# 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.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

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**3-24-6, Ueno**
**Taito-ku, Tokyo**
**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 METHOD**
**Target Organism(s) – *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** | **CERTIFICATION RENEWAL RECORD** |
|---|---|
| **September 16, 2022** | **New Approval** |
| **METHOD MODIFICATION RECORD** | **SUMMARY OF MODIFICATION** |
| **NONE** | **NONE** |
| **Under this AOAC *Performance Tested Methods*℠ License Number, 092201 this method is distributed by:** | **Under this AOAC *Performance Tested Methods*℠ License Number, 092201 this method is distributed as:** |
| **NONE** | **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 | *Bacillus cereus* | CRA 84 | Meatloaf | +[b] | + |
| 2 | *Bacillus cereus* | CRA 193 | Environmental | + | + |
| 3 | *Bacillus cereus* | CRA 1549 | Dried milk | + | + |
| 4 | *Bacillus cereus* | CRA 1731 | Chocolate ice cream | + | + |
| 5 | *Bacillus cereus* | CRA 1740 | Cream cake | + | + |
| 6 | *Bacillus cereus* | CRA 1741 | Flour | + | + |
| 7 | *Bacillus cereus* | CRA 1749 | Cream cake | + | + |
| 8 | *Bacillus cereus* | CRA 1964 | Milk/cream | + | + |
| 9 | *Bacillus cereus* | CRA 4110 | Contaminated flask | + | + |
| 10 | *Bacillus cereus* | CRA 6295 | Flavoring | + | + |
| 11 | *Bacillus cereus* | CRA6452 | Flour | + | + |
| 12 | *Bacillus cereus* | CRA7616 | Dairy | + | + |
| 13 | *Bacillus cereus* | CRA 8711 | Infant formula | + | + |
| 14 | *Bacillus cereus* | CRA 16100 | Flavor | + | + |
| 15 | *Bacillus cereus* | CRA 16101 | Flavor | + | + |
| 16 | *Bacillus cereus* | CRA 16381 | Environmental | + | + |
| 17 | *Bacillus cereus* | CRA 16438 | Environmental | + | + |
| 18 | *Bacillus cereus* | CRA 16563 | Unknown | + | + |
| 19 | *Bacillus cereus* | CRA 16564 | Food poisoning | + | + |
| 20 | *Bacillus cereus* | CRA 16565 | Pharmaceutical | + | + |
| 21 | *Bacillus cereus* | CRA 16566 | Unknown | + | + |
| 22 | *Bacillus cereus* | CRA 16569 | Meatloaf | + | + |
| 23 | *Bacillus cereus* | CRA 16570 | Food poisoning | + | + |
| 24 | *Bacillus cereus* | CRA 16571 | Unknown | + | + |
| 25 | *Bacillus cereus* | CRA 16579 | Industrial | + | + |
| 26 | *Bacillus cereus* | CRA 16580 | Industrial | + | + |
| 27 | *Bacillus cereus* | CRA 16582 | Environmental | + | + |
| 28 | *Bacillus cereus* | CRA 16583 | Industrial | + | + |
| 29 | *Bacillus cereus* | CRA 16662 | Dried potato | + | + |
| 30 | *Bacillus cereus* | CRA 17010 | Mangoes | + | + |
| 31 | *Bacillus cereus* | CRA17011 | Water | + | + |
| 32 | *Bacillus cereus* | CRA 17012 | Milk | + | + |
| 33 | *Bacillus cereus* | CRA 17013 | Soil | + | + |
| 34 | *Bacillus cytotoxicus* | DSM 22905 | Vegetable puree | -[c] | -[d] |
| 35 | *Bacillus mycoides* | CRA 16597 | UHT custard | - | - |
| 36 | *Bacillus mycoides* | CRA 1522 | Dried milk | + | + |
| 37 | *Bacillus mycoides* | CRA 16646 | Soft drink factory | + | + |
| 38 | *Bacillus mycoides* | CRA 1510 | Dried milk | + | + |
| 39 | *Bacillus mycoides* | CRA 8504 | Food environment | + | + |
| 40 | *Bacillus pseudomycoides* | CRA 16382 | Soil | - | + |
| 41 | *Bacillus thuringiensis kurstaki* | CRA 17032 | Insecticide | + | + |
| 42 | *Bacillus thuringiensis aizawai* | CRA 17033 | Insecticide | + | + |
| 43 | *Bacillus thuringiensis israelensis* | CRA 17034 | Insecticide | + | + |
| 44 | *Bacillus thuringiensis* | CRA 16616 | Broccoli | + | + |
| 45 | *Bacillus thuringiensis* | CRA 16314 | Flour moth | + | + |
| 46 | *Bacillus thuringiensis* | CRA 1744 | Flour | + | + |
| 47 | *Bacillus thuringiensis* | CRA 16619 | Broccoli | + | + |
| 48 | *Bacillus weihenstephanensis* | CRA 16578 | Pasteurized milk | + | + |
| 49 | *Bacillus weihenstephanensis* | DSM 104135 | Soil | + | + |
| 50 | *Bacillus weihenstephanensis* | DSM 104109 | Soil | + | + |

[a]CRA = 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

[c]Unless otherwise noted, "-" indicates growth did not occur

[d]This 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 | *Allicyclobacillus acidoterrestris* | CRA 5331 | Apple juice | - | - |
| 2 | *Alicyclobacillus cycloheptanicus* | CRA 16823 | Soil | - | - |
| 3 | *Alicyclobacillus fastidiosus* | CRA 16831 | Apple juice | - | - |
| 4 | *Alicyclobacillus pomorum* | CRA 16830 | Fruit juice | - | - |
| 5 | *Aneurinibacillus aneurinolyticus* | CRA 7751 | Flavor | - | - |
| 6 | *Anoxybacillus flavithermus* | CRA 17047 | Food isolate | - | - |
| 7 | *Bacillus amyloliquefaciens* | CRA 6317 | Crumpets | - | -[c] |
| 8 | *Bacillus circulans* | CRA 16584 | Cream | - | - |
| 9 | *Bacillus coagulans* | CRA 10205 | Evaporated milk | + | + |
| 10 | *Bacillus fusiformis* | CRA 16652 | Soft drinks | - | - |
| 11 | *Bacillus laterosporus* | CRA 1523 | Dried milk | + | + |
| 12 | *Bacillus licheniformis* | CRA 6335 | Pesto | - | - |
| 13 | *Bacillus megaterium* | CRA 16512 | Soil | - | - |
| 14 | *Bacillus oceanisediminis* | CRA 17220 | Food isolate | - | - |
| 15 | *Bacillus pumilus* | CRA 16594 | Industrial isolate | - | - |
| 16 | *Bacillus psychrodurans* | CRA 16694 | Soil | - | - |
| 17 | *Bacillus smithii* | CRA 7240 | Pineapple | - | - |
| 18 | *Bacillus sonorensis* | CRA 17231 | Food isolate | - | - |
| 19 | *Bacillus sphaericus* | CRA 7950 | Flavoring | - | - |
| 20 | *Bacillus subtilis* | CRA 14161 | Milk shake | - | -[c] |
| 21 | *Brevibacillus brevis* | CRA 7748 | Flavor | + | + |
| 22 | *Brevibacillus parabrevis* | CRA 7757 | Flavor | - | - |
| 23 | *Leuconostoc mesenteroides* | CRA 16022 | Soft ham | - | - |
| 24 | *Listeria ivanovii* | CRA 1123 | Soft cheese | - | - |
| 25 | *Lysinibacillus sphaericus* | CRA 7746 | Unknown | - | -[c] |
| 26 | *Paenibacillus amylolyticus* | CRA 16606 | Barley | - | - |
| 27 | *Paenibacillus macerans* | CRA 16488/DSM 357 | Unknown | - | - |
| 28 | *Paenibacillus pabuli* | CRA 16605 | Barley | - | - |
| 29 | *Paenibacillus polymyxa* | CRA 7747 | Food isolate | + | + |
| 30 | *Staphylococcus aureus* | CRA 1224 | Margarine | - | - |
| 31 | *Bacillus coagulans* | CRA 17185 | Industrial isolate | - | - |
| 32 | *Bacillus laterosporus* | CRA 1515 | Dried milk | + | + |
| 33 | *Paenibacillus polymyxa* | CRA 16386/ATCC 43865 | Unknown | - | - |

[a]CRA = 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)

[b]Unless otherwise noted, "-" indicates growth did not occur; "+" indicates growth occurred with typical morphology and confirmation on SBA.

[c]Strain 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)**

| | | | CompactDry "Nissui" BC | | ISO 7932:2004 | | | 95 % CI[e] | | 90 % CI | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Matrix | Contamination level | n[a] | Mean $Log_{10}$ CFU[b]/g | $s_r$[c] | Mean $Log_{10}$ CFU/g | $s_r$ | DOM[d] | 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 | 054 | -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 |

[a]n = number of replicate test portions
[b]CFU = Colony-forming units
[c]$s_r$ = Standard deviation of repeatability
[d]DOM = Difference of Means
[e]CI = Confidence Interval for DOM
[f]LCL = Lower confidence limit for DOM
[g]UCL = Upper confidence limit for DOM

**REFERENCES CITED**

1. 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 Methods*℠ certification number 092201. Approved September 16, 2022
2. 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.