CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

111902

The AOAC Research Institute hereby certifies the method known as:

CompactDry "Nissui" ETC

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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Expiration Date December 31, 2023

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3-24-6, Ueno
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METHOD NAME
CompactDry "Nissui" ETC

CATALOG NUMBER
54056 (1400 plates)

INDEPENDENT LABORATORY
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APPLICABILITY OF METHOD
Target Organism – *Enterococcus* spp. Both the reference method and this method have limitations of the species detected.

Matrixes – fresh chilled shipping cream (17% fat), fresh chilled custard (7%), pre-washed bagged iceberg lettuce leaves; prewashed bagged flat leaf parsley; cheddar and bacon deli pasta salad (14% fat); frozen raw ground 100% beef patties (20% fat); fresh cooked and peeled prawns (1% fat); pre-packaged egg, cress, and mayo sandwich on wheat germ bread (31% fat); fresh raw packaged beef steak (7% fat); and chilled tuna paté containing tuna, mayo and cream cheese (13% fat)

Performance claims – The study data detected no statistical difference between the CompactDry "Nissui" ETC method and the reference methods.

REFERENCE METHOD
Nordic Committee on Food Analysis (2011) Method 68, *Enterococcus*, Determination in Foods and Feeds (2)

ORIGINAL CERTIFICATION DATE
November 27, 2019

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
None

SUMMARY OF MODIFICATION
None

Under this AOAC *Performance Tested Methods*℠ License Number, 111902 this method is distributed by:
1. **Hardy Diagnostics**
2. **R-Biopharm AG**

Under this AOAC *Performance Tested Methods*℠ License Number, 111902 this method is distributed as:
1. **Compact Dry ETC**
2. **Compact Dry ETC**

PRINCIPLE OF THE METHOD (1)
Compact Dry 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 diluted sample into the center of the self-diffusible medium. The CompactDry "Nissui" ETC method contains a chromogenic medium and selective agent for the detection and enumeration of *Enterococcus* spp., which appear as blue/ blue green colonies after incubation for 20–24 h at 37 ± 1°C.

DISCUSSION OF THE VALIDATION STUDY (1)
The results of this study indicate that the CompactDry "Nissui" ETC method can be used for rapid and accurate enumeration of *Enterococcus* spp. in a variety of food commodities. It shows similar repeatability and selectivity to the reference method. There were a number of *Enterococcus* spp. not detected by either method. However, in the study there were 50 strains tested which represented 23 different species, and of these the candidate method detected 12 of the 23 species whereas the reference method only detected 8 of the different species. The method was shown to be robust to changes in sample volume, incubation temperature, and incubation time. The manufacturing is consistent from lot to lot and the data presented support a shelf life of 18 months.
The CompactDry "Nissui" ETC method offers a time saving of 1 to 2 days 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 advantages in reduction of storage space, waste disposal and required incubator space.

Table 3. Inclusivity testing of *Enterococcus* species (1)

No.	Species	Source[a]		Origin	Compact Dry ETC	NMKL 68
		CRA	Other culture collection code			
1	*Enterococcus aquamarinus*	16813	NCIMB 14241	Sea water	-[b]	-
2	*Enterococcus avium*	16862	NCIMB 701605	n/a	+[c]	+
3	*Enterococcus casseliflavus*	16811	NCTC 12361	Plants	+	+
4	*Enterococcus cecorum*	16849	NCTC 12421	Raw chicken	-	-
5	*Enterococcus columbae*	16851	NCIMB 13013	Pigeon liver	-	-
6	*Enterococcus dispar*	16850	NCIMB 13000	Human isolate	-	-
7	*E. dispar*	16864	NCIMB 702829	Human isolate	-	-
8	*Enterococcus durans*	16464	NCTC 662	Milk	+	-
9	*E. durans*	16810	NCTC 8130	Cheese	-	+
10	*Enterococcus faecalis*	5395	n/a	Industrial isolate	+	+
11	*E. faecalis*	5447	NCIMB775	n/a	+	+
12	*E. faecalis*	5723	n/a	Industrial isolate	+	+
13	*E. faecalis*	6369	NCIMB1993	n/a	+	+
14	*E. faecalis*	6635	n/a	Industrial isolate	+	+
15	*E. faecalis*	7068	n/a	Industrial isolate	+	+
16	*E. faecalis*	7296	n/a	Industrial isolate	+	+
17	*E. faecalis*	7297	n/a	Industrial isolate	+	+
18	*E. faecalis*	16049	NCIMB 13280	Human isolate	+	+
19	*E. faecalis*	1513	n/a	Dried milk powder	+	+
20	*E. faecalis*	1528	n/a	Dried milk powder	+	+
21	*E. faecalis*	4113	NCTC 775	n/a	+	+
22	*E. faecalis*	4132	n/a	Cheese	+	+
23	*E. faecalis*	16408	n/a	Industrial isolate	+	+
24	*E. faecalis*	16481	ATCC 29212	Human isolate	+	+
25	*E. faecalis*	1528	n/a	Dried milk	+	+
26	*Enterococcus faecium*	16844	NCIMB 2699	Cheese	+	+
27	*E. faecium*	16845	NCIMB 2702	Cheese	+	+
28	*E. faecium*	16846	NCIMB 700580	Commercial milk	+	+
29	*E. faecium*	16847	NCIMB 700594	Cheddar cheese	+	+
30	*E. faecium*	16848	NCIMB 9645	Grass silage	+	+
31	*E. faecium*	7865	n/a	Industrial isolate	+	+
32	*E. faecium*	16465	ATCC 8459	Cheese	+	+
33	*E. faecium*	16856	NCIMB 700502	Dried milk powder	+	+
34	*E. faecium*	16866	NCIMB12672	Undercooked sausage	+	+
35	*Enterococcus flavescens*	16855	NCIMB 13326	Bird	-	-
36	*Enterococcus gallinarum*	16861	NCIMB 701229	n/a	+	-
37	*Enterococcus haemoperoxidus*	16858	NCIMB 14071	Water	+	-
38	*Enterococcus hirae*	15939	ATCC 8043	n/a	+	+
39	*E. hirae*	16809	n/a	Industrial isolate	+	+
40	*Enterococcus malodoratus*	16860	NCIMB 700846	Gouda Cheese	+	-
41	*Enterococcus mundtii*	16812	NCTC 12363	Soil	+	+
42	*Enterococcus porcinus*	16857	NCIMB 13634	Pig	-	+
43	*Enterococcus pseudoavium*	16852	NCIMB 13084	Cow udder	-	-
44	*E. pseudoavium*	16869	NCIMB 2366		+	+
45	*Enterococcus sacharolyticus*	16863	NCIMB 702614	Bovine	-	-
46	*Enterococcus seriolicida*	16854	NCIMB 13208	Bird	-	-
47	*Enterococcus solitarus*	16867	NCIMB 12902	Human isolate	-	-
48	*Enterococcus sulfureus*	16853	NCIMB13117	Plant	-	-
49	*Enterococcus thailandicus*	16859	NCIMB 14560	Sausage	+	-
50	*Enterococcus xiangfangensis*	16865	NCIMB 14834	Pickle	-	-

[a]CRA = Campden Culture Collection (Campden BRI, Chipping Campden, UK); NCIMB = National Collections of Industrial, Marine and Food Bacteria (Aberdeen, Scotland, UK); NCTC = National Collection of Type Cultures (Public Health England, Porton Down, Salisbury, UK); ATCC = American Type Culture Collection (Manassas, VA, USA); n/a = not applicable.

[b]- indicates no growth

[c]+ indicates growth

Table 4. Exclusivity testing of non-*Enterococcus* species (1)

No.	Species	Source (CRA)[a]	Origin	Non-selective culture			Compact Dry ETC	NMKL 68
				Media[b]	Temp.	Growth		
1	*Bacillus cereus*	1549	Not known	NB	30 ± 1°C	+[c]	-[d]	-
2	*Bacillus subtilis*	16597	UHT custard	NB	30 ± 1°C	+	-	-
3	*Brochothrix thermospacta*	16019	Not known	NB	30 ± 1°C	+	-	-
4	*Carnobacterium divergens*	2072	Industrial isolate	MRSB	30 ± 1°C	+	-	-
5	*Citrobacter freundii*	1266	Sausage	NB	37 ± 1°C	+	-	-
6	*Enterobacter agglomerans*	490	Raw mince	NB	37 ± 1°C	+	-	-
7	*Enterobacter cloacae*	4772	Environmental	NB	37 ± 1°C	+	-	-
8	*Erwinia herbicola*	5442	Industrial isolate	NB	37 ± 1°C	+	-	-
9	*Escherichia coli*	545	Raw mince	NB	37 ± 1°C	+	-	-
10	*Hafnia alvei*	3996	Chicken giblets	NB	37 ± 1°C	+	-	-
11	*Lactobacillus brevis*	3169	Sileage	MRSB	30 ± 1°C	+	-	-
12	*Lactobacillus casei*	533	Industrial isolate	MRSB	30 ± 1°C	+	-	-
13	*Lactobacillus gasseri*	6804	Human	MRSB	30 ± 1°C	+	+	+
14	*Lactococcus lactis*	5396	Food factory isolate	MRSB	30 ± 1°C	+	-	-
15	*Leuconostoc mesenteroides*	16022	Ham	MRSB	30 ± 1°C	+	-	-
16	*Listeria innocua*	115	Beefburger	TSB	30 ± 1°C	+	-	-
17	*Listeria monocytogenes*	1105	Raw milk	TSB	37 ± 1°C	+	-	-
18	*Micrococcus luteus*	3503	Tea factory	TSB	37 ± 1°C	+	-	-
19	*Proteus mirabilis*	586	Poultry	NB	37 ± 1°C	+	-	-
20	*Pseudomonas aeruginosa*	8299	NCIMB 10753	NB	37 ± 1°C	+	-	-
21	*Pseudomonas fluorescens*	5361	Environmental	NB	30 ± 1°C	+	-	-
22	*Salmonella* Enteritidis	3505	Fish cakes	NB	37 ± 1°C	+	-	-
23	*Serratia liquefaciens*	504	Raw mince	NB	37 ± 1°C	+	-	-
24	*Staphylococcus aureus*	1224	Margarine	TSB	37 ± 1°C	+	-	-
25	*Staphylococcus carnosus*	4134	Fermented sausage	TSB	37 ± 1°C	+	-	-
26	*Staphylococcus hominis*	529	Milk powder	TSB	37 ± 1°C	+	-	-
27	*Streptococcus cremoris*	534	Raw mince	TSB	37 ± 1°C	+	-	-
28	*Streptococcus cremoris*	556	Raw mince	TSB	37 ± 1°C	+	-	-
29	*Streptococcus lactis*	1487	Raw mince	TSB	37 ± 1°C	+	-	-
30	*Streptococcus thermophilus*	5683	Industrial isolate	TSB	42 ± 1°C	+	-	-
31	*Streptococcus thermophilus*	16868	NCIMB 8510	TSB	42 ± 1°C	+	-	-

[a]CRA = Campden Culture Collection (Campden BRI, Chipping Campden, UK)
[b]NB = Nutrient Broth (Oxoid CM0001); MRSB = DeMan, Rogosa and Sharpe Broth (Oxoid CM0359); TSB = Tryptone Soy Broth (Oxoid CM0129)
[c]+ indicates growth
[d]- indicates no growth

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

Matrix (organism)	Cont. level	N	Compact Dry ETC			NMKL 68			DOM[a]	95% CI[b]		r^2
			Mean	s_r	RSD_r, %	Mean	s_r	RSD_r, %		LCL[c]	UCL[d]	
Cream (*Enterococcus mundtii* CRA16812)	Low	5	2.592	0.056	2.16	2.586	0.078	3.02	0.007	-0.109	0.123	0.998
	Medium	5	3.936	0.076	1.93	4.041	0.062	1.53	-0.106	-0.243	0.031	
	High	5	6.712	0.070	1.04	6.871	0.076	1.11	-0.159	-0.209	-0.110	
Custard (*Enterococcus mundtii* CRA16812)	Low	5	2.448	0.112	4.58	2.627	0.048	1.83	-0.179	-0.341	-0.017	0.997
	Medium	5	3.797	0.055	1.45	4.038	0.069	1.71	-0.240	-0.363	-0.118	
	High	5	6.700	0.060	0.90	6.819	0.068	1.00	-0.119	-0.194	-0.044	
Lettuce (*Enterococcus faecium* CRA9645)	Low	5	2.533	0.066	2.61	2.305	0.182	7.90	0.228	0.041	0.415	0.993
	Medium	5	3.721	0.054	1.45	3.766	0.099	2.63	-0.045	-0.187	0.097	
	High	5	6.651	0.176	2.65	6.607	0.154	2.33	0.044	-0.073	0.161	
Parsley (*Enterococcus faecium* CRA9645)	Low	5	2.533	0.064	2.53	2.446	0.145	5.93	0.087	-0.135	0.309	0.997
	Medium	5	3.869	0.073	1.89	3.819	0.057	1.49	0.050	-0.012	0.112	
	High	5	6.975	0.169	2.42	6.782	0.146	2.15	0.193	0.114	0.271	
Prawns (*Enrterococcus casseliflavus* CRA 16811)	Low	5	2.473	0.107	4.33	2.246	0.135	6.01	0.227	0.050	0.404	0.994
	Medium	5	3.546	0.186	5.25	3.425	0.225	6.57	0.121	-0.060	0.303	
	High	5	6.625	0.138	2.08	6.572	0.098	1.49	0.054	-0.157	0.265	
Tuna pate (*Enrterococcus casseliflavus* CRA 16811)	Low	5	2.386	0.130	5.45	2.273	0.211	9.28	0.113	-0.144	0.370	0.993
	Medium	5	3.802	0.055	1.45	3.582	0.092	2.57	0.220	0.116	0.324	
	High	5	6.661	0.222	3.33	6.637	0.144	2.17	0.025	-0.154	0.203	
Beef steak (*Enterococcus psuedoavium* CRA16869)	Low	5	2.837	0.066	2.33	2.766	0.209	7.56	0.071	-0.145	0.287	0.997
	Medium	5	3.917	0.374	9.55	3.886	0.299	7.69	0.031	-0.080	0.143	
	High	5	7.091	0.123	1.73	7.098	0.095	1.34	-0.007	-0.088	0.074	
Ground beef patties (*Enterococcus psuedoavium* CRA16869)	Low	5	2.653	0.233	8.78	2.612	0.203	7.77	0.041	-0.029	0.111	0.999
	Medium	5	3.977	0.245	6.16	3.964	0.222	5.60	0.013	-0.087	0.115	
	High	5	6.871	0.287	4.18	6.927	0.276	3.98	-0.056	-0.137	0.025	
Pasta salad (*Enterococcus hirae* CRA15939)	Low	5	2.730	0.079	2.89	2.641	0.222	8.41	0.089	-0.163	0.342	0.996
	Medium	5	3.940	0.044	1.12	3.932	0.062	1.58	0.008	-0.072	0.088	
	High	5	7.042	0.058	0.82	6.990	0.073	1.04	0.052	0.020	0.085	
Sandwiches (*Enterococcus hirae* CRA15939)	Low	5	2.808	0.033	1.18	2.611	0.102	3.91	0.197	0.107	0.287	0.997
	Medium	5	4.017	0.043	1.07	3.962	0.181	4.57	0.055	-0.127	0.237	
	High	5	6.830	0.068	1.00	6.949	0.095	1.37	-0.118	-0.216	-0.020	

[a]DOM = Difference of Means
[b]CI = Confidence Interval for DOM
[c]LCL = Lower confidence limit for DOM
[d]UCL = Upper confidence limit for DOM

REFERENCES CITED

1. Mizuochi, S. and Betts, G., CompactDry "Nissui" ETC for Enumeration of Enterococci in a Variety of Foods, AOAC *Performance Tested Methods*℠ certification number 111902.
2. Nordic Committee on Food Analysis (2011) Method 68, *Enterococcus*, Determination in Foods and Feeds, https://www.nmkl.org/index.php/en/publications/item/enterococcus-determination-in-foods-nmkl-68