CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

082201

The AOAC Research Institute hereby certifies the method known as:

CompactDry "Nissui" TCR

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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METHOD NAME
CompactDry "Nissui" TCR

CATALOG NUMBERS
06539 (40 plates); 06540 (240 plates)

INDEPENDENT LABORATORY
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APPLICABILITY OF METHOD
Target Organism – Mesophilic aerobic bacteria.

Matrixes – (USDA MLG 3.02) – Raw ground beef (80% lean, 50 g), raw ground pork (50 g), raw pork (50 g), raw chicken breast (50 g)
(USDA BAM Ch. 3) – raw shrimp (50 g), raw cod (50 g), bagged pre-washed shredded iceberg lettuce (50 g), bagged pre-washed mixed lettuce and vegetables (50 g)
(SMEDP Ch. 6) (11 mL) – pasteurized whole milk, nonfat dry milk, and pasteurized heavy cream

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

REFERENCE METHODS
U.S. Department of Agriculture Food Safety and Inspection Service (2015) *Microbiology Laboratory Guidebook, Chapter 3.02, Quantitative Analysis of Bacteria in Foods as Sanitary Indicators* (2)

U.S. Food and Drug Administration (2001) *Bacteriological Analytical Manual, Chapter 3, Aerobic Plate Count* (3)

Laird, D.T., Gambrel-Lenarz, S.A., Scher, F.M., Graham, T.E., Reddy, R., & Maturin, L.J., (2012) in Standard Methods for the Examination of Dairy Products, 17th Ed. Wehr, H.M., & Frank, J.F. (Ed), APHA Press, Washington, D.C., Chapter 6. (4)

ORIGINAL CERTIFICATION DATE
August 11, 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, 082201 this method is distributed by:
NONE

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NONE

PRINCIPLE OF THE METHOD (1)
The CompactDry "Nissui" TCR method for enumeration of mesophilic aerobic bacteria is a dry media sheet comprised of a culture medium, redox indicator, and a cold-soluble gelling agent. The medium is rehydrated by adding 1 mL of prepared sample, which diffuses throughout the plate. After correct incubation, colonies are counted to determine the mesophilic aerobic colony count in the sample.

DISCUSSION OF THE VALIDATION STUDY (1)
The results of the method developer studies indicate that the CompactDry "Nissui" TCR method at 35 ± 1°C and 32 ± 1°C (for dairy matrixes) at 24 h, 48 h and 72 h (for nonfat dry milk) of incubation can be used for rapid and accurate enumeration of mesophilic aerobic bacteria in a variety of food commodities, including raw ground beef, raw ground pork, raw pork, raw chicken breast, raw shrimp, raw cod, bagged pre-washed shredded iceberg lettuce, bagged pre-washed mixed lettuce and vegetables, pasteurized whole milk, nonfat dry milk, and pasteurized heavy cream. The results of the independent laboratory study verify the method performance of the CompactDry "Nissui" TCR method for enumeration of mesophilic aerobic bacteria in raw ground beef, raw ground pork, nonfat dry milk, and pasteurized heavy cream. In both studies, the CompactDry "Nissui" TCR method showed similar repeatability to the reference methods and equivalent mean results.
The CompactDry "Nissui" TCR method offers a time saving of 1 - 2 days over the reference methods. There is a reduction in the amount of technical labor required in preparation of agar. There are advantages in reduction of storage space, waste disposal and required incubator space.

Table 3. Method comparison data summary and statistics for CompactDry "Nissui" TCR at 24 h (1)

			CompactDry "Nissui" TCR		Reference Method[c]			95% CI[e]		90% CI	
Matrix[a]	Cont. level	n	Mean $\log_{10}$	s_r[b]	Mean $\log_{10}$	s_r	DOM[d]	LCL[f]	UCL[g]	LCL	UCL
Raw ground beef, 70% lean	Low	5	4.131	0.056	4.314	0.067	-0.183	-0.276	-0.091	-0.254	-0.112
	Medium	5	6.392	0.051	6.373	0.061	0.019	-0.026	0.064	-0.016	0.054
	High	5	8.884	0.042	8.957	0.062	-0.074	-0.140	-0.007	-0.125	-0.023
Raw ground beef, 80% lean (IL data[h])	Low	5	3.352	0.050	3.397	0.033	-0.045	-0.103	0.013	-0.090	-0.001
	Medium	5	5.348	0.044	5.466	0.050	-0.118	-0.159	-0.076	-0.149	-0.086
	High	5	7.429	0.049	7.431	0.063	-0.002	-0.093	0.089	-0.072	0.068
Raw ground pork, 75% lean	Low	5	4.169	0.043	4.326	0.070	-0.157	-0.213	-0.101	-0.200	-0.114
	Medium	5	6.319	0.056	6.711	0.086	-0.392	-0.498	-0.285	-0.474	-0.310
	High	5	8.400	0.033	8.543	0.080	-0.143	-0.242	-0.043	-0.219	-0.066
Raw ground pork, 75% lean (IL data)	Low	5	4.041	0.099	4.087	0.119	-0.046	-0.099	0.007	-0.087	-0.006
	Medium	5	6.134	0.112	6.114	0.084	0.020	-0.032	0.072	-0.020	0.060
	High	5	7.684	0.060	7.725	0.065	-0.042	-0.128	0.044	-0.108	0.024
Raw pork, 90% lean	Low	5	3.291	0.038	3.557	0.088	-0.265	-0.347	-0.184	-0.328	-0.203
	Medium	5	6.870	0.077	6.864	0.074	0.006	-0.029	0.040	-0.021	0.032
	High	5	8.344	0.050	8.357	0.035	-0.013	-0.042	0.017	-0.035	0.010
Raw chicken breast	Low	5	3.263	0.046	3.275	0.064	-0.012	-0.105	0.081	-0.083	0.059
	Medium	5	5.712	0.139	5.613	0.077	0.099	-0.068	0.265	-0.029	0.227
	High	5	8.009	0.040	8.140	0.014	-0.131	-0.177	-0.085	-0.166	-0.096
Raw shrimp, heads off	Low	5	4.198	0.023	4.343	0.064	-0.145	-0.236	-0.054	-0.214	-0.075
	Medium	5	5.719	0.072	5.770	0.083	-0.051	-0.164	0.062	-0.138	0.036
	High	5	7.123	0.062	7.024	0.036	0.099	0.014	0.184	0.034	0.164
Raw cod filet	Low	5	5.048	0.079	5.163	0.144	-0.115	-0.308	0.079	-0.263	0.034
	Medium	5	6.562	0.067	6.658	0.085	-0.096	-0.250	0.057	-0.214	0.022
	High	5	8.522	0.086	8.661	0.087	-0.139	-0.224	-0.055	-0.204	-0.075
Bagged pre-washed shredded iceberg lettuce	Low	5	4.156	0.056	4.451	0.148	-0.296	-0.431	-0.160	-0.400	-0.192
	Medium	5	5.327	0.058	5.687	0.090	-0.359	-0.509	-0.209	-0.474	-0.244
	High	5	6.337	0.080	6.409	0.149	-0.072	-0.169	0.025	-0.147	0.002
Bagged pre-washed mixed lettuce and vegetables	Low	5	5.175	0.090	5.090	0.066	0.085	0.018	0.152	0.034	0.137
	Medium	5	7.118	0.050	7.011	0.053	0.107	0.053	0.162	0.065	0.149
	High	5	8.097	0.062	8.029	0.090	0.068	0.000	0.137	0.016	0.121
Pasteurized whole milk 3.9% fat (35 ± 1°C)	Low	5	3.249	0.040	3.336	0.087	-0.087	-0.221	0.047	-0.190	0.016
	Medium	5	4.729	0.058	4.727	0.130	0.002	-0.093	0.097	-0.071	0.075
	High	5	7.450	0.023	7.575	0.058	-0.125	-0.203	-0.046	-0.185	-0.065
Pasteurized whole milk 3.9% fat (32 ± 1°C)	Low	5	3.281	0.051	3.336	0.087	-0.055	-0.177	0.068	-0.149	0.039
	Medium	5	4.746	0.102	4.727	0.130	0.018	-0.069	0.106	-0.049	0.085
	High	5	7.454	0.054	7.575	0.058	-0.121	-0.203	-0.039	-0.184	-0.058
Nonfat dry milk (35 ± 1°C)	Low	5	2.606	0.059	2.655	0.119	-0.048	-0.197	0.101	-0.163	0.066
	Medium	5	3.621	0.029	3.578	0.039	0.042	-0.009	0.093	0.003	0.081
	High	5	4.813	0.031	4.721	0.063	0.093	0.010	0.176	0.029	0.157
Nonfat dry milk (32 ± 1°C)	Low	5	2.603	0.113	2.655	0.119	-0.051	-0.205	0.102	-0.169	0.066
	Medium	5	3.618	0.164	3.578	0.039	0.039	-0.169	0.248	-0.121	0.199
	High	5	4.813	0.093	4.721	0.063	0.092	-0.010	0.195	0.013	0.171
Nonfat dry milk (35 ± 1°C) (IL data)	Low	5	2.707	0.050	2.656	0.072	0.051	-0.001	0.103	0.011	0.091
	Medium	5	3.606	0.075	3.541	0.079	0.065	0.022	0.108	0.032	0.098
	High	5	4.618	0.096	4.627	0.083	-0.010	-0.068	0.048	-0.054	0.035
Nonfat dry milk (32 ± 1°C) (IL data)	Low	5	2.738	0.046	2.656	0.072	0.082	-0.004	0.169	0.016	0.149
	Medium	5	3.635	0.072	3.541	0.079	0.094	0.055	0.133	0.064	0.124
	High	5	4.643	0.086	4.627	0.083	0.016	-0.031	0.063	-0.020	0.052
Past. heavy cream, 36.0% fat (35 ± 1°C)	Low	5	3.849	0.094	3.805	0.122	0.044	0.002	0.086	0.012	0.076
	Medium	5	4.287	0.145	4.308	0.125	-0.021	-0.062	0.019	-0.053	0.010
	High	5	8.119	0.096	8.149	0.064	-0.030	-0.093	0.034	-0.078	0.019
Past. heavy cream, 36.0% fat (32 ± 1°C)	Low	5	3.761	0.112	3.805	0.122	-0.044	-0.105	0.018	-0.091	0.003
	Medium	5	4.294	0.115	4.308	0.125	-0.014	-0.061	0.032	-0.050	0.021
	High	5	8.166	0.070	8.149	0.064	0.017	-0.034	0.068	-0.022	0.056
Past. Heavy cream 36.0% fat (35 ± 1°C) (IL data)	Low	5	2.954	0.030	2.967	0.033	-0.012	-0.024	0.000	-0.022	-0.003
	Medium	5	3.983	0.034	3.954	0.024	0.029	-0.040	0.099	-0.024	0.083
	High	5	4.955	0.043	4.965	0.021	-0.011	-0.085	0.064	-0.068	0.047
Past. Heavy cream 36.0% fat (32 ± 1°C) (IL data)	Low	5	2.990	0.016	2.967	0.033	0.024	-0.011	0.058	-0.003	0.050
	Medium	5	3.961	0.026	3.954	0.024	0.008	-0.032	0.048	-0.023	0.038
	High	5	4.950	0.036	4.965	0.021	-0.015	-0.057	0.027	-0.047	0.017

[a]Matrixes except for nonfat dry milk and pasteurized heavy cream (IL data) are naturally contaminated.

[b]s_r = standard deviation of replicates

[c]Reference methods are as follows: FSIS MLG 3.02 (ground beef, ground pork, pork, chicken); FDA BAM Ch3 (shrimp, cod, lettuce, lettuce and vegetables); APHA SMEDP Ch6 (liquid milk, dry milk, cream).

[d]DOM = Difference of means; $mean_{cand}$ - $mean_{ref}$

[e]CI = Confidence interval for DOM

[f]LCL = Lower confidence limit for DOM

[g]UCL = Upper confidence limit for DOM

[h]IL data = Independent laboratory data

Table 4. Method comparison data summary and statistics for CompactDry "Nissui" TCR at 48 h (1)

			CompactDry "Nissui" TCR		Reference Method[c]			95% CI[e]		90% CI	
Matrix[a]	Cont. level	n	Mean log_{10}	s_r[b]	Mean log_{10}	s_r	DOM[d]	LCL[f]	UCL[g]	LCL	UCL
Raw ground beef, 70% lean	Low	5	4.264	0.032	4.314	0.067	-0.050	-0.152	0.051	-0.128	0.028
	Medium	5	6.595	0.088	6.373	0.061	0.222	0.143	0.302	0.161	0.284
	High	5	9.043	0.050	8.957	0.062	0.086	0.006	0.166	0.024	0.147
Raw ground beef, 80% lean (IL data[h])	Low	5	3.429	0.052	3.397	0.033	0.032	-0.035	0.098	-0.019	0.083
	Medium	5	5.446	0.040	5.466	0.050	-0.020	-0.089	0.049	-0.073	0.033
	High	5	7.474	0.055	7.431	0.063	0.043	-0.053	0.140	-0.031	0.117
Raw ground pork, 75% lean	Low	5	4.281	0.046	4.326	0.070	-0.044	-0.123	0.035	-0.105	0.017
	Medium	5	6.630	0.099	6.711	0.086	-0.081	-0.202	0.040	-0.174	0.012
	High	5	8.505	0.060	8.543	0.080	-0.038	-0.169	0.092	-0.139	0.062
Raw ground pork, 75% lean (IL data)	Low	5	4.085	0.091	4.087	0.119	-0.001	-0.084	0.081	-0.065	0.062
	Medium	5	6.154	0.105	6.114	0.084	0.040	-0.004	0.084	0.006	0.074
	High	5	7.767	0.061	7.725	0.065	0.042	-0.048	0.132	-0.027	0.112
Raw pork, 90% lean	Low	5	3.450	0.026	3.557	0.088	-0.107	-0.189	-0.024	-0.170	-0.043
	Medium	5	6.889	0.082	6.864	0.074	0.025	-0.023	0.074	-0.012	0.062
	High	5	8.355	0.056	8.357	0.035	-0.002	-0.041	0.037	-0.032	0.028
Raw chicken breast	Low	5	3.334	0.018	3.275	0.064	0.059	-0.012	0.130	0.004	0.114
	Medium	5	5.723	0.160	5.613	0.077	0.109	-0.078	0.297	-0.034	0.253
	High	5	8.118	0.051	8.140	0.014	-0.022	-0.080	0.037	-0.066	0.023
Raw shrimp, heads off	Low	5	4.318	0.023	4.343	0.064	-0.025	-0.115	0.064	-0.094	0.043
	Medium	5	5.817	0.053	5.770	0.083	0.046	-0.041	0.134	-0.021	0.114
	High	5	7.142	0.068	7.024	0.036	0.118	0.033	0.202	0.053	0.182
Raw cod filet	Low	5	5.150	0.093	5.163	0.144	-0.013	-0.205	0.180	-0.160	0.135
	Medium	5	6.622	0.058	6.658	0.085	-0.036	-0.158	0.086	-0.129	0.058
	High	5	8.548	0.081	8.661	0.087	-0.113	-0.232	0.005	-0.204	-0.023
Bagged pre-washed shredded iceberg lettuce	Low	5	4.212	0.055	4.451	0.148	-0.239	-0.388	-0.090	-0.353	-0.125
	Medium	5	5.345	0.044	5.687	0.090	-0.342	-0.485	-0.199	-0.451	-0.232
	High	5	6.355	0.083	6.409	0.149	-0.054	-0.161	0.054	-0.136	0.029
Bagged pre-washed mixed lettuce and vegetables	Low	5	5.254	0.079	5.090	0.066	0.165	0.098	0.232	0.113	0.216
	Medium	5	7.111	0.053	7.011	0.053	0.100	0.035	0.166	0.050	0.150
	High	5	8.089	0.073	8.029	0.090	0.060	0.001	0.119	0.015	0.105
Pasteurized whole milk, 3.9% fat (35 ± 1°C)	Low	5	3.269	0.035	3.336	0.087	-0.067	-0.204	0.069	-0.172	0.037
	Medium	5	4.755	0.065	4.727	0.130	0.028	-0.059	0.114	-0.039	0.094
	High	5	7.456	0.026	7.575	0.058	-0.119	-0.198	-0.040	-0.179	-0.058
Pasteurized whole milk, 3.9% fat (32 ± 1°C)	Low	5	3.297	0.066	3.336	0.087	-0.039	-0.151	0.074	-0.125	0.048
	Medium	5	4.783	0.086	4.727	0.130	0.056	-0.037	0.149	-0.016	0.127
	High	5	7.443	0.031	7.575	0.058	-0.131	-0.217	-0.046	-0.197	-0.066
Nonfat dry milk (35 ± 1°C)	Low	5	2.611	0.056	2.655	0.119	-0.044	-0.189	0.102	-0.156	0.068
	Medium	5	3.616	0.032	3.578	0.039	0.038	-0.014	0.090	-0.002	0.077
	High	5	4.813	0.034	4.721	0.063	0.093	0.008	0.178	0.028	0.158
Nonfat dry milk (32 ± 1°C)	Low	5	2.607	0.114	2.655	0.119	-0.047	-0.195	0.101	-0.161	0.066
	Medium	5	3.625	0.157	3.578	0.039	0.047	-0.154	0.248	-0.107	0.201
	High	5	4.817	0.090	4.721	0.063	0.097	-0.005	0.198	0.018	0.175
Nonfat dry milk (35 ± 1°C) (IL data)	Low	5	2.700	0.035	2.656	0.072	0.044	-0.047	0.134	-0.026	0.113
	Medium	5	3.598	0.067	3.541	0.079	0.057	0.013	0.101	0.024	0.091
	High	5	4.615	0.112	4.627	0.083	-0.012	-0.073	0.048	-0.059	0.034
Nonfat dry milk (32 ± 1°C) (IL data)	Low	5	2.738	0.046	2.656	0.072	0.082	-0.004	0.169	0.016	0.149
	Medium	5	3.647	0.071	3.541	0.079	0.106	0.070	0.142	0.078	0.134
	High	5	4.640	0.092	4.627	0.083	0.013	-0.036	0.062	-0.025	0.051
Past. heavy cream, 36.0% fat (35 ± 1°C)	Low	5	3.849	0.093	3.805	0.122	0.044	0.003	0.085	0.012	0.076
	Medium	5	4.284	0.141	4.308	0.125	-0.025	-0.060	0.011	-0.052	0.003
	High	5	8.126	0.089	8.149	0.064	-0.022	-0.087	0.043	-0.072	0.028
Past. heavy cream, 36.0% fat (32 ± 1°C)	Low	5	3.761	0.112	3.805	0.122	-0.044	-0.105	0.018	-0.091	0.003
	Medium	5	4.289	0.118	4.308	0.125	-0.019	-0.053	0.014	-0.045	0.006
	High	5	8.166	0.070	8.149	0.064	0.017	-0.034	0.068	-0.022	0.056
Past. heavy cream, 36.0% fat (35 ± 1°C) (IL data)	Low	5	2.964	0.034	2.967	0.033	-0.003	-0.018	0.013	-0.015	0.009
	Medium	5	3.972	0.041	3.954	0.024	0.018	-0.053	0.089	-0.037	0.072
	High	5	4.941	0.044	4.965	0.021	-0.024	-0.102	0.053	-0.084	0.035
Past. heavy cream, 36.0% fat (32 ± 1°C) (IL data)	Low	5	2.969	0.030	2.967	0.033	0.002	-0.036	0.040	-0.027	0.031
	Medium	5	3.955	0.022	3.954	0.024	0.001	-0.041	0.043	-0.031	0.033
	High	5	4.935	0.044	4.965	0.021	-0.031	-0.068	0.007	-0.059	-0.002

[a]Matrixes except for nonfat dry milk and pasteurized heavy cream (IL data) are naturally contaminated.

[b]S_r = standard deviation of replicates

[c]Reference methods are as follows: FSIS MLG 3.02 (ground beef, ground pork, pork, chicken); FDA BAM Ch3 (shrimp, cod, lettuce, lettuce and vegetables); APHA SMEDP Ch6 (liquid milk, dry milk, cream).

[d]DOM = Difference of means; $mean_{cand}$ - $mean_{ref}$

[e]CI = Confidence interval for DOM

[f]LCL = Lower confidence limit for DOM

[g]UCL = Upper confidence limit for DOM

[h]IL data = Independent laboratory data

Table 5. Method comparison data summary and statistics for CompactDry "Nissui" TCR at 72 h (1)

			CompactDry "Nissui" TCR		Reference Method[b]			95% CI[d]		90% CI	
Matrix	Cont. level	n	Mean $\log_{10}$	s_r[a]	Mean $\log_{10}$	s_r	DOM[c]	LCL[e]	UCL[f]	LCL	UCL
Nonfat dry milk	Low	5	2.609	0.056	2.655	0.119	-0.046	-0.195	0.103	-0.160	0.069
(35 ± 1°C)	Medium	5	3.625	0.029	3.578	0.039	0.046	0.000	0.093	0.011	0.082
	High	5	4.812	0.034	4.721	0.063	0.091	0.008	0.175	0.028	0.155
Nonfat dry milk	Low	5	2.605	0.114	2.655	0.119	-0.050	-0.202	0.103	-0.167	0.068
(32 ± 1°C)	Medium	5	3.675	0.086	3.578	0.039	0.096	-0.017	0.209	0.010	0.183
	High	5	4.816	0.088	4.721	0.063	0.096	-0.004	0.195	0.019	0.172
Nonfat dry milk	Low	5	2.731	0.044	2.656	0.072	0.075	0.030	0.119	0.040	0.109
(35 ± 1°C)	Medium	5	3.621	0.072	3.541	0.079	0.080	0.039	0.121	0.048	0.112
(IL data[g])	High	5	4.625	0.100	4.627	0.083	-0.002	-0.052	0.047	-0.040	0.036
Nonfat dry milk	Low	5	2.756	0.061	2.656	0.072	0.099	-0.007	0.206	0.017	0.182
(32 ± 1°C)	Medium	5	3.656	0.072	3.541	0.079	0.115	0.072	0.159	0.082	0.149
(IL data)	High	5	4.651	0.084	4.627	0.083	0.024	-0.027	0.075	-0.015	0.063

[a] s_r = standard deviation of replicates
[b] Reference methods are APHA SMEDP Ch6.
[c] DOM = Difference of means; $mean_{cand}$ - $mean_{ref}$
[d] CI = Confidence interval for DOM
[e] LCL = Lower confidence limit for DOM
[f] UCL = Upper confidence limit for DOM
[g] IL data = Independent laboratory data

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