLIANCE

MICROVAL

HEREBY DECLARES THAT THE CERTIFICATION ASSESSMENT BY

LLOYD'S REGISTER QUALITY ASSURANCE

HAS DEMONSTRATED THAT

Compact Dry XBC for *Bacillus cereus*

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 7
The validation has been performed in accordance with EN ISO 16140:2003

as demonstrated by the reports of Campden BRI of the Method's Comparison Study and the Inter-Laboratory Study.

Certificate no.: 2011LR41

Validation date: 22 August 2013
Surveillance date: 13 December 2017
Expiry date: 31 December 2018

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

K.P. van der Mandelelaan 41a, 3062 MB Rotterdam, The Netherlands. Kvk nr.: 24247948

Lloyd's Register

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 XBC contains chromogenic medium and selective agents for the detection and enumeration of B. cereus, which according to the manufacturer's instructions appear as light blue/blue colonies after a total of 48h incubation at 30°C.

SCOPE

All human food products

RESTRICTION OF USE

None

REFERENCE METHOD

ISO 7932:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive Bacillus cereus – Colony count technique at 30°C.

RELATIVE ACCURACY, RELATIVE SENSITIVITY and RELATIVE SPECIFICITY

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

The tests were performed from in 2012 on five food categories shown in the Table below. In total 25 samples were naturally contaminated and 100 samples were artificially contaminated with B.cereus using seeding or spiking protocols at 5 contamination levels

Table of results (Cf table 1 standard EN-ISO 16140:2003)

Food category	Food Item	Correlation Coefficient	Regression equation	Range of contamination level tested (log_{10} cfu/g)
Meat products	Cooked meat	0.994	y = - 0.456 + 1.070 x	<0.5 to 6.04
Bakery Products	Cream cake	0.990	y = - 0.382 + 1.024 x	<0.5 to 4.79
Fruit and vegetable based products	Dried potato	0.994	y = 0.116 + 0.854 x	<0.5 to 8.64
Dairy products	Ice cream	0.994	y = - 0.072 + 0.950 x	<0.5 to 5.48
Other products	Pepper corns	0.994	y = - 0.631 + 1.032 x	<0.5 to 5.88
All	All	0.985	y = - 0.013 + 0.915 x	<0.5 to 8.64

Lloyd's Register

Relative accuracy
The tests were performed in 2012 on fifty seven samples of food items covering five food categories. Each food item was tested in duplicate giving a total of 114 test results. All samples were artificially contaminated with B.cereus using seeding or spiking protocols covering a contamination range of log10 cfu/g of <0.5 to 7.08.

Food Category	Food type	Correlation Coefficient	Repeatability limit ISO	Repeatability limit XBC	Bias
Meat and meat products	Cooked Deli meats	0.914	0.45	0.21	-0.34
	Raw chilled/frozen meat				
Fruit and Vegetable based products	Dried potato products	0.965	0.39	0.25	-0.08
	Fresh mushrooms				
Dairy products	Chilled dairy sauces	0.971	0.30	0.34	-0.41
	Dry milk powders				
Bakery products	Donuts	0.963	0.27	0.28	0.52
	Part baked bread				
Other products	Herbs/Spices	0.977	0.20	0.12	-0.63
	Chilled cooked rice				
	Dry cereal/flour				
TOTAL		0.959	0.33	0.26	-0.29

Conclusion

Comparison of the two methods using linear regression showed a correlation coefficient (r) values of between 0.990 and 0.994 for the 5 food categories and 0.985 for all foods. The repeatability limits were similar between the reference method and the XBC method. The data did show a negative bias indicating that on average counts were 0.29logs lower on XBC than the reference method. Overall, the results of the method comparison study showed the Compact Dry XBC method to be equivalent to the reference method for a range of foods.

DETECTION AND QUANTIFICATION LIMITS

The measurement protocol and samples described in the NOTE to Section 6.2.2.3 of ISO 16140 were used for the determination of detection and quantification limits. Actual counts for the target analytical doses of 30, 10 and 3 cfu/ml were 40, 20 and 4 cfu/ml, respectively. Each target dose was measured 10 times using XBC and the Critical level (LC), Detection limit (LOD) and Quantification limit (LOQ): determined

Results
Approximate theoretical ideal limits and associated powers deduced from ISO 16140 Annex P

Critical limit (LC)	0.7 cfu (power = 50%)
Detection Limit (LOD)	3 cfu (power = 95%), 5 cfu (power = 99%)
Determination (Quantification) Limit (LOQ)	100 cfu (coefficient of variation ≤10%)

K.P. van der Mandelelaan 41a, 3062 MB Rotterdam, The Netherlands. Kvk nr.: 24247948

INCLUSIVITY/EXCLUSIVITY

Implementation of alternative method only.

Thirty one strains of B.cereus were tested for inclusivity. Thirty of the 31 strains of B. cereus showed typical light blue/blue colonies on XBC, whilst one strain of B. cereus did not grow on X-BC. All strains gave typical pink colonies with halos on MYP (ISO 7932:2004).
Twenty two strains were tested for exclusivity. Five of the non-target strains grew on both X-BC and MYP, these being B. thuringiensis (3 strains), B. weihenstephanensis and B. pseudomycoides. The colonies were not typical on XBC. They were smaller and a more intensive blue colour on X-BC. They do not match the colony description of light blue/blue. The colonies on MYP showed typical morphology A further 3 strains produced atypical colonies on MYP only. These were L.monocytogenes, S.aureus and Lysinibacillus sphaericus. Colonies were yellow or very pale pink without halos and would not be confused with typical B.cereus colonies.

PRACTICABILITY

The Compact Dry XBC method provides a convenient alternative to the conventional culture method for the enumeration members of *B.cereus* in foods.

The Compact Dry XBC method employs a selective medium containing chromagens which enables easy recognition of colonies which appear as coloured colonies, typically light blue/blue. Plates can be observed at 24h but must be incubated for the full 48h before a final count can be made

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 XBC 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. Three laboratories commented that colonies were not particularly large enough or coloured enough to count at 24h but at 48h were much larger, and darker in colour and easier to count than the reference method which was prone to overgrowth after 48h.

INTERLABORATORY STUDY

The inter-laboratory study was conducted in March 2013 with 9 laboratories in 5 European countries. Samples of pasteurised milk were artificially contaminated with defined numbers of B.cereus to provide samples with the following contamination levels; low (102 cfu/ml), medium (103 CFU/ml) and high (104 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.

Results interlaboratory study:

Contamination level	Number of samples analysed	Reference method		Alternative method		
		Repeatability r	Reproducibility R	Repeatability r	Reproducibility R	Bias
Low (10^2)	18	0.0785	0.2110	0.1115	0.1115	- 0.2311
Medium (10^3)	18	0.1612	0.1612	0.1175	0.1399	- 0.4331
High (10^4)	18	0.2284	0.2717	0.2415	0.2415	- 0.5032

Lloyd's Register

Conclusion

There were no differences in the repeatability or reproducibility of the Compact Dry XBC method and the ISO method for enumeration of B.cereus. There was statistically significant evidence of an underlying bias between the two methods with the Compact Dry giving a lower count than the ISO method. The magnitude of the bias was on average -0.39 and was similar to that previously found in the Methods comparison study.

FINAL CONCLUSION

The results from the method comparison study and inter-laboratory study revealed that there were no substantial differences between the Compact Dry XBC method and the reference method ISO 7932:2004 for the enumeration of B.cereus. Compact Dry X-BC is therefore a suitable method for use with enumeration of presumptive B. cereus in all foods categories.

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

K.P. van der Mandelelaan 41a, 3062 MB Rotterdam, The Netherlands. Kvk nr.: 24247948