



CERTIFICATION

AOAC Research Institute *Performance Tested Methods*SM

Certificate No.
092201

The AOAC Research Institute hereby certifies the method known as:

CompactDry "Nissui" BC

manufactured by

NISSUI Pharmaceutical Co., Ltd.

3-24-6, Ueno

Taito-ku, Tokyo

Japan 110-8736

This method has been evaluated in the AOAC Research Institute *Performance Tested Methods*SM Program and found to perform as stated in the applicability of the method. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*SM certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

Scott Coates, Senior Director
Signature for AOAC Research Institute

Issue Date

January 10, 2023

Expiration Date

December 31, 2023

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SUBMITTING COMPANY

NISSUI Pharmaceutical Co., Ltd.
3-24-6, Ueno
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Japan 110-8736

METHOD NAME

CompactDry "Nissui" BC

CATALOG NUMBERS

06533 (40 plates); 06534 (240 plates)

INDEPENDENT LABORATORY

Campden BRI
Chipping Campden
Gloucestershire, United Kingdom

AOAC EXPERTS AND PEER REVIEWERS

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APPLICABILITY OF METHODTarget Organism – *Bacillus cereus*.

Matrixes – (10 g sample portions) - Panna cotta (with raspberries), double cream (50% fat), dried baby food (cereal-based with strawberry and raspberry flakes), dried vegetable soup mix, surimi seafood sticks, salmon pâté, sliced ham, pork liver pâté, sandwiches (ham and cheese on malted brown bread), and pasta salad (with chicken, bacon, and Caesar dressing).

Performance claims – Performance is equivalent to that of 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 (2)* for the matrixes tested.

REFERENCE METHOD

Anonymous (2004) ISO 7932:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive *Bacillus cereus* — Colony-count technique at 30 degrees C (2)

ORIGINAL CERTIFICATION DATE

September 16, 2022

CERTIFICATION RENEWAL RECORD

Renewed annually through December 2023.

METHOD MODIFICATION RECORD

NONE

SUMMARY OF MODIFICATION

NONE

Under this AOAC *Performance Tested Methods*SM License Number, 092201 this method is distributed by:

NONE

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NONE

PRINCIPLE OF THE METHOD (1)

CompactDry "Nissui" BC are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent. The film is rehydrated by inoculating 1 mL of sample into the center of the self-diffusible medium. The CompactDry "Nissui" BC culture medium contains nutrients, mannitol, selective agents, chromogenic enzyme substrate and gelling agent, for the detection and enumeration of *Bacillus cereus* after incubation at 30 ± 1°C for 24 ± 2 h. Colonies appear blue/pale blue and must be confirmed according to ISO 7932:2004 (2).

DISCUSSION OF THE VALIDATION STUDY (1)

The results of these studies indicate that the CompactDry "Nissui" BC method can be used for rapid and accurate enumeration of *Bacillus cereus* in a variety of food commodities, including panna cotta, double cream, dried baby food, dried vegetable soup mix, seafood sticks, salmon pâté, sliced ham, pork liver pâté, sandwich, and pasta salad. The CompactDry "Nissui" BC method shows similar repeatability to the ISO 7932:2004 reference method and equivalent mean results. The inclusivity and exclusivity results showed selectivity of the CompactDry "Nissui" BC with 47 of 50 inclusivity strains positive and 28 of 33 exclusivity strains negative. These results were similar on the reference method agar. Similar to the ISO reference method, it may also enumerate other members of the wider *B. cereus* group such as *Bacillus thuringiensis*, *Bacillus mycoides*, and *Bacillus weihenstephanensis*. The CompactDry "Nissui" BC method eliminates unstable and variable steps over the reference method. There is a reduction in the amount of technical labor required in preparation of agar and there is no need for confirmation procedures. There are additional advantages in reduction of storage space, waste disposal and required incubator space.

Table 1. Inclusivity testing on CompactDry "Nissui" BC and MYP Agar (1)

No.	Species	Source ^a	Origin	CD BC	MYP
1	<i>Bacillus cereus</i>	CRA 84	Meatloaf	+ ^b	+
2	<i>Bacillus cereus</i>	CRA 193	Environmental	+	+
3	<i>Bacillus cereus</i>	CRA 1549	Dried milk	+	+
4	<i>Bacillus cereus</i>	CRA 1731	Chocolate ice cream	+	+
5	<i>Bacillus cereus</i>	CRA 1740	Cream cake	+	+
6	<i>Bacillus cereus</i>	CRA 1741	Flour	+	+
7	<i>Bacillus cereus</i>	CRA 1749	Cream cake	+	+
8	<i>Bacillus cereus</i>	CRA 1964	Milk/cream	+	+
9	<i>Bacillus cereus</i>	CRA 4110	Contaminated flask	+	+
10	<i>Bacillus cereus</i>	CRA 6295	Flavoring	+	+
11	<i>Bacillus cereus</i>	CRA6452	Flour	+	+
12	<i>Bacillus cereus</i>	CRA7616	Dairy	+	+
13	<i>Bacillus cereus</i>	CRA 8711	Infant formula	+	+
14	<i>Bacillus cereus</i>	CRA 16100	Flavor	+	+
15	<i>Bacillus cereus</i>	CRA 16101	Flavor	+	+
16	<i>Bacillus cereus</i>	CRA 16381	Environmental	+	+
17	<i>Bacillus cereus</i>	CRA 16438	Environmental	+	+
18	<i>Bacillus cereus</i>	CRA 16563	Unknown	+	+
19	<i>Bacillus cereus</i>	CRA 16564	Food poisoning	+	+
20	<i>Bacillus cereus</i>	CRA 16565	Pharmaceutical	+	+
21	<i>Bacillus cereus</i>	CRA 16566	Unknown	+	+
22	<i>Bacillus cereus</i>	CRA 16569	Meatloaf	+	+
23	<i>Bacillus cereus</i>	CRA 16570	Food poisoning	+	+
24	<i>Bacillus cereus</i>	CRA 16571	Unknown	+	+
25	<i>Bacillus cereus</i>	CRA 16579	Industrial	+	+
26	<i>Bacillus cereus</i>	CRA 16580	Industrial	+	+
27	<i>Bacillus cereus</i>	CRA 16582	Environmental	+	+
28	<i>Bacillus cereus</i>	CRA 16583	Industrial	+	+
29	<i>Bacillus cereus</i>	CRA 16662	Dried potato	+	+
30	<i>Bacillus cereus</i>	CRA 17010	Mangoes	+	+
31	<i>Bacillus cereus</i>	CRA17011	Water	+	+
32	<i>Bacillus cereus</i>	CRA 17012	Milk	+	+
33	<i>Bacillus cereus</i>	CRA 17013	Soil	+	+
34	<i>Bacillus cytotoxicus</i>	DSM 22905	Vegetable puree	- ^c	- ^d
35	<i>Bacillus mycoides</i>	CRA 16597	UHT custard	-	-
36	<i>Bacillus mycoides</i>	CRA 1522	Dried milk	+	+
37	<i>Bacillus mycoides</i>	CRA 16646	Soft drink factory	+	+
38	<i>Bacillus mycoides</i>	CRA 1510	Dried milk	+	+
39	<i>Bacillus mycoides</i>	CRA 8504	Food environment	+	+
40	<i>Bacillus pseudomycoides</i>	CRA 16382	Soil	-	+
41	<i>Bacillus thuringiensis kurstaki</i>	CRA 17032	Insecticide	+	+
42	<i>Bacillus thuringiensis aizawai</i>	CRA 17033	Insecticide	+	+
43	<i>Bacillus thuringiensis israelensis</i>	CRA 17034	Insecticide	+	+
44	<i>Bacillus thuringiensis</i>	CRA 16616	Broccoli	+	+
45	<i>Bacillus thuringiensis</i>	CRA 16314	Flour moth	+	+
46	<i>Bacillus thuringiensis</i>	CRA 1744	Flour	+	+
47	<i>Bacillus thuringiensis</i>	CRA 16619	Broccoli	+	+
48	<i>Bacillus weihenstephanensis</i>	CRA 16578	Pasteurized milk	+	+
49	<i>Bacillus weihenstephanensis</i>	DSM 104135	Soil	+	+
50	<i>Bacillus weihenstephanensis</i>	DSM 104109	Soil	+	+

^aCRA = Campden Culture Collection (Campden BRI, Chipping Campden, UK); DSM = DSMZ German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany)

^b"+" indicates growth occurred with typical morphology and confirmation on SBA

^cUnless otherwise noted, "-" indicates growth did not occur

^dThis strain showed typical colonies on MYP, but did not confirm on SBA, so was deemed confirmed negative

Table 2. Exclusivity testing on CompactDry "Nissui" BC and MYP agar with confirmation on SBA (1)

No.	Species	Source ^a	Origin	CD BC ^b	MYP ^b
1	<i>Allicyclobacillus acidoterrestris</i>	CRA 5331	Apple juice	-	-
2	<i>Alicyclobacillus cycloheptanicus</i>	CRA 16823	Soil	-	-
3	<i>Alicyclobacillus fastidiosus</i>	CRA 16831	Apple juice	-	-
4	<i>Alicyclobacillus pomorum</i>	CRA 16830	Fruit juice	-	-
5	<i>Aneurinibacillus aneurinolyticus</i>	CRA 7751	Flavor	-	-
6	<i>Anoxybacillus flavithermus</i>	CRA 17047	Food isolate	-	-
7	<i>Bacillus amyloliquefaciens</i>	CRA 6317	Crumpets	-	- ^c
8	<i>Bacillus circulans</i>	CRA 16584	Cream	-	-
9	<i>Bacillus coagulans</i>	CRA 10205	Evaporated milk	+	+
10	<i>Bacillus fusiformis</i>	CRA 16652	Soft drinks	-	-
11	<i>Bacillus laterosporus</i>	CRA 1523	Dried milk	+	+
12	<i>Bacillus licheniformis</i>	CRA 6335	Pesto	-	-
13	<i>Bacillus megaterium</i>	CRA 16512	Soil	-	-
14	<i>Bacillus oceanisediminis</i>	CRA 17220	Food isolate	-	-
15	<i>Bacillus pumilus</i>	CRA 16594	Industrial isolate	-	-
16	<i>Bacillus psychrodurans</i>	CRA 16694	Soil	-	-
17	<i>Bacillus smithii</i>	CRA 7240	Pineapple	-	-
18	<i>Bacillus sonorensis</i>	CRA 17231	Food isolate	-	-
19	<i>Bacillus sphaericus</i>	CRA 7950	Flavoring	-	-
20	<i>Bacillus subtilis</i>	CRA 14161	Milk shake	-	- ^c
21	<i>Brevibacillus brevis</i>	CRA 7748	Flavor	+	+
22	<i>Brevibacillus parabrevis</i>	CRA 7757	Flavor	-	-
23	<i>Leuconostoc mesenteroides</i>	CRA 16022	Soft ham	-	-
24	<i>Listeria ivanovii</i>	CRA 1123	Soft cheese	-	-
25	<i>Lysinibacillus sphaericus</i>	CRA 7746	Unknown	-	- ^c
26	<i>Paenibacillus amylolyticus</i>	CRA 16606	Barley	-	-
27	<i>Paenibacillus macerans</i>	CRA 16488/DSM 357	Unknown	-	-
28	<i>Paenibacillus pabuli</i>	CRA 16605	Barley	-	-
29	<i>Paenibacillus polymyxa</i>	CRA 7747	Food isolate	+	+
30	<i>Staphylococcus aureus</i>	CRA 1224	Margarine	-	-
31	<i>Bacillus coagulans</i>	CRA 17185	Industrial isolate	-	-
32	<i>Bacillus laterosporus</i>	CRA 1515	Dried milk	+	+
33	<i>Paenibacillus polymyxa</i>	CRA 16386/ATCC 43865	Unknown	-	-

^aCRA = Campden Culture Collection (Campden BRI, Chipping Campden, UK); DSM = DSMZ German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany); ATCC = American Type Culture Collection (Manassas, VA, USA)

^bUnless otherwise noted, "-" indicates growth did not occur; "+" indicates growth occurred with typical morphology and confirmation on SBA.

^cStrain showed typical growth on MYP, but the colonies did not confirm on SBA, so were deemed confirmed negative.

Table 7. Method comparison data summary and statistics (1)

Matrix	Contamination level	n ^a	CompactDry "Nissui" BC		ISO 7932:2004		DOM ^d	95 % CI ^e		90 % CI	
			Mean Log ₁₀ CFU ^b /g	s _r ^c	Mean Log ₁₀ CFU/g	s _r		LCL ^f	UCL ^g	LCL	UCL
Panna cotta	Low	5	2.41	0.202	2.61	0.296	-0.19	-0.44	0.06	-0.38	0.00
	Medium	5	3.82	0.112	3.83	0.230	0.00	-0.40	0.39	-0.31	0.30
	High	5	5.50	0.402	5.38	0.089	0.12	-0.30	0.53	-0.20	0.44
Double cream	Low	5	3.00	0.275	3.11	0.203	-0.11	-0.32	0.11	-0.27	0.06
	Medium	5	4.30	0.120	4.51	0.287	-0.21	-0.57	0.15	-0.48	0.06
	High	5	5.76	0.097	5.82	0.133	-0.06	-0.26	0.14	-0.21	0.09
Dried baby food	Low	5	4.58	0.091	4.74	0.154	-0.16	-0.35	0.03	-0.31	-0.02
	Medium	5	5.68	0.147	5.76	0.178	-0.08	-0.25	0.10	-0.21	0.06
	High	5	5.45	0.172	5.44	0.046	0.01	-0.18	0.21	-0.14	0.16
Dried vegetable soup mix	Low	5	4.55	0.108	4.42	0.372	0.14	-0.26	0.54	-0.17	0.44
	Medium	5	5.63	0.093	5.66	0.181	-0.02	-0.31	-0.27	-0.24	0.20
	High	5	6.94	0.154	6.94	0.248	-0.01	-0.36	0.35	-0.28	0.27
Seafood sticks	Low	5	1.19	0.262	1.28	0.272	-0.09	-0.50	0.32	-0.40	0.23
	Medium	5	3.88	0.130	3.92	0.119	-0.04	-0.16	0.08	-0.13	0.05
	High	5	5.27	0.120	5.45	0.093	-0.18	-0.25	-0.10	-0.24	-0.12
Salmon pâté	Low	5	2.57	0.104	2.59	0.087	-0.02	-0.19	0.15	-0.15	0.11
	Medium	5	3.50	0.305	3.42	0.307	0.08	-0.25	0.42	-0.18	0.34
	High	5	5.34	0.318	5.49	0.340	-0.15	-0.26	-0.04	-0.24	-0.06
Sliced ham	Low	5	1.45	0.131	1.51	0.150	-0.07	-0.37	0.24	-0.30	0.17
	Medium	5	3.61	0.139	3.65	0.090	-0.04	-0.15	0.06	-0.12	0.04
	High	5	5.06	0.184	5.18	0.159	-0.11	-0.31	0.08	-0.27	0.04
Pork liver pâté	Low	5	1.44	0.287	1.34	0.349	0.10	-0.12	0.33	-0.07	0.28
	Medium	5	2.68	0.181	3.11	0.234	-0.43	-0.61	-0.26	-0.57	-0.30
	High	5	4.56	0.106	4.71	0.091	-0.15	-0.17	-0.13	-0.16	-0.13
Sandwiches	Low	5	2.15	0.070	1.86	0.400	0.29	-0.16	0.74	-0.06	0.63
	Medium	5	4.04	0.356	4.18	0.346	-0.14	-0.23	-0.06	-0.21	-0.08
	High	5	5.66	0.207	5.72	0.200	-0.06	-0.17	0.05	-0.14	0.03
Pasta salad	Low	5	2.06	0.065	1.85	0.243	0.21	-0.02	0.44	0.03	0.39
	Medium	5	3.82	0.078	4.01	0.058	-0.19	-0.22	-0.16	-0.21	-0.17
	High	5	5.40	0.075	5.49	0.104	-0.10	-0.24	0.05	-0.21	0.01

^an = number of replicate test portions^bCFU = Colony-forming units^cs_r = Standard deviation of repeatability^dDOM = Difference of Means^eCI = Confidence Interval for DOM^fLCL = Lower confidence limit for DOM^gUCL = Upper confidence limit for DOM

REFERENCES CITED

- Hosokawa, S., Yamazaki, T., and Toyota, K., Validation of the CompactDry "Nissui" BC for Rapid Enumeration of *Bacillus cereus* in a Variety of Foods, AOAC Performance Tested MethodsSM certification number 092201. Approved September 16, 2022
- Anonymous (2004) ISO 7932:2004 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of presumptive *Bacillus cereus* — Colony-count technique at 30 degrees C, <https://www.iso.org/standard/38219.html>, accessed September 2022.