CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

110401

The AOAC Research Institute hereby certifies the method known as:

Compact Dry CF

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 10, 2023

Expiration Date December 31, 2023

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MODIFICATION JUNE 2015: Shingo Mizuochi and Maria Nelson

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CURRENT COMPANY ADDRESS
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METHOD NAME
Compact Dry CF

CATALOG NUMBERS
06744, 06745

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Modification June 2015
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APPLICABILITY OF METHOD
Target organism – Coliforms

Matrixes – OMA 966.24 - Raw ground pork, raw pork, raw lamb, raw veal, and ground beef
June 2015 Modification – ISO 4832:2006; ISO 16649-1:2001 – cooked chicken, pre-washed bagged shredded iceberg lettuce, frozen cod fillets, instant non-fat dry milk, pasteurized milk (2 %)

Performance claims – The study data detected no statistical difference between the Compact Dry CF method and the reference methods.

REFERENCE METHODS
Official Methods of Analysis (2005) 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD. Method 966.24 (Most Probable Number Method) (2)

ISO 4832:2006, Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms -- Colony-count technique (4)

ORIGINAL CERTIFICATION DATE
November 17, 2004

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

METHOD MODIFICATION RECORD
1. **June 2015 Level 2**
2. **February 2019 Level 2**

SUMMARY OF MODIFICATION
1. **Matrix extension approval.**
2. **Shelf life increase to 24 months and corporate address change.**

Under this AOAC *Performance Tested Methods*℠ License Number, 110401 this method is distributed by:
1. **Hardy Diagnostics**
2. **R-Biopharm AG**

Under this AOAC *Performance Tested Methods*℠ License Number, 110401 this method is distributed as:
1. **Compact Dry CF**
2. **Compact Dry CF**

PRINCIPLE OF THE METHOD (1)
The test method is a plate count unit facilitating the rapid determination of coliform bacterial loads of raw meats. The plates are pre-sterilized, contained nutrients supplemented with selective substances, chromogenic enzyme substrate and a cold water-soluble gelling agent. The medium should be re-hydrated with 1 ml of (diluted) sample material with a diffuse automatically and can be incubated. Full medium size in the plate is 20 cm^2. Gelling agent is allowed to solidify, plates are incubated, and then coliforms are counted.

DISCUSSION OF THE VALIDATION STUDY (1)
This method validation study demonstrated that the Compact Dry CF methodology and the reference conventional culture method produced comparable coliform count results. Therefore, Compact Dry CF plates could be a convenient alternative for routine microbiological food testing. Observations during this study have shown that the Compact Dry CF plates are easy to use, with a minimal amount of analyst time needed for a single quantitative test. The time from preparation media to setting up was 250 min per 20 samples for AOAC methodology. The time for same procedures was 150 min per 20 samples for Compact Dry CF methodology. The equipment requirement and overall cost of the assay is less than that of the AOAC methodology. There is no secondary transfer into multiple tubes or additional biochemical analyses. The amount of time required to read the Compact Dry CF is a bit longer than reading successive tubes and biochemicals from the AOAC methodology, but the plates are read anywhere from one to nine days prior to when the result would be completed from the AOAC methodology, allowing for faster reaction time and less down time for the producer or originator of the sample. There are economical and safety advantages to having a more rapid response time.
In conclusion, the Compact Dry CF, using the newly developed dry sheet medium technology, is a convenient alternative for routine microbiological food testing.

Table 1. Compact Dry CF Inclusivity Study (coliform bacteria) (1)

Strain	Test number	Color reaction	Negative
Citrobacter amalonaticus	1	B	
Citrobacter freundii	4	B	
Citrobacter koseri	2	B	
Enterobacter aerogenes	4	B	
Enterobacter amnigenus	4	B	
Enterobacter asburiae	1	B	
Enterobacter cancerogenus	1	B	
Enterobacter cloacae	4	B	
Enterobacter gergoviae	1	BG	
Enterobacter intermedium	2	BG	
Enterobacter sakazakii	1	BG	
Escherichia blattae	1	W	1
Eschericia coli	9	B	
Eschericia coli O157:H7	2	B	
Eschericia coli O111	2	B	
Escherichia fergusonii	2	B	
Eschericia hermanii	1	B	
Klebsiella oxytoca	4	B	
Klebsiella ozaenae	2	B	
Klebsiella pneumoniae	2	B	
Klebsiella terrigena	1	LB	
	51		1

B: Blue , BG: Blue/Green, LB: Light Blue, W: White.

Table 2. Compact Dry CF Exclusivity Study(Non-coliform bacteria) (1)

Strain	Test number	Color reaction	Positive
Achromobacter xylosoxidans subsp. *denitrificans*	1	sW	
Achromobacter xylosoxidans subsp. *xylosoxidans*	1	sW	
Acinetobacter baumanii	1	W	
Acinetobacter calcoaceticus	2	W /-	
Aeromonas hydrophila	2	WG/-	1
Alcaligenes faecalis	1	sW	
Bacillus cereus	2	-	
Edwardsiella tarda	3	W	
Lactobacillus lactis	1	-	
Micrococcus luteus	1	-	
Micrococcus lylae	1	-	
Moraxella nonliquefaciens	1	-	
Moraxella ovis	1	-	
Proteus mirabilis	1	br	
Proteus vulgaris	2	br	
Providencia alcalifaciens	2	br	
Pseudomonas aeruginosa	1	W	
Pseudomonas alcaligenes	1	-	
Pseudomonas diminuta	2	-	
Pseudomonas fluorescens	1	-	
Pseudomonas mendocina	1	W	
Pseudomonas pseudoalcaligenes	1	sW	
Pseudomonas putida	1	-	
Pseudomonas stutzeri	1	W	
Pseudomonas vesicularis	1	-	
Salmonella Choleraesuis	2	W	
Salmonella Typhimurium	1	W	
Shigella flexineri	2	W	
Shigella boydii	1	G	1
Staphylococcus aureus	3	-	
Streptococcus agalactiae	1	-	
Streptococcus bovis	1	-	
Streptococcus canis	1	-	
Streptococcus equines	1	-	
Streptococcus pneumoniae	1	-	
Streptococcus pyogenes	1	-	
Streptococcus salivarius	1	-	
Streptococcus sanguis	1	-	
Streptococcus uberis	1	-	
Serratia marcescens	1	B	1
	52		3

B: Blue , G: Green, br: Brown, W: White, WG: White Green, -:non growth, s: small.

Table 3. Compact Dry CF Method Comparison (Raw Ground Pork) (1)

Coliform LEVEL		Compact Dry CF		AOAC 966.24	
		Coliform		Coliform	
		cfu/g	log_{10}cfu/g	cfu/g	log_{10}cfu/g
10-100 cfu/g	1	5	0.70	9.1	0.96
	2	15	1.18	23	1.36
	3	30	1.48	43	1.63
	4	15	1.18	23	1.36
	5	10	1.00	3.6	0.56
	Mean	15	1.11	20.34	1.17
	Sr	9.35	0.28	15.28	0.42
	RSDr%	62.36	25.76	75.13	35.86
100-1000 cfu/g	1	240	2.38	230	2.36
	2	200	2.30	430	2.63
	3	270	2.43	230	2.36
	4	100	2.00	150	2.18
	5	180	2.26	93	1.97
	Mean	198	2.27	226.6	2.30
	Sr	64.96	0.17	127.58	0.25
	RSDr%	32.81	7.37	56.30	10.74
1000-10000cfu/g	1	1800	3.26	2300	3.36
	2	1800	3.26	4300	3.63
	3	2200	3.34	4300	3.63
	4	1800	3.26	2400	3.38
	5	1500	3.18	2400	3.38
	Mean	1820	3.26	3140	3.48
	Sr	249.00	0.06	1059.72	0.14
	RSDr%	13.68	1.81	33.75	4.09
Uninoculated	1	<10	<1	<3	<0.48
	2	<10	<1	<3	<0.48
	3	<10	<1	<3	<0.48
	4	<10	<1	<3	<0.48
	5	<10	<1	<3	<0.48
	Mean	<10	<1	<3	<0.48
	Sr	-	-	-	-
	RSDr%	-	-	-	-

Table 5. Compact Dry CF Method Comparison (Raw Pork) (1)

Coliform LEVEL		Compact Dry CF		AOAC 966.24	
		Coliform		Coliform	
		cfu/g	log_{10}cfu/g	cfu/g	log_{10}cfu/g
10-100cfu/g	1	90	1.95	43	1.64
	2	145	2.16	150	2.18
	3	140	2.15	39	1.59
	4	140	2.15	150	2.18
	5	75	1.88	93	1.97
	Mean	118	2.06	95	1.91
	Sr	32.90	0.13	54.53	0.28
	RSDr%	27.88	6.45	57.40	14.89
100-1000cfu/g	1	360	2.56	460	2.66
	2	340	2.53	930	2.97
	3	580	2.76	460	2.66
	4	680	2.83	460	2.66
	5	290	2.46	430	2.63
	Mean	450	2.63	548	2.72
	Sr	170	0.16	213.94	0.14
	RSDr%	37.78	6.07	39.04	5.17
1000-10000cfu/g	1	4100	3.61	4300	3.63
	2	5750	3.76	2400	3.38
	3	1000	3.00	1500	3.18
	4	1500	3.18	1500	3.18
	5	1620	3.21	2400	3.38
	Mean	2794	3.35	2420	3.35
	Sr	2044.18	0.32	1143.24	0.19
	RSDr%	73.16	9.54	47.24	5.64
Uninoculated	1	<10	<1	<3	<0.48
	2	<10	<1	<3	<0.48
	3	<10	<1	<3	<0.48
	4	<10	<1	<3	<0.48
	5	<10	<1	<3	<0.48
	Mean	<10	<1	<3	<0.48
	Sr	-	-	-	-
	RSDr%	-	-	-	-

Table 7. Compact Dry CF Method Comparison (Raw Lamb) (1)

Coliform LEVEL		Compact Dry CF		AOAC 966.24	
		Coliform		Coliform	
		cfu/g	log_{10}cfu/g	cfu/g	log_{10}cfu/g
10-100 cfu/g	1	80	1.90	43	1.63
	2	20	1.30	23	1.36
	3	20	1.30	9.1	0.96
	4	20	1.30	9.1	0.96
	5	35	1.54	23	1.36
	Mean	35	1.47	21.44	1.26
	Sr	25.98	0.26	13.91	0.29
	RSDr%	74.23	17.96	64.89	23.27
100-1000cfu/g	1	265	2.42	240	2.38
	2	235	2.37	240	2.38
	3	160	2.20	430	2.63
	4	265	2.423	150	2.18
	5	380	2.58	430	2.63
	Mean	261	2.40	298	2.44
	Sr	79.17	0.13	125.98	0.19
	RSDr%	30.33	5.61	42.27	7.98
1000-10000cfu/g	1	5300	3.72	4600	3.66
	2	2485	3.40	1100	3.04
	3	6400	3.81	4300	3.63
	4	2995	3.48	2100	3.32
	5	1390	3.14	2300	3.36
	Mean	3714	3.51	2880	3.40
	Sr	2071.13	0.27	1507.32	0.25
	RSDr%	55.77	7.57	52.34	7.48
Uninoculated	1	<10	<1	<3	<0.48
	2	<10	<1	<3	<0.48
	3	<10	<1	<3	<0.48
	4	<10	<1	<3	<0.48
	5	<10	<1	<3	<0.48
	Mean	<10	<1	<3	<0.48
	Sr	-	-	-	-
	RSDr%	-	-	-	-

Table 9. Compact Dry CF Method Comparison (Raw Veal) (1)

Coliform LEVEL		Compact Dry CF Coliform		AOAC 966.24 Coliform	
		cfu/g	log_{10}cfu/g	cfu/g	log_{10}cfu/g
10-100 cfu/g	1	5	0.70	9.1	0.96
	2	15	1.18	23	1.36
	3	30	1.48	43	1.63
	4	15	1.18	23	1.36
	5	10	1.00	3.6	0.56
	Mean	15	1.11	20.34	1.17
	Sr	9.35	0.28	15.28	0.42
	RSDr%	62.36	25.76	75.13	35.86
100-1000 cfu/g	1	240	2.38	230	2.36
	2	200	2.30	430	2.63
	3	270	2.43	230	2.36
	4	100	2.00	150	2.18
	5	180	2.26	93	1.97
	Mean	198	2.27	226.6	2.30
	Sr	64.96	0.17	127.58	0.25
	RSDr%	32.81	7.37	56.303	10.74
1000-10000cfu/g	1	1800	3.26	2300	3.36
	2	1800	3.26	4300	3.63
	3	2200	3.34	4300	3.63
	4	1800	3.26	2400	3.38
	5	1500	3.18	2400	3.38
	Mean	1820	3.26	3140	3.48
	Sr	249.00	0.06	1059.72	0.14
	RSDr%	13.68	1.81	33.75	4.09
Uninoculated	1	<10	<1	<3	<0.48
	2	<10	<1	<3	<0.48
	3	<10	<1	<3	<0.48
	4	<10	<1	<3	<0.48
	5	<10	<1	<3	<0.48
	Mean	<10	<1	<3	<0.48
	Sr	-	-	-	-
	RSDr%	-	-	-	-

DISCUSSION OF THE MODIFICATION APPROVED JUNE 2015 (3)

For this matrix extension study, the Compact Dry CF was evaluated against ISO 4832. In the single laboratory matrix studies, statistical differences were indicated between the Compact Dry CF and ISO 4832 for two of the five contamination levels tested for lettuce, and for the low contamination levels of cooked chicken, frozen fish, and milk powder. For lettuce, the ISO reference method recorded higher results than the Compact Dry CF in the lower contamination level, and the Compact Dry CF recorded higher results in the higher contamination level. For the other three levels, mean differences were small (<0.5) and CIs were within (-0.5, 0.5). For the low levels of cooked chicken, frozen fish, and milk powder, statistical differences were indicated, but only a few colonies were recovered in each of these matrixes at each of these levels, so the significance is not considered to be relevant. Using Grubb's test, statistical outliers were detected in one contamination level of cooked chicken and one contamination level of 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 value was >0.94 for all matrixes.

In the multi-laboratory study on pasteurized milk, no statistical differences were found. Due to shipping and scheduling issues, two laboratories plus the organizing laboratory initiated testing one day later than the other 11 laboratories. Because the organizing laboratory discovered a potential for die-off during sample storage, data from the laboratories (Laboratories 12–14) starting one day late were not included in the statistical analysis. Across 11 data sets, the mean differences between the Compact Dry CF and ISO 4832 were less than 0.10 $\log_{10}$ at each contamination level, and the CIs were less than (-0.2, 0.2), well within the (-0.5, 0.5) acceptance criterion. The s_r, RSD_r, s_R, and RSD_R values were slightly smaller for the Compact Dry CF compared to ISO 4832. The r^2 value was 0.99. The results from both the single laboratory and multi-laboratory studies indicate that the Compact Dry CF gives consistent results, comparable to the reference method, within and across laboratories.

Table 2. Single laboratory matrix study: Compact Dry CF vs ISO 4832 (3)

Matrix	Cont. level	Compact Dry CF			ISO 4832			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	0.000	0.000	NA[i]	0.000	0.000	NA	0.000	0.000	0.000	0.96
	2	1.010	0.388	38.4	0.774	0.552	71.3	0.236	-0.256	0.729	
	3	2.533	0.117	4.62	2.448	0.134	5.47	0.085	0.035	0.136	
	4	3.493	0.126	3.61	3.428	0.248	7.23	0.064	-0.046	0.175	
	5	4.523	0.153	3.38	4.353	0.133	3.06	0.169	0.044	0.294	
Lettuce	1	0.000	0.000	NA	0.000	0.000	NA	0.000	0.000	0.000	0.97
	2	1.981	0.529	26.7	2.216	0.531	24.0	-0.234	-0.627	0.159	
	3	3.933	0.167	4.25	4.026	0.153	3.80	-0.093	-0.146	-0.039	
	4	4.761	0.196	4.12	4.241	0.202	4.76	0.520	-0.358	0.683	
	5	5.086	0.084	1.65	4.955	0.126	2.54	0.131	-0.055	0.206	
Frozen fish	1	1.181	0.469	39.7	1.748	0.296	16.9	-0.567	-0.855	-0.279	0.96
	2	2.105	0.153	7.27	2.169	0.180	8.30	-0.064	-0.184	0.056	
	3	3.089	0.086	2.78	3.098	0.116	3.74	-0.009	-0.071	0.052	
	4	4.035	0.121	3.00	4.090	0.135	3.30	-0.055	-0.138	0.028	
	5	5.048	0.171	3.39	5.097	0.179	3.51	-0.049	-0.090	-0.008	
Milk powder	1	0.577	0.617	107	0.000	0.000	NA	0.577	-0.136	1.018	0.94
	2	1.887	0.912	48.3	2.041	0.735	36.0	-0.155	-0.496	0.187	
	3	2.598	0.164	6.31	2.719	0.236	8.68	-0.121	-0.230	-0.012	
	4	3.880	0.044	1.13	3.972	0.079	1.99	-0.092	-0.144	-0.039	
	5	4.872	0.140	2.87	4.951	0.177	3.58	-0.080	-0.123	-0.036	

[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.

[i]Not applicable.

REFERENCES CITED

1. NISSUI PHARMACEUTICAL CO., LTD, Evaluation of the Compact Dry CF, AOAC *Performance Tested Methods*℠ certification number 110401.
2. *Official Methods of Analysis* (2005) 18th Ed. AOAC INTERNATIONAL, Gaithersburg, MD. Method 966.24 (Most Probable Number Method)
3. Mizuochi, S. and Nelson, M., Evaluation of Compact Dry CF: Matrix Extension, AOAC *Performance Tested Methods*℠ certification number 110401, approved June 2015.
4. ISO 4832:2006, *Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms -- Colony-count technique,* http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=34524, accessed 2007