



CERTIFICATION

AOAC[®] Performance TestedSM

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[®] *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 (December 15, 2021 – December 31, 2026). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

Scott Coates

Scott Coates, Senior Director
Signature for AOAC Research Institute

December 15, 2021

Date

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METHOD AUTHORS Original Validation: NISSUI PHARMACEUTICAL CO., LTD. Modification June 2015: Shingo Mizuochi and Maria Nelson		SUBMITTING COMPANY Nissui Pharmaceutical Co., Ltd. 3-23-9, Ueno Taito-ku, Tokyo Japan 110-8736	CURRENT COMPANY ADDRESS NISSUI Pharmaceutical Co., Ltd. 3-24-6, Ueno Taito-ku, Tokyo Japan 110-8736
METHOD NAME(S) Compact Dry CF		CATALOG NUMBERS 06744, 06745	
INDEPENDENT LABORATORY Q.Laboratories 1400 Harrison Ave Cincinnati, OH 45214 USA.	Modification June 2015 Campden BRI Station Road Chipping Campden Gloucestershire, GL55 6LD, UK	AOAC EXPERTS AND PEER REVIEWERS Original Validation: Wallace Andrews ¹ , Joseph Odumeru ² , Yataro Kokubo ³ ¹ Retired USDA FDA CFSAN, College Park, MD, USA ² University of Guelph, Guelph, Ontario, CANADA ³ Japan Food Hygiene Association, JAPAN June 2015 Modification: Yi Chen ⁴ , Yvonne Salfinger ⁵ , Maria Cristina Fernandez ⁶ ⁴ USDA FDA CFSAN, College Park, MD, USA ⁵ Consultant, Denver, CO, USA ⁶ University of Buenos Aires, Buenos Aires, Argentina ⁷ Brodsky Consultants, Ontario, Canada: February 2019 modification	
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 - Company Dry CF is equivalent to the reference method (AOAC OMA 966.24).		REFERENCE METHODS Official Methods of Analysis (2005) 18 th 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 2022.	
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® <i>Performance Tested</i> SM License Number, 110401 this method is distributed by: 1. Hardy Diagnostics 2. R-Biopharm AG		Under this AOAC® <i>Performance Tested</i> SM 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 cm². 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
<i>Citrobacter amalonaticus</i>	1	B	
<i>Citrobacter freundii</i>	4	B	
<i>Citrobacter koseri</i>	2	B	
<i>Enterobacter aerogenes</i>	4	B	
<i>Enterobacter amnigenus</i>	4	B	
<i>Enterobacter asburiae</i>	1	B	
<i>Enterobacter cancerogenus</i>	1	B	
Enterobacter cloacae	4	B	
Enterobacter gergoviae	1	BG	
<i>Enterobacter intermedium</i>	2	BG	
<i>Enterobacter sakazakii</i>	1	BG	
Escherichia blattae	1	W	1
<i>Escherichia coli</i>	9	B	
<i>Escherichia coli</i> O157:H7	2	B	
<i>Escherichia coli</i> O111	2	B	
<i>Escherichia fergusonii</i>	2	B	
<i>Escherichia hermannii</i>	1	B	
<i>Klebsiella oxytoca</i>	4	B	
<i>Klebsiella ozaenae</i>	2	B	
<i>Klebsiella pneumoniae</i>	2	B	
<i>Klebsiella terrigena</i>	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
<i>Achromobacter xylosoxidans</i> subsp. <i>denitrificans</i>	1	sW	
<i>Achromobacter xylosoxidans</i> subsp. <i>xylosoxidans</i>	1	sW	
<i>Acinetobacter baumannii</i>	1	W	
<i>Acinetobacter calcoaceticus</i>	2	W /-	
<i>Aeromonas hydrophila</i>	2	WG/-	1
<i>Alcaligenes faecalis</i>	1	sW	
<i>Bacillus cereus</i>	2	-	
<i>Edwardsiella tarda</i>	3	W	
<i>Lactobacillus lactis</i>	1	-	
<i>Micrococcus luteus</i>	1	-	
<i>Micrococcus lylae</i>	1	-	
<i>Moraxella nonliquefaciens</i>	1	-	
<i>Moraxella ovis</i>	1	-	
<i>Proteus mirabilis</i>	1	br	
<i>Proteus vulgaris</i>	2	br	
<i>Providencia alcalifaciens</i>	2	br	
<i>Pseudomonas aeruginosa</i>	1	W	
<i>Pseudomonas alcaligenes</i>	1	-	
<i>Pseudomonas diminuta</i>	2	-	
<i>Pseudomonas fluorescens</i>	1	-	
<i>Pseudomonas mendocina</i>	1	W	
<i>Pseudomonas pseudoalcaligenes</i>	1	sW	
<i>Pseudomonas putida</i>	1	-	
<i>Pseudomonas stutzeri</i>	1	W	
<i>Pseudomonas vesicularis</i>	1	-	
<i>Salmonella Choleraesuis</i>	2	W	
<i>Salmonella Typhimurium</i>	1	W	
<i>Shigella flexneri</i>	2	W	
<i>Shigella boydii</i>	1	G	1
<i>Staphylococcus aureus</i>	3	-	
<i>Streptococcus agalactiae</i>	1	-	
<i>Streptococcus bovis</i>	1	-	
<i>Streptococcus canis</i>	1	-	
<i>Streptococcus equines</i>	1	-	
<i>Streptococcus pneumoniae</i>	1	-	
<i>Streptococcus pyogenes</i>	1	-	
<i>Streptococcus salivarius</i>	1	-	
<i>Streptococcus sanguis</i>	1	-	
<i>Streptococcus uberis</i>	1	-	
<i>Serratia marcescens</i>	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		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	56.303	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 log₁₀ 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	

^aMean of five replicate portions, plated in duplicate, after logarithmic transformation: $\text{Log}_{10}[\text{CFU}/g + (0.1)]$.

^bRepeatability standard deviation.

^cRelative standard deviation for repeatability.

^dMean difference between the candidate and reference methods.

^eConfidence interval.

^f95% Lower confidence limit for difference of means.

^g95% Upper confidence limit for difference of means.

^hSquare of correlation coefficient.

ⁱNot applicable.

REFERENCES CITED

1. NISSUI PHARMACEUTICAL CO., LTD, Evaluation of the Compact Dry CF, AOAC® *Performance Tested*SM 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*SM 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