NordVal International / NMKL
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NordVal International Certificate

Issued for:	HyServe Compact Dry X-BC Method for the Enumeration of *Bacillus cereus* in Foods
NordVal No:	045
First approval date:	3 September 2015
Valid until:	~~3 September 2017~~ 1 May 2018 (temporarily)

HyServe Compact Dry X-BC

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

Supplied by:
HyServe GmbH & Co. KG,
Hechenrainerstr 24,
82449 Uffing,
Germany

fulfils the requirements of the NordVal Validation Protocol. The reference method was 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.

NordVal International has reviewed the method and the validation studies conducted by CCFRA Technology Limited, Chipping Campden, UK. The studies have been conducted according to ISO 16140:2003. The results document that there is a negative bias with the alternative method, however it is not statistically significant, and hence there are no statistical difference in the performances between Compact Dry X-BC using incubation conditions 30 ± 1°C for 48 ± 2h and the ISO 7932:2004.

This certificate has been temporarily prolonged until 1 May 2018, as results from a complementary study, to comply with ISO 16140-2:2016, are awaited.

Date: 1 September 2015

Yours sincerely,

Sven Qvist
Chair of NordVal International

Hilde Skår Norli
NMKL Secretary General

PRINCIPLE OF THE METHOD

Compact Dry X-BC method contains a ready-to-use dry chromogenic medium, and selective agents for the detection and enumeration of *Bacillus cereus,* which form blue colonies. An aliquot of 1 ml of an appropriate dilution is plated onto Compact Dry X-BC plate. The incubation conditions tested with satisfactory results in the study were 30 ± 1°C for 48 ± 2h. The method was not accepted for incubation at 24h only, due to too high negative bias.

FIELD OF APPLICATION

The method has been tested on the enumeration *Bacillus cereus* in foods.

RESULTS OF THE COMPARISONs STUDIES

Two comparisons studies were carried out in 2012 by CCFRA Technology Limited, Chipping Campden, UK.

Selectivity; inclusivity and exclusivity

Inclusivity: Thirty of the 31 strains of *Bacillus cereus* showed typical blue colonies on Compact Dry X-SA. (One strain of *B. cereus* did not grow on X-BC). The size of the colonies was approximately 1 to 2mm in diameter. Some strains had pale blue colonies and others had darker blue colonies. All strains gave typical pink colonies with haloes on MYP.

Exclusivity: 22 strains were tested. 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 either media. They were smaller and a more intensive blue colour on X-BC and showed typical morphology on MYP. It is possible that laboratories less familiar with the correct colony morphologies would count these organisms as *B. cereus* on either media.

Precision and compliance between Compact Dry X-BC and the reference method

The method was tested in two comparison studies; noted as study 1 and study 2. Five different food categories; meat, fruit /vegetable, dairy, bakery and other products, were studied. All samples were artificially contaminated with the exception of the dried potato in study 1, which was naturally contaminated. The levels of contamination ranged from <5 cfu/g to 10^7 cfu/g.

In study 1, five levels of contamination were used for each food category to cover a minimum, a central, a maximum and two intermediary levels. For each level, 5 parallels were analysed, which gives a total of 125 samples in study 1.

In study 2, three different food types per category were studied. For each sample, two parallels were analysed, which gives a total of 176 samples.

Meat products

Study 1
Five levels and five samples at each level were analysed for meat products. The results are given in Table 1.

Table 1: The mean (in log cfu/g), standard deviation (in log cfu/g) and the bias (in log cfu/g) compared to the reference method obtained for meat samples at different levels

Level	ISO 7932:2004			X-BC 48h			
	N	Mean	SD	N	Mean	SD	Bias
1	5	nd		5	nd		
2	5	3,18	0,021	5	2,88	0,121	-0,31
3	5	3,98	0,046	5	3,93	0,042	-0,05
4	5	5,07	0,054	5	4,96	0,031	-0,11
5	5	5,96	0,053	5	5,89	0,034	-0,07

The results obtained by the alternative method are equivalent to the results obtained by the reference method as the results of the alternative method fall within the confidence levels of the results obtained by the reference method (Figure 1A). The lowest quantified level with satisfactory precision is about 3 log cfu/g.
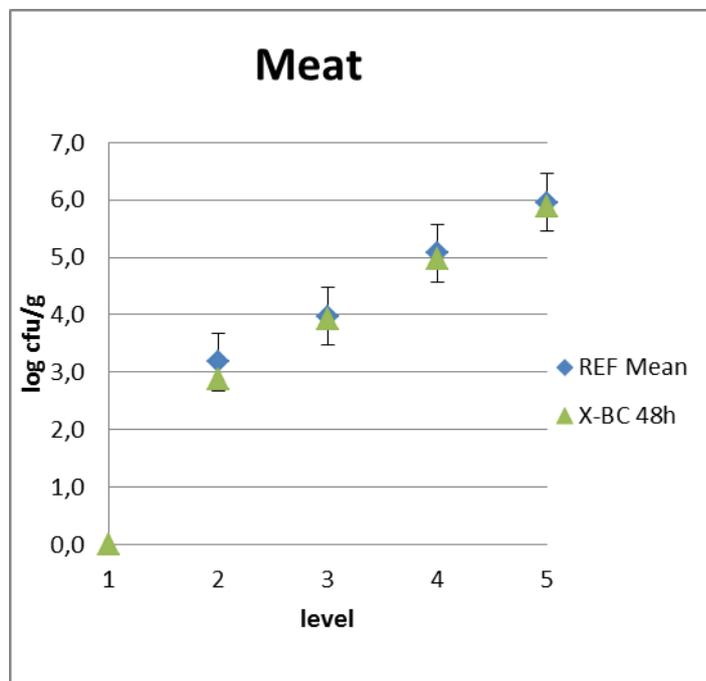

Figure 1 A   Results in meat in study 1

<u>Study 2</u>

12 samples of meat products, including liver sausage, sliced beef, sliced chicken, sliced turkey, honey roast ham, liver pork, frozen minced lamb, frozen minced beef, frozen beef steak were analysed in duplicates. The levels ranged from 2-7 log cfu/g, and the average of the bias was -0.22 log cfu/g. For levels at 3.4 log cfu/g and higher, the results of the alternative method and the reference method are equivalent as the results obtained by the alternative method fall within the confidence levels of the results obtained using the reference method. The results are shown in figure 1B.
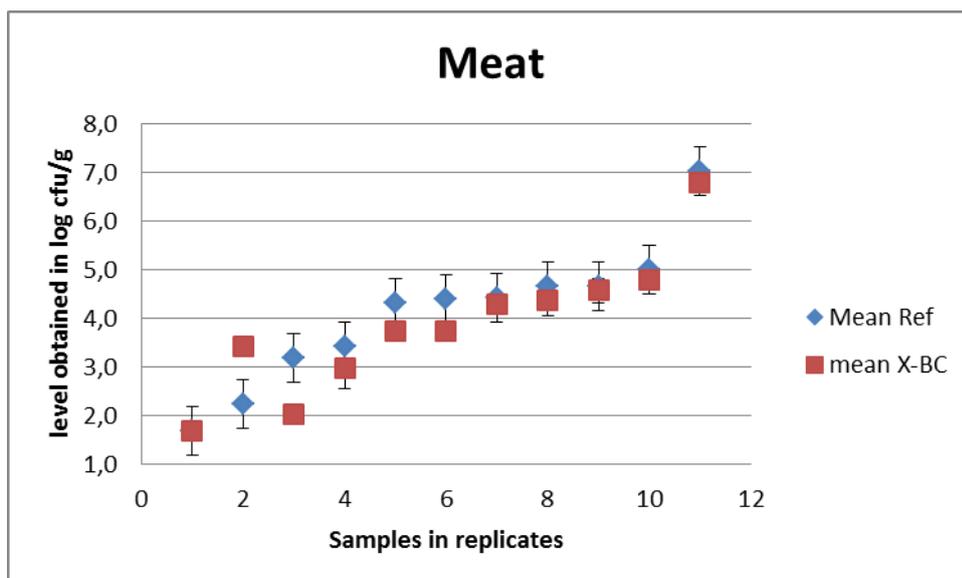
Figure 1B   Results in meat in study 2

Fruit and vegetables

Study 1

Five levels and five samples at each level were analysed for dried potato and peppercorns, respectively. The results are given in Table 2.

Table 2: The mean (in log cfu/g), standard deviation (in log cfu/g) and the bias (in log cfu/g) compared to the reference method obtained for fruit and vegetable samples at different levels

Food	Level	N	Mean	SD	N	Mean	SD	Bias
		ISO 7932:2004			X-BC 48h			
Dried potato	1	5	1,07	0,234	2			
	2	5	4,43	0,078	5	3,72	0,018	-0,70
	3	5	5,43	0,097	5	4,81	0,193	-0,62
	4	5	6,47	0,134	5	5,70	0,056	-0,77
	5	5	8,41	0,161	5	7,30	0,071	-1,11
Pepper-corns	1	5	Nd		5	Nd		
	2	5	3,09	0,112	5	2,51	0,112	-0,58
	3	5	3,96	0,065	5	3,54	0,065	-0,42
	4	5	5,01	0,085	5	4,54	0,085	-0,47
	5	5	5,76	0,093	5	5,29	0,093	-0,47

The results shown in figure 2A and 2B indicate that there is a negative bias for both products (an average of -0.6 log cfu/g), especially for dried potato. This is not satisfactory. The levels are high, and it has been reported that it is much easier to count the colonies on the X-BC plates than on the plates used in the reference method. On X-BC, the colonies appear as discrete colonies.
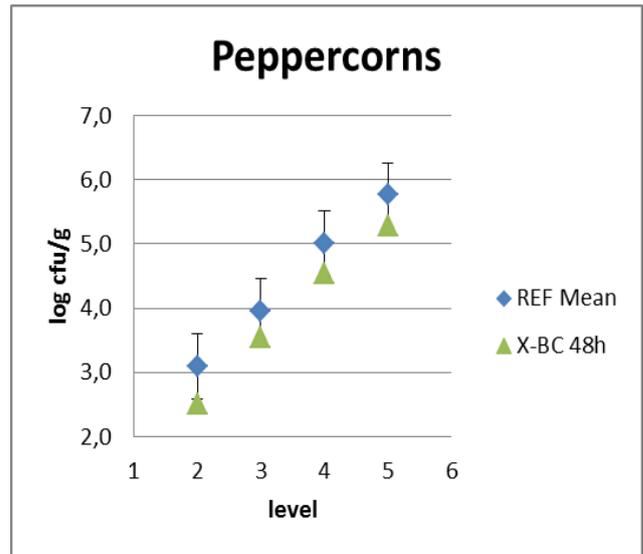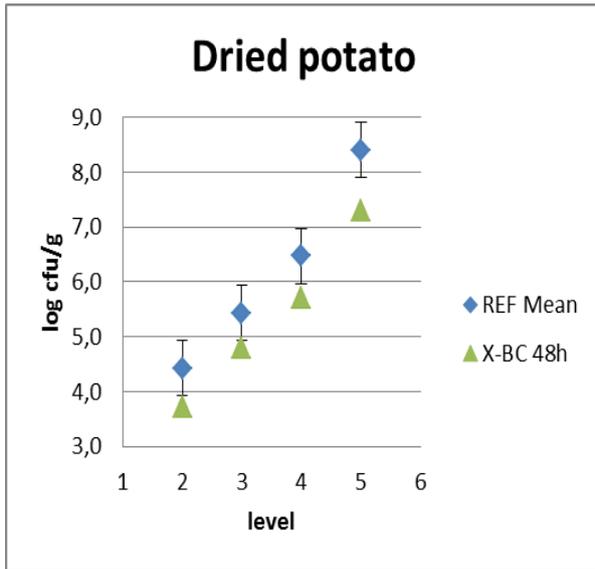
Figure 2A Results in dried potato in study 1   *Figure 2B Results in peppercorns in study 1*

Study 2

13 samples of Fruit and Vegetables, including pink oyster, smash buttery potato, smash potato, oak lane instant mash, tesco value mash, tesco instant mash, button mushrooms, yellow oyster, shimejo were analysed in duplicate. The mean bias was only -0.16 log cfu/g, which is satisfactory. The results are shown in figure 2C.
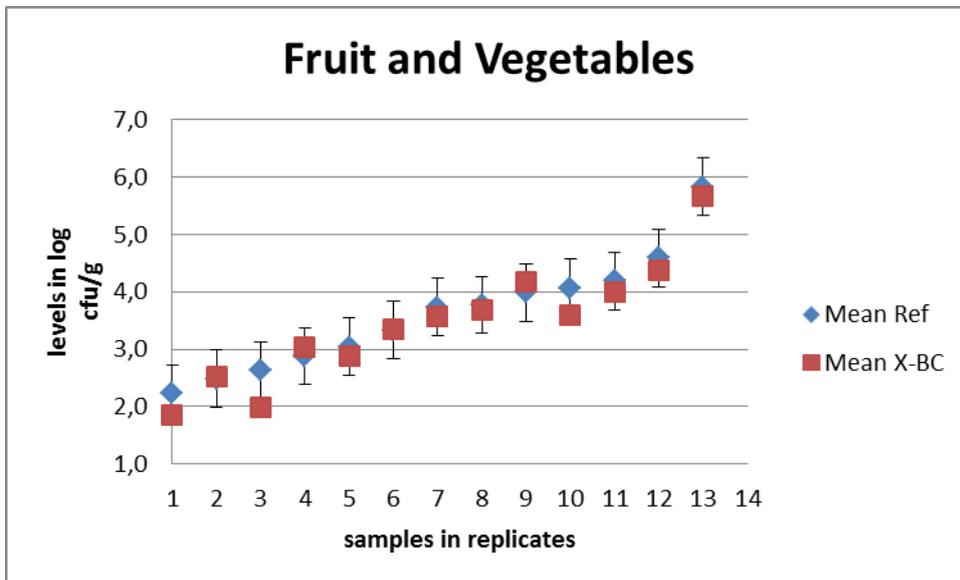

Figure 2C Results in fruits and vegetables in study 2

Based on study 2, it can be concluded that the method is also applicable to fruit and vegetables. The lowest validated level with satisfactory precision is about 2 log cfu/g.

Bakery products

Study 1
Five levels and five samples at each level were analysed for cream cake. The results are given in Table 3.

Table 3: The mean (in log cfu/g), standard deviation (in log cfu/g) and the bias (in log cfu/g) compared to the reference method obtained for bakery samples at different levels

Food	Level	ISO 7932:2004			X-BC 48h			
		N	Mean	SD	N	Mean	SD	Bias
Cream cake	1	5	nd		5	nd		
	2	5	1,72	0,268	5	1,47	0,232	-0,25
	3	5	2,84	0,091	5	2,40	0,061	-0,44
	4	5	3,73	0,053	5	3,44	0,098	-0,29
	5	5	4,70	0,064	5	4,50	0,022	0,20

The results obtained by the alternative method are equivalent to the results obtained by the reference method as the results fall within the confidence levels of the results obtained by the reference method (Figure 3A). The lowest quantified level with satisfactory precision is about 1.5 log cfu/g.
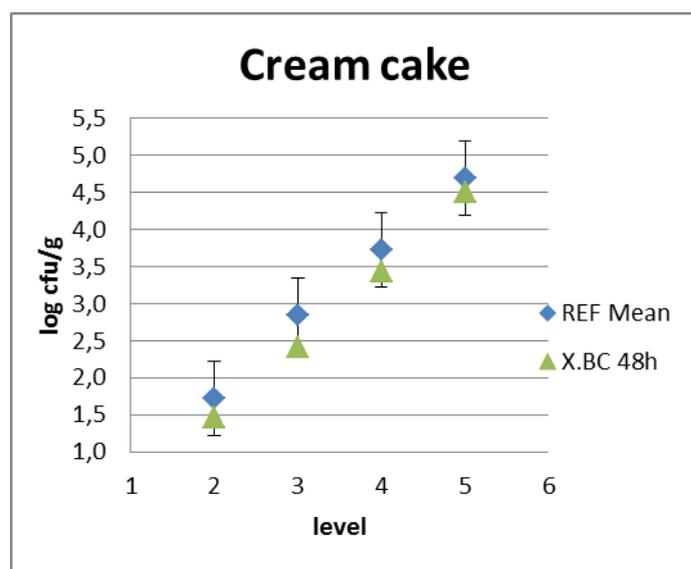

Figure 3 A   Results in cream cake in study 1

Study 2

11 samples of bakery products, including part baked baguette, garlic baguette, part baked ciabatta, brown seed rolls, brown seed baguettes, custard donuts, jam donuts, brown seed baguettes were analysed in duplicates. The mean of the bias was -0.31 log cfu/g.
All results are satisfactory, except for one sample, sample 7. This is illustrated in figure 3 B.
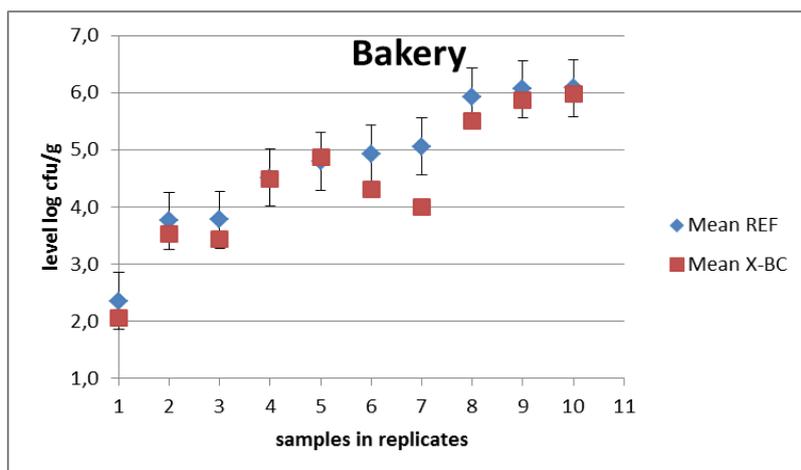
Figure 3 B   Results in bakery products in study 2

The results in study 1 and 2 show that the method is satisfactory for levels of 1.5 log cfu/g and higher for bakery products.

Dairy products

Study 1
Five levels and five samples at each level were analysed for ice cream. The results are given in Table 4.

Table 4: The mean (in log cfu/g), standard deviation (in log cfu/g) and the bias (in log cfu/g) compared to the reference method obtained for ice cream at different levels

		ISO 7932:2004			X-BC 48h			
Food	Level	N	Mean	SD	N	Mean	SD	Bias
Ice-cream	1	5	nd		5	nd		
	2	5	2,73	0,049	5	2,49	0,047	-0,23
	3	5	3,73	0,048	5	3,47	0,048	-0,26
	4	5	4,60	0,075	5	4,43	0,075	-0,18
	5	5	5,37	0,082	5	4,94	0,082	-0,43

The results obtained by the alternative method are equivalent to the results obtained by the reference method as the results obtained by the alternative method fall within the confidence levels of the results obtained using the reference method (Figure 4A). The lowest quantified level with satisfactory precision is about 2.5 log cfu/g.
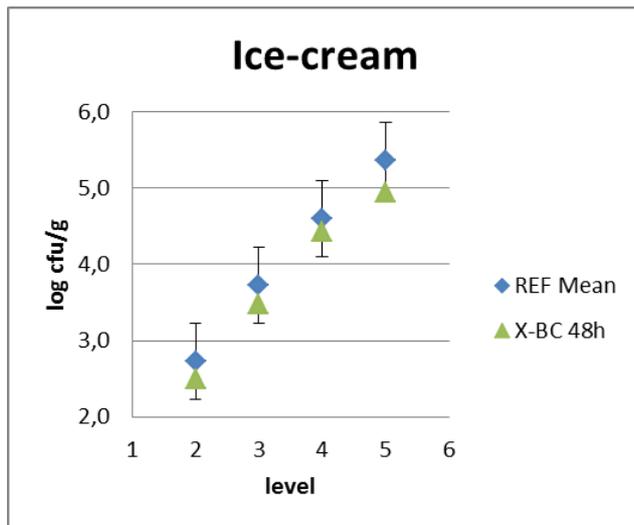
Figure 4 A   Results in ice cream in study 1

13 samples of dairy products, including cheese sauce, value skimmed milk, skimmed milk, marvel skimmed milk, coffee mate, instant skimmed, green pesto sauce, carbonara sauce, creamy basil sauce, mushroom sauce in duplicates were analysed. The mean bias was -0.27 log cfu/g. The results are shown in Figure 4B. All the samples except one obtained satisfactory results.
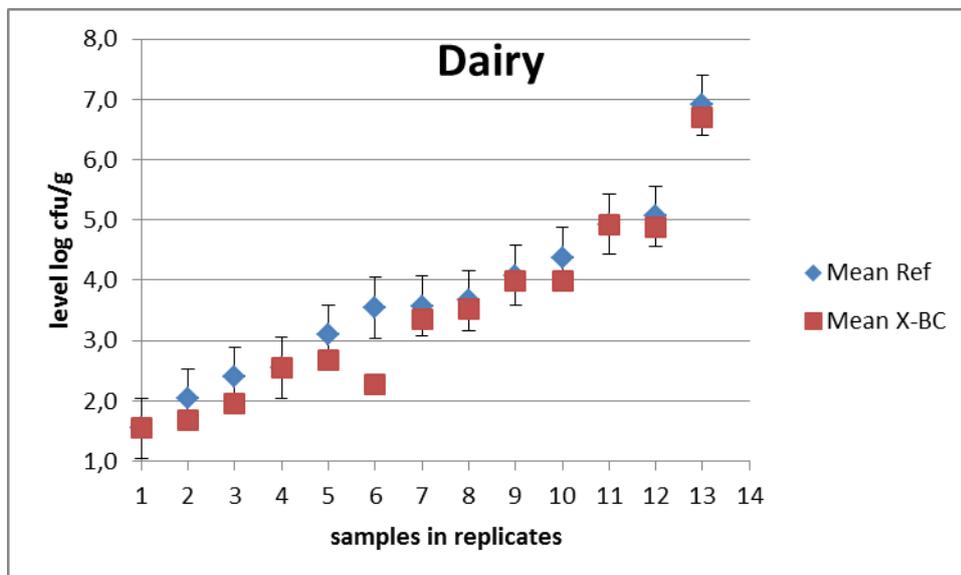

Figure 4 B Results in dairy products in study 2

The lowest validated level with satisfactory precision is about 1.5 log cfu/g.

Other products

Study 2

In addition 12 samples of other products, including pilau rice, special fried rice, egg fried rice, takeaway pilau, teco egg fried, lemon rice, morrocon cous cous, tomato/garlic cous cpis, batter mix, dried mix herbs, flour were analysed in duplicates. The average of the bias was -0.3 log cfu/g. The results are illustrated in table 5B. 10 of the 12 samples obtained
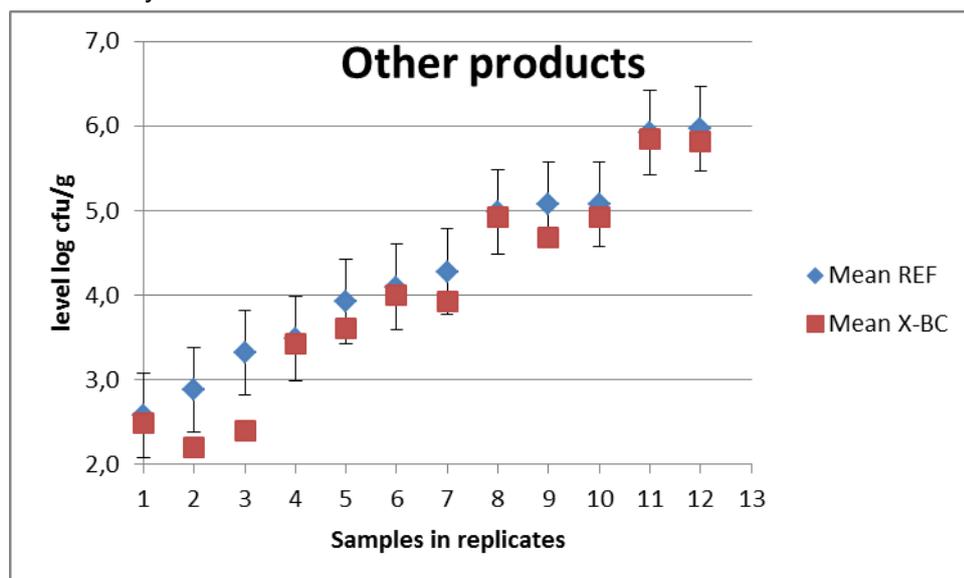
satisfactory results.

Figure 5B Results in other products in study 2

Conclusions of the comparison study

A total of 301 samples (125 in study 1 and 176 in study 2, respectively) have been analysed with both the reference and the alternative method. The alternative method has an advantage in forming more discrete colonies than the reference method, and hence is easier to read. However, there seems to be a negative bias compared against the reference method. With the exception of a few samples of different category and the results obtained from fruit and vegetables in study 1, the bias is not statistically significant. For all the other samples, the results obtained by the alternative method fall within the corresponding confidence levels of the reference method.

RESULTS OF THE COLLABORATIVE STUDY

Nine laboratories participated. They analysed three levels of a dairy product with both the reference and the alternative method. The results are given in table 6 and illustrated in figure 6.

Table 6. The median (in log cfu/g), standard deviation (in log cfu/g) and the bias (in log cfu/g) compared to the reference method

	ISO 7932:2004				X-BC 48h			
Level	median	Sr	SR	2sR	median	Sr	SR	Bias
Low	2.56	0.079	0.211	0.422	2.29	0.112	0.112	-0.27
medium	3.70	0.161	0.161	0.322	3.31	0.118	0.140	-0.39
high	5.05	0.228	0.272	0.543	4.52	0.242	0.242	-0.53

The results show that the precision is satisfactory for both the alternative and the reference method. The results obtained using the alternative methods fall within the confidence level of the results obtained using the reference method. For medium level, that is not completely true as the standard deviation of the reproducibility, SR, is so small. However, the confidence levels of the two methods are overlapping. It can be concluded that there is no significant difference in the results obtained with the alternative and the reference method.
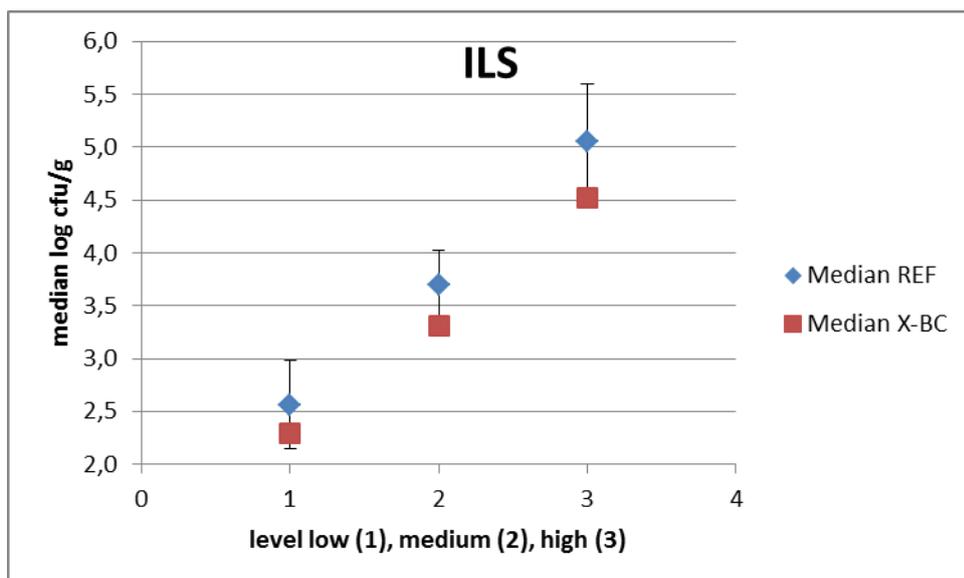
Figure 5 The results of the collaborative study/interlaboratory study (ILS)

CONCLUSION:

According to the comparison and the collaborative studies, the Compact Dry X-BC method and the reference method (ISO 7932:2004) provide equivalent results for the enumeration of *Bacillus cereus*. The levels tested with satisfactory precision are 1.5 log cfu/g and higher depending on the matrix.