



CERTIFICATION

AOAC[®] Performance TestedSM

Certificate No.

092002

The AOAC Research Institute hereby certifies the test kit known as:

CompactDry "Nissui" YMR

manufactured by

NISSUI Pharmaceutical Co., Ltd.

3-23-9, Ueno

Taito-ku, Tokyo

Japan 110-8736

This method has been evaluated in the AOAC[®] *Performance Tested Methods*SM Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC[®] Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*SM certification mark along with the statement - "THIS METHOD'S PERFORMANCE WAS REVIEWED BY AOAC RESEARCH INSTITUTE AND WAS FOUND TO PERFORM TO THE MANUFACTURER'S SPECIFICATIONS" - on the above mentioned method for a period of one calendar year from the date of this certificate (September 25, 2020 – December 31, 2021). Renewal may be granted at the end of one year 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

October 09, 2020

Date

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KIT NAME(S) CompactDry "Nissui" YMR	CATALOG NUMBERS 06777, 6778
INDEPENDENT LABORATORY Campden BRI Chipping Campden Gloucestershire, UK	AOAC EXPERTS AND PEER REVIEWERS Yi Chen ¹ , Yvonne Salfinger ² , Maria Cristina Fernandez ³ ¹ FDA CFSAN, College Park, MD, USA ² Consultant, Tallahassee, FL, USA ³ Consultant, Buenos Aires, Argentina
APPLICABILITY OF METHOD Target Organism(s) – Yeasts and molds Matrices – (10 g samples) - cooked, peeled, chilled, cold water prawns with heads off (1.3% salt); beetroot deli salad (66% beetroot, 11% carrot in a vinaigrette dressing 1.3% fat, 0.1% salt); chilled tuna pâté (23% fat, 0.9% salt); fermented strawberry yogurt drink (1.8% fat containing <i>L. acidophilus</i> , <i>Bifidobacterium</i> and <i>L. casei</i>); spinach and ricotta quiche (14.3% fat, 0.3% salt); egg custard tarts (pastry cases filled with sweet egg custard, 12.8% fat, 14.1% sugar); fruit and vegetable smoothie (cucumber, lemon, kale, spinach, pear, apple); full fat soft cream cheese (24% fat); fresh chilled egg mayonnaise salad sandwich on malted brown sliced bread (6.3% fat, 0.9% salt); and deli pasta salad (7.4% fat 0.7% salt) containing cooked chicken (11%), bacon (2.5%) and blanched sweet corn (17%) Performance claims - Performance was found to be equivalent to that of ISO 21527-1:2008, <i>Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of yeasts and molds - Colony count technique in products with water activity greater than 0.95</i> (2) for the matrixes tested.	REFERENCE METHOD ISO 21527-1:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0.95 (2)
ORIGINAL CERTIFICATION DATE September 25, 2020	CERTIFICATION RENEWAL RECORD New Approval 2020
METHOD MODIFICATION RECORD NONE	SUMMARY OF MODIFICATION NONE
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PRINCIPLE OF THE METHOD (1)

CompactDry "Nissui" YMR 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" YMR method contains a special spread sheet containing nutrients, chloramphenicol to inhibit bacterial growth, and a chromogenic enzyme substrate, X-phos, for the detection and enumeration of yeasts and molds after incubation at 25 ± 1°C for 3 days. While most colonies are some shade of green/blue, any colored colony should be counted. In addition, mold colonies may have a diffuse or cottony appearance with blue/green or other color.

DISCUSSION OF THE VALIDATION STUDY (1)

The results of this study indicate that the CompactDry “Nissui” YMR method can be used for rapid and accurate enumeration of yeast and mold species in a variety of food commodities, including cooked prawns, deli vegetable salad, tuna pâté, fermented yogurt drink, spinach and ricotta quiche, egg custard tarts, fruit and vegetable smoothie, cream cheese, egg salad sandwich, and deli pasta salad. The CompactDry “Nissui” YMR method shows slightly better repeatability than the ISO 21527-1:2008 reference method and equivalent mean results. The inclusivity and exclusivity results showed excellent selectivity of the CompactDry “Nissui” YMR with all 51 inclusivity strains positive and all 32 exclusivity strains negative.

The method was shown to be robust to changes in sample volume, incubation temperature and incubation time. While the data demonstrated no significant difference when incubation temperature was varied between 23-28°C and incubation time was varied between 48-72 h, for best results it is recommended to incubate at 25±1°C for 72±3 h. The product consistency and stability study demonstrated no significant lot-to-lot variation or loss of performance over a 24-month shelf life.

The CompactDry “Nissui” YMR method offers a time saving of 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 additional advantages in reduction of storage space, waste disposal and required incubator space.

Table 1. Inclusivity testing of Yeast and Mold species on CompactDry “Nissui” YMR (1)

No.	Species	Type	Source ^a	Origin	Growth at 72 h ^b	Morphology at 72 h
1	<i>Alternaria alternata</i>	Mold	IFO 31188	living leaf, <i>Stevia rebaudiana</i>	+	blue green - dark green, unclear edge
2	<i>Aspergillus brasiliensis</i>	Mold	NBRC 9455	Blueberry, <i>Vaccinium</i> sp.	+	white - blue green unclear edge, black spots in center
3	<i>Aspergillus flavus</i>	Mold	NBRC 6343	Shoe sole	+	pale blue green atypical, black spots in hypha
4	<i>Aspergillus fumigatus</i>	Mold	NBRC 33022	unknown	+	pale blue green rather unclear colony
5	<i>Aspergillus niger</i>	Mold	NBRC 105649	Leather	+	deep blue green; black spots in center
6	<i>Aspergillus oryzae</i>	Mold	NBRC 5375	unknown	+	blue green; pale brown center
7	<i>Aspergillus terreus</i>	Mold	NBRC 6346	Haversack	+	White; not clearly formed edge
8	<i>Aspergillus versicolor</i>	Mold	NBRC 4098	tobacco	+	pale blue green; not clearly formed edge
9	<i>Aureobasidium pullulans</i>	Yeast	NBRC 6353	unknown	+	blue - dark green
10	<i>Candida albicans</i>	Yeast	NBRC 1594	Clinical bronchomycosis	+	white - pale green
11	<i>Candida apicola</i>	Yeast	NBRC 10261	intestine of bee	+	blue green
12	<i>Candida lactis-condensi</i>	Yeast	NBRC 1286	fermenting condensed milk	+	blue green; blue green edge
13	<i>Chaetomium globosum</i>	Mold	NBRC 6347	Stored cotton	+	blue green
14	<i>Cladosporium cladosporioides</i>	Mold	NBRC 6348	unknown	+	pale blue green - blue green; not clearly formed colony
15	<i>Cladosporium halotolerans</i>	Mold	NBRC 4460	Air	+	white - pale blue green; not clearly formed colony
16	<i>Debaryomyces hansenii</i>	Yeast	IFO 0026	beef-and-pork sausage	+	white
17	<i>Debaryomyces maramus</i>	Yeast	NBRC 0668	Air	+	white
18	<i>Fusarium oxysporum</i>	Mold	NBRC 7155	unknown	+	pale blue green; deep blue green center
19	<i>Fusarium solani</i>	Mold	NBRC 5232	unknown	+	blue green; cottony center
20	<i>Geotrichum candidum</i>	Yeast	NBRC 4598	unknown	+	pale greenish white; cottony
21	<i>Hormoconis resinae</i>	Mold	NBRC 100535	unknown	+	very pale blue green; atypical growth, not clearly formed colony
22	<i>Monascus purpureus</i>	Mold	NBRC 32316	red rice	+	blue green; pale brown center
23	<i>Moniliella acetoabutans</i>	Yeast	NBRC 9482	sweet fruit sauce	+	blue green
24	<i>Myrothecium verrucaria</i>	Mold	NBRC 6113	Deteriorated baled cotton	+	blue green; pale brown center
25	<i>Neosartorya fischeri</i>	Mold	IFO 8789	rubber tire scrap	+	pale blue green - blue green; not clearly formed edge
26	<i>Paecilomyces variotii</i>	Mold	NBRC 33284	unknown	+	pale blue green - blue green

27	<i>Penicillium aurantiogriseum</i>	Mold	NBRC 7733	Rotting grain of <i>Zea mays</i>	+	blue green
28	<i>Penicillium brevicompactum</i>	Mold	NBRC 5727	soil	+	blue green; white - pale brown center
29	<i>Penicillium chrysogenum</i>	Mold	IFO 32030	cheese	+	blue green; white cottony center
30	<i>Penicillium citrinum</i>	Mold	NBRC 6352	unknown	+	yellow green; white cottony center
31	<i>Penicillium funiculosum</i>	Mold	NBRC 100958	Mercury-treated fabric	+	pale white; not clearly formed colony
32	<i>Penicillium martensii</i>	Mold	NBRC 8142	unknown	+	Green; not clearly formed edge
33	<i>Penicillium ochrochloron</i>	Mold	NBRC 4612	unknown	+	blue green; not clearly formed edge
34	<i>Penicillium pinophilum</i>	Mold	NBRC 33285	unknown	+	white - very pale blue green; not clearly formed colony
35	<i>Phialophora fastigiata</i>	Mold	IFO 6850	unknown	+	blue green; not clearly formed edge
36	<i>Phoma herbarum</i>	Mold	NBRC 107643	Polyester straw on drinking pot	+	white - very pale blue green; not clearly formed edge
37	<i>Pichia anomala</i>	Yeast	IFO 10213	unknown	+	blue green (nearly green)
38	<i>Pseudocochliobolus lunatus</i>	Mold	NBRC 30883	leaf of sudangrass 'Greenleaf'	+	pale blue green; not clearly formed edge
39	<i>Rhizopus oryzae</i>	Mold	NBRC 31005	Radio set	+	partly pale yellow brown; atypical growth
40	<i>Rhodotorula acuta</i>	Yeast	IFO 1912	grape must	+	blue green; pale blue green edge
41	<i>Rhodotorula glutinis</i>	Yeast	NBRC 1125	Air	+	blue green; pale blue green edge
42	<i>Rhodotorula mucilaginosa</i>	Yeast	NBRC 0889	unknown	+	pale blue green; very pale blue green edge
43	<i>Saccharomyces cerevisiae</i>	Yeast	NBRC 101557	Fermenting sake mash	+	white - pale green
44	<i>Scopulariopsis brevicaulis</i>	Mold	NBRC 100536	Chrysalis of silkworm	+	pale blue green - blue green; not clearly formed colony
45	<i>Torulaspora delbrueckii</i>	Yeast	IFO 1180	grape must	+	blue green (nearly green); pale blue green edge
46	<i>Trichoderma citrinoviride</i>	Mold	IFO 31137	Soil in organic layer	+	pale blue green; not clearly formed edge, deep blue green center
47	<i>Trichoderma virens</i>	Mold	NBRC 6355	Soil	+	blue green - yellow green; atypical growth
48	<i>Trichophyton mentagrophytes</i>	Mold	IFO 6202	unknown	+	pale blue green
49	<i>Trichosporon asahii</i>	Yeast	NBRC 103889	case of trichosporia cutis	+	blue green
50	<i>Zygosaccharomyces bailii</i>	Yeast	NBRC 1098	unknown	+	pale blue green - blue green; very pale blue green edge
51	<i>Zygosaccharomyces rouxii</i>	Yeast	NBRC 1960	cane sugar	+	white - pale blue green; very pale blue green edge

^aIFO = Campden Culture Collection (Campden BRI, Chipping Campden, UK); NBRC = National Institute of Technology and Evaluation Biological Resource Center (Tokyo, Japan)

Table 2. Exclusivity testing of non-Yeast and non-Mold species on CompactDry "Nissui" YMR (1)

No.	Species	Source ^a	Origin	Growth ^b
1	<i>Acinetobacter baumannii</i>	JCM 6841	Urine	-
2	<i>Acinetobacter calcoaceticus</i>	ATCC 19606	unknown	-
3	<i>Alcaligenes faecalis</i>	IFO 13111	unknown	-
4	<i>Bacillus cereus</i>	IFO 13494	unknown	-
5	<i>Bacillus licheniformis</i>	NBRC 12200	unknown	-
6	<i>Bacillus subtilis</i>	NBRC 3134	unknown	-
7	<i>Burkholderia cepacia</i>	NBRC 15124	10% benzalkonium chloride	-

			solution	
8	<i>Citrobacter freundii</i>	IFO 12681	unknown	-
9	<i>Enterobacter aerogens</i>	ATCC 13048	Sputum, South Carolina Dept. of Health and Environmental Control	-
10	<i>Enterobacter cloacae</i> subsp. <i>Cloacae</i>	NBRC 13535	Spinal fluid	-
11	<i>Enterococcus faecalis</i>	NBRC 12965	Citrus juice	-
12	<i>Enterococcus faecium</i>	ATCC 19434	unknown	-
13	<i>Escherichia coli</i>	NBRC 3301	Human feces	-
14	<i>Escherichia coli</i>	NBRC 3972	Feces	-
15	<i>Flavobacterium odoratum</i>	ATCC 4651	unknown	-
16	<i>Kocuria rhizophila</i>	ATCC 9341	Soil	-
17	<i>Lactobacillus casei</i>	NBRC 15883	Cheese	-
18	<i>Lactococcus lactis</i>	NS 6938	human	-
19	<i>Listeria monocytogenes</i>	VTU 206	unknown	-
20	<i>Micrococcus luteus</i>	NBRC 3333	unknown	-
21	<i>Morganella morganii</i>	ATCC 25830	Patient with summer diarrhea	-
22	<i>Paenibacillus polymyxa</i>	NBRC 15309	unknown	-
23	<i>Proteus mirabilis</i>	IFO 3849	unknown	-
24	<i>Pseudomonas aeruginosa</i>	NBRC 13275	Outer ear infection	-
25	<i>Pseudomonas stutzeri</i>	ATCC 17587	Bile	-
26	<i>Rhodococcus equi</i>	IFO 14956	Lung abscess of foal	-
27	<i>Salmonella</i> Typhimurium	ATCC 14028	Tissue, animal - pools of heart and liver from 4-week-old chickens	-
28	<i>Serratia marcescens</i> subsp. <i>Marcescens</i>	NBRC 102204	Pond water	-
29	<i>Shigella flexneri</i>	ATCC 12022	unknown	-
30	<i>Staphylococcus aureus</i> subsp. <i>Aureus</i>	NBRC 12732	unknown	-
31	<i>Staphylococcus epidermidis</i>	ATCC 35984	Catheter sepsis, Tennessee	-
32	<i>Streptococcus pyogenes</i>	JCM 5674	Scarlet fever	-

^aJCM = Japan Collection of Microorganisms (RIKEN BioResource Center, Ibaraki, Japan); ATCC = American Type Culture Collection (Manassas, VA, USA); IFO = Campden Culture Collection (Campden BRI, Chipping Campden, UK); NBRC = National Institute of Technology and Evaluation Biological Resource Center (Tokyo, Japan); VTU = Visvesvaraya Technological University (Bangalore, India)

^b"-" indicates growth did not occur.

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

Matrix	Contamination level	n ^a	CompactDry "Nissui" YMR				ISO 21527-1:2008			DOM ^d	95 % CI ^e		90 % CI	
			Mean Log ₁₀ CFU ^b /g	s _r	RSD _r ^c , %		Mean Log ₁₀ CFU/g	s _r	RSD _r , %		LCL ^f	UCL ^g	LCL	UCL
Fermented yogurt drink	Low	5	2.828	0.048	1.70		2.819	0.046	1.63	0.009	-0.076	0.094	0.056	0.074
	Medium	5	4.851	0.029	0.60		4.818	0.022	0.46	0.033	-0.007	0.073	0.003	0.064
	High	5	5.769	0.078	1.35		5.776	0.095	1.64	-0.008	-0.135	0.120	-0.106	0.090
Cream cheese	Low	5	2.767	0.107	3.87		2.629	0.057	2.17	0.139	0.010	0.268	0.040	0.238
	Medium	5	4.778	0.058	1.21		4.841	0.064	1.32	-0.064	-0.095	-0.032	-0.088	-0.039
	High	5	5.660	0.103	1.82		5.716	0.096	1.68	-0.057	-0.131	0.018	-0.114	0.001
Egg custard tarts	Low	5	2.420	0.049	2.02		2.370	0.157	6.62	0.050	-0.145	0.244	-0.099	0.199
	Medium	5	2.963	0.102	3.44		3.247	0.122	3.76	-0.284	-0.310	-0.257	-0.304	-0.263
	High	5	4.645	0.115	2.48		4.837	0.063	1.30	-0.192	-0.359	-0.025	-0.320	-0.063
Spinach and ricotta quiche	Low	5	2.396	0.050	2.09		2.343	0.062	2.65	0.053	-0.060	0.166	-0.034	0.139
	Medium	5	2.963	0.102	3.44		3.247	0.122	6.67	-0.284	-0.310	-0.257	-0.304	-0.263
	High	5	4.510	0.107	2.37		4.668	0.202	4.33	-0.158	-0.353	0.037	-0.308	-0.009
Deli vegetable salad	Low	5	2.180	0.090	4.13		2.457	0.151	6.15	-0.277	-0.534	-0.019	-0.475	-0.079
	Medium	5	3.968	0.066	1.66		3.934	0.057	1.45	0.033	-0.050	0.116	-0.030	0.097
	High	5	5.976	0.029	0.49		5.922	0.065	1.10	0.053	-0.027	0.133	-0.008	0.115
Fruit and vegetable smoothie	Low	5	3.427	0.057	1.66		3.537	0.109	3.08	-0.111	-0.246	0.024	-0.214	-0.007
	Medium	5	4.718	0.115	2.44		4.846	0.118	2.43	-0.128	-0.220	-0.035	-0.119	-0.057
	High	5	5.758	0.106	1.84		5.880	0.131	2.23	-0.122	-0.205	-0.039	-0.186	-0.059
Cooked prawns	Low	5	1.466	0.118	8.05		1.159	0.221	19.1	0.307	-0.013	0.627	0.061	0.553
	Medium	5	4.202	0.138	3.28		4.265	0.124	2.91	-0.063	-0.260	0.133	-0.215	0.088
	High	5	5.539	0.113	2.04		5.603	0.114	2.03	-0.064	-0.153	0.025	-0.133	0.004
Tuna pâté	Low	5	2.454	0.040	1.63		2.275	0.194	8.53	0.179	-0.080	0.438	-0.020	0.378
	Medium	5	4.208	0.136	3.23		4.279	0.187	4.37	-0.070	-0.209	0.069	-0.177	0.036
	High	5	5.485	0.094	1.71		5.394	0.082	1.52	0.091	-0.081	0.263	-0.041	0.223
Egg salad sandwiches	Low	5	2.459	0.161	6.55		2.436	0.134	5.50	0.022	-0.062	0.107	-0.043	0.087
	Medium	5	3.930	0.232	5.90		3.933	0.150	3.81	-0.003	-0.199	0.193	-0.153	0.147
	High	5	5.322	0.075	1.41		5.383	0.095	1.76	-0.060	-0.255	0.134	-0.210	0.089
Deli pasta salad	Low	5	2.991	0.060	2.01		3.120	0.091	2.92	-0.129	-0.304	0.046	-0.263	0.006
	Medium	5	3.833	0.065	1.70		3.810	0.121	3.18	0.024	-0.133	0.180	-0.097	0.144
	High	5	5.050	0.179	3.54		5.233	0.053	1.01	-0.183	-0.400	0.033	-0.350	-0.017

^an = number of replicate test portions

^bCFU = Colony-forming units

^fRSD_r = Relative standard deviation of repeatability

^gDOM = Difference of Means

^eCI = Confidence Interval for DOM

^fLCL = Lower confidence limit for DOM

^gUCL = Upper confidence limit for DOM

REFERENCES CITED

1. Mizuochi, S., Validation of the CompactDry “Nissui” YMR for Enumeration of Yeasts and Molds in a Variety of Foods, AOAC® *Performance Tested*SM certification number 092002.
2. ISO 21527-1:2008 Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of yeasts and moulds — Part 1: Colony count technique in products with water activity greater than 0.95
<https://www.iso.org/standard/38275.html> accessed June 2020