CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

010404

The AOAC Research Institute hereby certifies the method known as:

Compact Dry "Nissui" Total Count

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

Issue Date January 9, 2023

Expiration Date December 31, 2023

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SUBMITTING COMPANY
NISSUI Pharmaceutical Co., Ltd.
3-24-6, Ueno
Taito-ku, Tokyo
Japan 110-8736

METHOD NAME
Compact Dry "Nissui" Total Count

CATALOG NUMBERS
Nissui 06740 (40 plates), 06741 (240 plates)

INDEPENDENT LABORATORY ORIGINAL VALIDATION
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APPLICABILITY OF METHOD
Target Organism – Aerobic microbial cells

Matrixes – raw meat (50 g) (raw ground pork, raw pork, raw lamb, raw veal, raw ground beef)
MODIFICATION APRIL 2015 – cooked chicken (10 g), lettuce (10 g), milk powder (10 g), frozen fish (10 g), pasteurized milk (1 mL)
MODIFICATION DECEMBER 2020 – raw ground beef (50 g), raw chicken breast (50 g)

Performance claims – The study data detected no statistical difference between the Compact Dry "Nissui" Total Count method and the reference methods.

REFERENCE METHODS
Official Methods of Analysis 19th Ed., AOAC INTERNATIONAL, Rockville, MD, Method 966.23, Microbiological Methods (2)
ISO 4ISO 4833:2003, Microbiology of food and animal feeding stuffs – Horizontal Method for the enumeration of microorganisms – Colony-count technique at 30°C. (3)
FSIS MLG 3.02 Quantitative Analysis of Bacteria in Foods as Sanitary Indicators: Chapter 3.6 Aerobic Plate Count (6)

ORIGINAL CERTIFICATION DATE
February 2004

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
1. **April 2015 Level 2**
2. **February 2019 Level 2**
3. **December 2020 Level 3**

SUMMARY OF MODIFICATION
1. **Matrix extension to include cooked chicken, pre-washed bagged shredded iceberg lettuce, frozen cod fillets, instant non-fat dry milk, pasteurized milk (2%).**
2. **Shelf life increased to 24 months and corporate address change.**
3. **Matrix extension for raw chicken breast enumeration at 24 ± 2 h and 48 ± 3 h. Added claim for raw ground beef at 24 ± 2 h.**

Under this AOAC *Performance Tested Methods*SM License Number, 010404 this method is distributed by:
1. **Hardy Diagnostics**
2. **R-Biopharm AG**

Under this AOAC *Performance Tested Methods*SM License Number, 010404 this method is distributed as:
1. **Compact Dry TC**
2. **Compact Dry TC**

PRINCIPLE OF THE METHOD (1)
Compact Dry TC qualifies as a rapid method kit for determining aerobic colony counts in foods. The plates are presterilized and contain culture medium and a cold-soluble gelling agent. The medium is rehydrated by inoculating 1 mL diluted sample into the center of the self-diffusible medium and allowing the solution to diffuse by capillary action. The plates can then be incubated and the colonies counted without any additional steps. The Compact Dry TC method was validated with 5 different raw meats. The performance tests were conducted at 35° and 30°C. In all required performance studies, no apparent differences were observed between the Compact Dry TC method and the Standard Pour Plate method (AOAC Official Method **966.23**) for the detection level of aerobic microorganisms. For the accuracy claim (n = 60), a correlation factor of r^2 35 = 0.9977 (35˚C) and r^2 30 = 0.9932 (30˚C) could be assigned, as stated in the application for "Performance Tested Method℠" Quality consistency and storage robustness studies, showed no significant variations in plate count results with different production lots or plates of diverse storage age.

DISCUSSION OF THE VALIDATION STUDY (1)

The retested results, as instructed by AOAC RI with exactly the same sample preparation procedure as AOAC Official Method **966.23** showed equal results on both the Compact Dry TC Method and AOAC Official Method **966.23**. As evidenced by attached data of 5 replicates for 3 levels (4 levels for raw veal) of 5 kinds of raw meat (n = 80) through internal and independent validation study, r^2 as correlation coefficient for both methods was 0.9965. This means that the retested results are the same as the original, and therefore discussion on the original report must be valid.

Regarding the measured value, for only the lowest level, the value between Compact Dry TC and AOAC Official Method **966.23** differed from each other more than the range of the standard deviation, and by one-way ANOVA(P< 0.05) in independent validation studies. This phenomenon could not be observed with the other bacterial load levels and with the other 4 matrixes (raw ground pork, raw pork, raw lamb, and raw veal) by internal validation study. For all remaining levels and samples by internal validation study, the standard deviation of analogous samples delivered comparable values for the Compact Dry TC method and the AOAC Official Method **966.23**. Consequently, the results from the Compact Dry TC method for the repeatability study correspond with the results from pour plate methods.

Using an incubation temperature of 30°C instead of the recommended 35˚C, which is the more common incubation temperature for total aerobic count estimations in the food regulations of the European Community, the results of the repeatability, accuracy, lot consistency, and lot stability obtained from the different matrixes were similar to those at 35°C. Using yogurt in the repeatability study, the 30˚C incubation series exhibited major differences. The counts of the dilutions 104 and 103 measured with Compact

Dry TC were significantly lower than those with the pour plate method. Dilution 2.5 x 10^2 and 10^2 did not give any count with the Compact Dry TC technique. Because those results with yogurt could not be observed in the accuracy, lot consistency, and lot stability study at 30˚C incubation, the results of the repeatability study might be attributed to internal errors.

The big advantages of the Compact Dry TC system are the reduced hands-on time and economical usage, as confirmed by the independent laboratory. In terms of plate preparation, inoculation, and reading the result, the Compact Dry TC system was easier and quicker than the conventional pour plate technique. Reading the plates was faster with the Compact Dry TC system, with the TTC indicator speeding up counting. It was observed that food particles, when present, did not appear to absorb the indicator. Food particles in the pour plate made reading plates and counting colonies relatively more difficult. For the Compact Dry TC system, less training is required than for the pour plate technique. Instructions on the use of the Compact Dry TC are clear and unambiguous. The Compact Dry TC system would also bring advantages in reduced storage space, waste disposal, and required incubator space. The long shelf life of the product also has benefits compared to ready-prepared agar, which has a limited shelf life and therefore requires more logistical planning.

Table 1. AOAC method comparison (raw ground pork) (1)

			Compact Dry TC aerobic bacteria		AOAC **966.23** aerobic bacteria	
Level	Spike, CFU/g	Replicate	CFU/g	Log_{10} CFU/g	CFU/g	log_{10} CFU/g
About 10^2 CFU/g Lot 1	0[a]	1	525	2.72	500	2.70
		2	680	2.83	670	2.83
		3	430	2.63	415	2.62
		4	635	2.80	635	2.80
		5	365	2.56	355	2.55
		Mean	527	2.71	515	2.70
		s_r	133	0.11	136	0.12
		RSD_r, %	25.2	4.18	26.5	4.37
About 10^4 CFU/g Lot 2	0[a]	1	78000	4.89	70500	4.85
		2	80000	4.90	76000	4.88
		3	77000	4.89	78000	4.89
		4	99000	5.00	96000	4.98
		5	55000	4.74	42500	4.63
		Mean	77800	4.88	72600	4.85
		s_r	15600	0.09	19400	0.13
		RSD_r, %	20.1	1.87	26.7	2.72
About 10^6 CFU/g Lot 3	0[a]	1	6400000	6.81	5850000	6.77
		2	9100000	6.96	8850000	6.95
		3	9350000	6.97	9150000	6.96
		4	6800000	6.83	6400000	6.81
		5	9300000	6.97	8250000	6.92
		Mean	8190000	6.91	7700000	6.88
		s_r	1460000	0.08	1490000	0.09
		RSD_r, %	17.8	1.17	19.3	1.27

[a] Naturally contaminated samples.

Table 2. AOAC method comparison (raw pork) (1)

			Compact Dry TCaerobic bacteria		AOAC **966.23** aerobic bacteria	
Level	Spike, CFU/g	Replicate	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
About 10^2 CFU/g Lot 1	0[a]	1				
			125	2.10	165	2.22
		2	180	2.26	170	2.23
		3	100	2.00	90	1.95
		4	145	2.16	100	2.00
		5	120	2.08	170	2.23
		Mean	134	2.12	139	2.13
		s_r	30.3	0.10	40.4	0.14
		RSD_r, %	22.6	4.52	29.0	6.46
About 10^4 CFU/g Lot 2	0[a]	1	37500	4.57	37500	4.57
		2	35000	4.54	35000	4.54
		3	96500	4.98	96500	4.98
		4	16100	4.21	15800	4.20
		5	18350	4.26	10400	4.02
		Mean	40690	4.51	39040	4.46
		s_r	32600	0.31	34200	0.37
		RSD_r, %	80.2	6.85	87.6	8.38
About 10^6 CFU/g Lot 3	0[a]	1	3700000	6.57	3500000	6.54
		2	2855000	6.46	2840000	6.45
		3	7800000	6.89	7550000	6.88
		4	1210000	6.08	1300000	6.11
		5	1545000	6.19	1775000	6.25
		Mean	3420000	6.44	3393000	6.45
		s_r	2640000	0.32	2480000	0.29
		RSD_r, %	77.3	4.98	73.1	4.56

[a] Naturally contaminated samples.

Table 3. AOAC method comparison (raw lamb) (1)

			Compact Dry TCaerobic bacteria		AOAC **966.23** aerobic bacteria	
Level	Spike, CFU/g	Replicate	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
About 10^2 CFU/g Lot 1	0[a]	1				
			255	2.41	225	2.35
		2	370	2.57	360	2.56
		3	455	2.66	410	2.61
		4	145	2.16	130	2.11
		5	840	2.92	965	2.98
		Mean	413	2.54	418	2.52
		s_r	266	0.28	325	0.32
		RSD_r, %	64.4	11.2	77.8	12.8
About 10^4 CFU/g Lot 2	0[a]	1	19450	4.29	18900	4.28
		2	13400	4.13	13600	4.13
		3	83500	4.92	79000	4.90
		4	15000	4.18	14200	4.15
		5	10300	4.01	9700	3.99
		Mean	28330	4.31	27100	4.29
		s_r	31000	0.36	29200	0.36
		RSD_r, %	109	8.33	108	8.28
About 10^6 CFU/g Lot 3	0[a]	1	5850000	6.77	4800000	6.68
		2	1610000	6.21	1120000	6.05
		3	2020000	6.31	2025000	6.31
		4	3700000	6.57	3500000	6.54
		5	1290000	6.11	1320000	6.12
		Mean	2890000	6.39	2553000	6.34
		s_r	1900000	0.27	1565118.206	0.27
		RSD_r, %	65.5	4.23	61.30506096	4.26

[a] Naturally contaminated samples.

Table 4. AOAC method comparison (raw veal) (1)

			Compact Dry TCaerobic bacteria		AOAC **966.23** aerobic bacteria	
Level	Spike, CFU/g	Replicate	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
0 CFU/g Lot 1	None	1	5	0.70	5	0.70
		2	10	1.00	5	0.70
		3	20	1.30	10	1.00
		4	10	1.00	5	0.70
		5	10	1.00	10	1.00
		Mean	11	1.00	7	0.82
		s_r	5.48	0.21	2.74	0.16
		RSD_r, %	49.8	21.3	39.1	20.1
About 10^2 CFU/g Lot 1	250	1	260	2.41	256	2.41
		2	224	2.35	238	2.38
		3	170	2.23	172	2.24
		4	292	2.47	284	2.45
		5	220	2.34	230	2.36
		Mean	233.2	2.36	236	2.37
		s_r	45.9	0.09	41.4	0.08
		RSD_r, %	19.7	3.75	17.5	3.44
About 10^4 CFU/g Lot 2	25000	1	23200	4.37	23200	4.37
		2	11800	4.07	11900	4.08
		3	13700	4.14	13580	4.13
		4	56000	4.75	53800	4.73
		5	14680	4.17	14800	4.17
		Mean	23900	4.30	23500	4.29
		s_r	18500	0.27	17500	0.27
		RSD_r, %	77.4	6.39	74.7	6.21
About 10^6 CFU/g Lot 3	1500000	1	1340000	6.13	1320000	6.12
		2	1780000	6.25	1540000	6.19
		3	860000	5.93	1210000	6.08
		4	1100000	6.04	1290000	6.11
		5	1540000	6.19	1340000	6.13
		Mean	1324000	6.11	1340000	6.13
		s_r	361000	0.12	122000	0.04
		RSD_r, %	27.2	2.03	9.12	0.63

Table 6. Method comparison (independent laboratory data) (1)

			Compact Dry TCaerobic bacteria		AOAC **966.23** aerobic bacteria	
Level	Spike, CFU/g	Replicate	CFU/g	Log_{10} CFU/g	CFU/g	Log_{10} CFU/g
About 10^4 CFU/g Lot 1	0[a]	1	15300	4.18	25400	4.40
		2	9600	3.98	24650	4.39
		3	10900	4.04	20200	4.31
		4	11100	4.05	27950	4.45
		5	10600	4.03	20700	4.32
		Mean	11500	4.06	23780	4.37
		s_r	2200	0.08	3280	0.06
		RSD_r, %	19.1	1.89	13.8	1.38
About 10^6 CFU/g Lot 2	0[a]	1	1590000	6.20	1300000	6.11
		2	1660000	6.22	1335000	6.13
		3	1870000	6.27	1780000	6.25
		4	1110000	6.05	980000	5.99
		5	1850000	6.27	1680000	6.23
		Mean	1616000	6.20	1415000	6.14
		s_r	307000	0.09	321000	0.10
		RSD_r, %	19.0	1.49	22.7	1.68
About 10^8 CFU/g Lot 3	0[a]	1	93000000	7.97	86500000	7.94
		2	125000000	8.10	111500000	8.05
		3	164000000	8.21	128000000	8.11
		4	120000000	8.08	104000000	8.02
		5	190000000	8.28	158500000	8.20
		Mean	138400000	8.13	117700000	8.06
		s_r	38400000	0.12	27250000	0.10
		RSD_r, %	27.7	1.50	23.2	1.22

[a] Naturally contaminated samples.

DISCUSSION OF MODIFICATION STUDY APPROVED APRIL 2015 (4)

For this matrix extension study, the Compact Dry TC was evaluated at two time points, 48 ± 3 h, which is recommended by Nissui and 72 ± 3 h, which is consistent with ISO 4833. Results from each time point were compared to ISO 4833 and to each other. Statistical analyses indicate that there were no significant differences in enumeration of total aerobic counts in cooked chicken, lettuce, frozen fish, milk powder and pasteurized milk between the two time points tested, and thus 48 h is still recommended.

In the single laboratory matrix studies for cooked chicken, lettuce, frozen fish, and milk powder, statistical differences were found between the Compact Dry TC and ISO 4833 for two levels each out of five levels tested for lettuce and frozen fish. In both matrixes, the differences were found in the mid and high contamination levels. For the 48 h Compact Dry TC time point, the mean log_{10} differences were >0.5 (0.702 and 0.624 for lettuce, and 0.708 and 0.522 for frozen fish), and although the CIs were small [(0.601, 0.802) and (0.529, 0.719) for lettuce, and (0.538, 0.879) and (0.448, 0.595) for fish], the CIs were outside (-0.5, 0.5) acceptance criterion. In each case, the reference method recorded higher results than the Compact Dry TC. For the other three levels tested for lettuce and fish, mean differences were small (<0.5) and CIs were within (-0.5, 0.5). No significant differences were found in cooked chicken and milk powder. Statistical outliers were detected using Grubb's test in cooked chicken and lettuce, but no justifiable cause was noted in the study for removing the outliers, and so all data were included in the analysis. The r^2 was >0.97 for all matrixes.

In the multi-laboratory study on pasteurized milk, no statistical differences were found between any of the method comparisons after Laboratory 4 was removed from the analysis for unexpectedly high counts in the uncontaminated samples. Due to shipping and scheduling issues, four laboratories initiated testing one day later than the other ten laboratories. An initial review of the data indicated no significant differences in results between the two start dates, so all laboratories (with the exception of Laboratory 4) were included in the statistical analysis. Across 14 data sets, the mean differences between Compact Dry TC at 48 and 72 h were -0.001 to 0.001 (r^2 = 1.0), and the mean differences between the Compact Dry TC at 48 h and ISO 4833 were -0.60 to 0.101 (r^2 = 0.98). The CIs on the mean differences were well within the (-0.5, 0.5) acceptance criterion, and even smaller (less than -0.2, 0.2) for the contaminated samples. The s_r, RSD_r, s_R, and RSD_R were equally small. These data indicate that the Compact Dry TC gives consistent results, comparable to the reference method, across laboratories.

MODIFICATION DATA APPROVED APRIL 2015 (4)

Table 1. Single laboratory matrix study: Compact Dry TC 48 h vs ISO 4833 (4)

Matrix	Cont. level	Compact Dry TC 48 h			ISO 4833			Mean diff.[d]	95% CI[e]		r^{2h}
		Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Cooked chicken	1	0.281	0.594	211	0.357	0.757	212	0.076	-0.070	0.219	
	2	2.399	0.304	12.7	2.572	0.517	20.1	0.173	-0.113	0.459	
	3	3.309	0.364	11.0	3.383	0.357	10.6	0.074	-0.015	0.163	0.99
	4	5.056	0.284	5.62	5.404	0.133	2.46	0.348	0.207	0.490	
	5	6.173	0.252	4.08	6.194	0.209	3.37	0.021	-0.036	0.077	
Lettuce	1	2.346	0.663	28.3	2.663	0.682	25.6	0.317	0.156	0.478	
	2	3.359	0.709	21.1	3.437	0.346	10.1	0.078	-0.230	0.387	
	3	4.491	0.324	7.21	5.193	0.352	6.78	0.702	0.601	0.802	0.98
	4	5.443	0.201	3.69	5.638	0.244	4.28	0.196	0.129	0.263	
	5	7.010	0.143	2.04	7.634	0.134	1.76	0.624	0.529	0.719	
Frozen fish	1	2.953	0.178	6.03	3.089	0.158	5.11	0.136	0.016	0.256	
	2	3.924	0.121	3.08	4.052	0.091	2.25	0.128	0.056	0.200	
	3	4.791	0.191	3.99	5.499	0.139	2.53	0.708	0.538	0.879	0.97
	4	5.854	0.135	2.31	6.246	0.091	1.46	0.392	0.326	0.459	
	5	6.432	0.066	1.03	6.953	0.090	1.29	0.522	0.448	0.595	
Milk powder	1	0.645	0.845	131	1.012	0.736	72.7	0.367	-0.067	0.800	
	2	2.064	0.139	6.73	2.232	0.157	7.03	0.168	0.082	0.253	
	3	4.026	0.134	3.33	3.993	0.076	1.90	-0.033	-0.102	0.036	0.98
	4	5.010	0.110	2.20	4.926	0.103	2.09	-0.084	-0.149	-0.018	
	5	5.709	0.132	2.31	5.639	0.171	3.03	-0.070	-0.127	-0.012	

[a]Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f].
[b]Repeatability standard deviation.
[c]Relative standard deviation for repeatability.
[d]Mean difference between the candidate and reference methods.
[e]Confidence interval.
[f]95% Lower confidence limit for difference of means.
[g]95% Upper confidence limit for difference of means.
[h]Square of correlation coefficient.

Table 2. Single laboratory matrix study: Compact Dry TC 72 h vs ISO 4833 (4)

Matrix	Cont. level	Compact Dry TC 72 h			ISO 4833			Mean diff.[d]	95% CI[e]		r²[h]
		Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Cooked chicken	1	0.281	0.594	211	0.357	0.757	212	0.076	-0.068	0.219	0.99
	2	2.734	0.636	23.4	2.572	0.517	20.1	-0.163	-0.358	0.033	
	3	3.323	0.369	11.1	3.383	0.357	10.6	0.060	-0.033	0.153	
	4	5.063	0.280	5.53	5.404	0.133	2.46	0.342	0.203	0.480	
	5	6.179	0.249	4.03	6.194	0.209	3.37	0.015	-0.040	0.070	
Lettuce	1	2.496	0.593	23.8	2.663	0.682	25.6	0.168	0.040	0.296	0.98
	2	3.393	0.732	21.6	3.437	0.346	10.1	0.044	-0.277	0.365	
	3	4.782	0.246	5.14	5.193	0.352	6.78	0.411	0.309	0.513	
	4	5.527	0.200	3.62	5.638	0.244	4.28	0.111	0.034	0.188	
	5	7.117	0.182	2.56	7.634	0.134	1.76	0.517	0.419	0.615	
Frozen fish	1	3.000	0.155	5.17	3.089	0.158	5.11	0.090	-0.019	0.198	0.98
	2	3.924	0.121	3.08	4.052	0.091	2.25	0.128	0.056	0.199	
	3	4.822	0.174	3.61	5.499	0.139	2.53	0.677	0.518	0.838	
	4	5.860	0.133	2.27	6.246	0.091	1.46	0.386	0.323	0.450	
	5	6.474	0.065	1.00	6.953	0.090	1.29	0.479	0.414	0.544	
Milk powder	1	0.881	0.792	89.9	1.012	0.736	72.7	0.130	-0.190	0.450	0.99
	2	2.105	0.154	7.32	2.232	0.157	7.03	0.127	0.026	0.277	
	3	4.027	0.133	3.30	3.993	0.076	1.90	-0.034	-0.103	0.035	
	4	5.010	0.110	2.20	4.926	0.103	2.09	-0.084	-0.149	-0.018	
	5	5.720	0.131	2.29	5.639	0.171	3.03	-0.081	-0.142	-0.019	

[a]Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f].
[b]Repeatability standard deviation.
[c]Relative standard deviation for repeatability.
[d]Mean difference between the candidate and reference methods.
[e]Confidence interval.
[f]95% Lower confidence limit for difference of means.
[g]95% Upper confidence limit for difference of means.
[h]Square of correlation coefficient.

Table 3. Single laboratory matrix study: Compact Dry TC 48 h vs Compact Dry 72 h (4)

Matrix	Cont. level	Compact Dry TC 48 h			Compact Dry TC 72 h			Mean diff.[d]	95% CI[e]		r^{2h}
		Mean[a]	s_r[b]	RSD_r[c]	Mean	s_r	RSD_r		LCL[f]	UCL[g]	
Cooked chicken	1	0.281	0.594	211	0.281	0.594	211	0.00	0.00	0.00	
	2	2.399	0.304	12.7	2.734	0.636	23.4	0.336	-0.079	0.750	
	3	3.309	0.364	11.0	3.323	0.369	11.1	0.014	-0.001	0.029	0.99
	4	5.056	0.284	5.62	5.063	0.280	5.53	0.007	0.002	0.011	
	5	6.173	0.252	4.08	6.179	0.249	4.03	0.006	0.003	0.009	
Lettuce	1	2.346	0.663	28.3	2.496	0.593	23.76	0.149	0.065	0.233	
	2	3.359	0.709	21.1	3.393	0.732	21.6	0.034	0.007	0.061	
	3	4.491	0.324	7.21	4.782	0.246	5.14	0.291	0.200	0.381	0.99
	4	5.443	0.201	3.69	5.527	0.200	3.62	0.085	0.001	0.169	
	5	7.010	0.143	2.04	7.117	0.182	2.56	0.107	0.031	0.183	
Frozen fish	1	2.953	0.178	6.03	3.000	0.155	5.17	0.046	0.026	0.067	
	2	3.924	0.121	3.08	3.924	0.121	3.08	0.000	-0.001	0.000	
	3	4.791	0.191	3.99	4.822	0.174	3.61	0.031	0.000	0.063	1.00
	4	5.854	0.135	2.31	5.860	0.133	2.27	0.006	0.002	0.010	
	5	6.432	0.066	1.03	6.474	0.065	1.00	0.043	-0.022	0.107	
Milk powder	1	0.645	0.845	131	0.881	0.792	89.9	0.236	-0.123	0.596	
	2	2.064	0.139	6.73	2.105	0.154	7.32	0.041	0.008	0.074	
	3	4.026	0.134	3.33	4.027	0.133	3.30	0.001	0.000	0.003	0.99
	4	5.010	0.110	2.20	5.010	0.110	2.20	0.000	0.000	0.001	
	5	5.709	0.132	2.31	5.720	0.131	2.29	0.011	0.002	0.020	

[a]Mean of five replicate portions, plated in duplicate, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f].
[b]Repeatability standard deviation.
[c]Relative standard deviation for repeatability.
[d]Mean difference between the candidate and reference methods.
[e]Confidence interval.
[f]95% Lower confidence limit for difference of means.
[g]95% Upper confidence limit for difference of means.
[h]Square of correlation coefficient.

Table 4. Multi-laboratory study: Summary of pasteurized milk results by laboratory for each contamination level for each method (4)

Laboratory	Uncontaminated			Low level			Medium level			High level		
	CD TC[a] 48 h	CD TC 72 h	ISO 4833	CD TC 48 h	CD TC 72 h	ISO 4833	CD TC 48 h	CD TC 72 h	ISO 4833	CD TC 48 h	CD TC 72 h	ISO 4833
1	0.000[b]	0.000	0.734	2.557	2.560	2.698	3.533	3.538	3.697	4.701	4.703	4.723
2	0.000	0.000	0.000	2.638	2.638	2.832	3.726	3.726	3.825	4.727	4.727	4.860
3	0.521	0.591	0.000	2.684	2.689	2.806	3.763	3.771	3.973	4.803	4.808	4.947
4	2.340	2.365	2.192	2.930	2.937	2.992	3.872	3.909	3.943	4.829	4.852	5.049
5	0.000	0.000	0.000	2.599	2.599	2.762	3.728	3.740	3.805	4.740	4.748	4.814
6	0.000	0.000	0.000	2.795	2.810	2.694	3.737	3.766	3.758	4.811	4.816	4.832
7	0.000	0.000	0.000	2.749	2.749	2.852	3.831	3.831	3.953	4.823	4.825	4.945
8	0.000	0.000	0.000	2.588	2.588	2.770	3.627	3.627	3.917	4.761	4.761	4.826
9	0.260	0.260	0.000	2.751	2.751	2.803	3.790	3.790	3.888	4.904	4.904	4.963
10	0.000	0.260	0.000	2.618	2.618	2.788	3.688	3.688	3.724	4.710	4.710	4.616
11[c]	0.000	0.000	0.000	2.703	2.705	2.752	3.726	3.726	3.820	4.793	4.793	4.833
12[c]	0.000	0.000	0.000	2.748	2.753	2.730	3.812	3.734	3.854	4.983	4.940	4.979
13[c]	0.000	0.000	0.000	2.779	2.779	2.825	3.876	3.878	3.896	4.853	4.858	4.864
14[d]	0.260	0.260	0.000	2.700	2.700	2.810	3.739	3.739	3.789	4.835	4.836	4.833
14[c,d]	0.260	0.260	0.000	2.700	2.700	2.745	3.774	3.774	3.833	4.716	4.716	4.701

[a]Compact Dry TC.
[b]Results are reported for each laboratory as a mean of 2 replicate portions, plated in duplicate, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f].
[c]Sample analysis initiated one day later than scheduled start date.
[d]Organizing Laboratory.

Table 6. Multi-laboratory study: Method comparison of pasteurized milk results for each contamination level across 14 data sets[a] (4)

Contam. level	Compact Dry TC 48 h vs ISO 4833						Compact Dry TC 72 h vs ISO 4833						Compact Dry TC 48 h vs 72 h					
	Mean[b] CD TC 48 h	Mean ISO 4833	Mean diff.[c]	LCL[d]	UCL[e]	r^{2f}	Mean CD TC 72 h	Mean ISO 4833	Mean diff.	LCL	UCL	r^2	Mean CD TC 48 h	Mean CD TC 72 h	Mean diff.	LCL	UCL	r^2
Uncont.	0.112	0.052	-0.060	-0.270	0.080	0.98	0.117	0.052	-0.065	-0.281	0.079	0.98	0.112	0.117	0.005	-0.006	0.018	1.0
Low	2.686	2.776	0.090	0.046	0.129		2.688	2.776	0.088	0.043	0.129		2.686	2.688	0.002	-0.003	0.006	
Mid	3.737	3.838	0.101	0.064	0.131		3.738	3.838	0.100	0.065	0.134		3.737	3.738	0.001	-0.011	0.008	
High	4.797	4.838	0.041	0.011	0.069		4.796	4.838	0.042	0.012	0.070		4.797	4.796	-0.001	-0.008	0.006	

[a]Laboratory 4 removed from statistical analysis due to unexpectedly high counts in the uncontaminated samples. See Table 4.
[b]Overall mean across laboratories for each method, after logarithmic transformation: Log_{10}[CFU/g + (0.1)f].
[c]Mean difference between methods.
[d]95% Lower confidence limit for difference of means.
[e]95% Upper confidence limit for difference of means.
[f]Square of correlation coefficient.

DISCUSSION OF MODIFICATION STUDY APPROVED DECEMBER 2020 (5)

The results of this study indicate that the CompactDry "Nissui" TC method can be used for rapid and accurate enumeration of mesophilic aerobic bacteria in raw ground beef and raw chicken breast at 24 h of incubation. The CompactDry "Nissui" TC method showed similar repeatability to the MLG 3.02 reference method and equivalent mean results. Enumeration was equivalent between 24 h and 48 h for the CompactDry "Nissui" TC.

The CompactDry "Nissui" TC method offers a time saving of 1 day over the reference method and produces a mesophilic aerobic bacterial count in 24 ± 2 h. Advantages of the CompactDry "Nissui" TC method include reductions in storage space, media preparation, incubator space, and waste disposal.

Table 4. Method comparison data summary and statistics for 24 h incubation of candidate method (5)

Matrix	Cont. level	N	CompactDry "Nissui" TC, 24 h			MLG 3.02			DOM[a]	95 % CI[b]		90 % CI[b]		Equivalence
			Mean Log_{10} CFU/g	s_r	RSD_r, %	Mean Log_{10} CFU/g	s_r	RSD_r, %		LCL[c]	UCL[d]	LCL[c]	UCL[d]	
Raw ground beef, 95% lean (naturally contaminated)	Low	5	2.189	0.120	5.48	2.368	0.109	4.60	-0.180	-0.389	0.030	-0.340	-0.019	Passed
	Med	5	5.519	0.214	3.88	5.881	0.177	3.01	-0.362	-0.495	-0.229	-0.464	-0.260	Passed
	High	5	7.399	0.174	2.35	7.577	0.068	0.90	-0.177	-0.357	0.002	-0.315	-0.040	Passed
Raw chicken breast, 98% lean (naturally contaminated)	Low	5	3.458	0.061	1.76	3.390	0.035	1.03	0.068	-0.041	0.177	-0.016	0.151	Passed
	Med	5	5.691	0.447	7.85	5.788	0.303	5.23	-0.097	-0.350	0.156	-0.292	0.097	Passed
	High	5	7.487	0.334	4.46	7.333	0.260	3.55	0.153	-0.018	0.324	0.022	0.284	Passed

[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

Table 5. Method comparison data summary and statistics for 48 h incubation of candidate method (5)

			CompactDry "Nissui" TC, 48 h			MLG 3.02				95 % CI[b]		90 % CI[b]		
Matrix	Cont. level	N	Mean Log_{10} CFU/g	s_r	RSD_r, %	Mean Log_{10} CFU/g	s_r	RSD_r, %	DOM[a]	LCL[c]	UCL[d]	LCL[c]	UCL[d]	Equivalence
Raw ground beef, 95% lean (naturally contaminated)	Low	5	2.552	0.145	5.68	2.368	0.109	4.60	0.184	0.016	0.352	0.055	0.313	Passed
	Med	5	5.694	0.202	3.55	5.881	0.177	3.01	-0.186	-0.310	-0.063	-0.281	-0.091	Passed
	High	5	7.478	0.122	1.63	7.577	0.068	0.90	-0.099	-0.215	0.018	-0.188	-0.009	Passed
Raw chicken breast, 98% lean (naturally contaminated)	Low	5	3.685	0.065	1.76	3.390	0.035	1.03	0.295	0.199	0.391	0.222	0.369	Passed
	Med	5	5.728	0.457	7.98	5.788	0.303	5.23	-0.060	-0.343	0.222	-0.277	0.156	Passed
	High	5	7.518	0.319	4.24	7.333	0.260	3.54	0.185	0.013	0.356	0.053	0.317	Passed

[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

Table 6. Method comparison data summary and statistics for 24 h vs. 48 h incubation of candidate method (5)

			CompactDry "Nissui" TC, 24 h			CompactDry "Nissui" TC, 48 h				95 % CI[b]		90 % CI[b]		
Matrix	Cont. level	N	Mean Log_{10} CFU/g	s_r	RSD_r, %	Mean Log_{10} CFU/g	s_r	RSD_r, %	DOM[a]	LCL[c]	UCL[d]	LCL[c]	UCL[d]	Equivalence
Raw ground beef, 95% lean (naturally contaminated)	Low	5	2.189	0.120	5.48	2.552	0.145	5.68	-0.363	-0.465	-0.262	-0.441	-0.286	Passed
	Med	5	5.519	0.214	3.88	5.694	0.202	3.55	-0.176	-0.206	-0.146	-0.199	-0.153	Passed
	High	5	7.399	0.174	2.35	7.478	0.122	1.63	-0.079	-0.157	0.000	-0.139	-0.019	Passed
Raw chicken breast, 98% lean (naturally contaminated)	Low	5	3.458	0.061	1.76	3.685	0.065	1.76	-0.228	-0.295	-0.161	-0.279	-0.176	Passed
	Med	5	5.691	0.447	7.85	5.728	0.457	7.98	-0.037	-0.081	0.007	-0.070	-0.003	Passed
	High	5	7.487	0.334	4.46	7.518	0.319	4.24	-0.032	-0.083	0.020	-0.071	0.008	Passed

[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. Kodaka, H., Mizuochi, S., Teramura, H., and Nirazuka, T., Comparison of the Compact Dry TC Method with the Standard Pour Plate Method (AOAC Official Method 966.23) for Determining Aerobic Colony Counts in Food Samples, AOAC *Performance Tested Methods*^{SM} certification number 010404.
2. *Official Methods of Analysis* 19th Ed., AOAC INTERNATIONAL, Rockville, MD, Method **966.23**, *Microbiological Methods*, http://www.eoma.aoac.org, accessed 2004
3. ISO 4833:2003, *Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C,* http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=34524, accessed 2006
4. Mizuochi, S., Evaluation of Compact Dry TC: Matrix Extension, AOAC *Performance Tested Methods*^{SM} certification number 010404. Modification approved April 2015.
5. Toyota, K., Modification of the CompactDry "Nissui" TC for Enumeration of Aerobic Bacteria at 24 Hours in raw Ground Beef and Raw Chicken Breast, AOAC *Performance Tested Methods*^{SM} certification number 010404. Approved December 2020.
6. FSIS MLG 3.02 Quantitative Analysis of Bacteria in Foods as Sanitary Indicators: Chapter 3.6 Aerobic Plate Count https://www.fsis.usda.gov/wps/wcm/connect/f2162091-af72-4888-997b-718d6592bcc9/MLG-3.pdf?MOD=AJPERES accessed July 17, 2020