CERTIFICATION

AOAC Research Institute

Performance Tested Methods[SM]

Certificate No.

081001

The AOAC Research Institute hereby certifies the method known as:

Compact Dry X-SA

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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METHOD NAME
Compact Dry X-SA

CATALOG NUMBERS
06746, 06747

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APPLICABILITY OF METHOD
Target organism – *Staphylococcus aureus*

Matrixes – Frozen prawns, cooked ham, unpasteurized cow's milk, cream pastries, & chilled fresh pasta

Performance claims - This method is an alternative method to the standard method enabling determination *S. aureus* counts in foods after 24 ± 2h incubation.

REFERENCE METHOD
BS EN ISO 6888-1:1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium. (2)

ORIGINAL CERTIFICATION DATE
August 13, 2010

CERTIFICATION RENEWAL RECORD
Renewed annually through December 2023.

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

SUMMARY OF MODIFICATION
1. **Shelf life extension to 21 months and corporate address change.**

Under this AOAC *Performance Tested Methods℠* License Number, 081001 this method is distributed by:
1. **Hardy Diagnostics**
2. **R-Biopharm AG**

Under this AOAC *Performance Tested Methods℠* License Number, 081001 this method is distributed as:
1. **Compact Dry X-SA**
2. **Compact Dry X-SA**

PRINCIPLE OF THE METHOD (1)
Compact Dry (Nissui Pharmaceutical Co. Ltd.; supplied by HyServe Gmbh & Co. KG) are ready-to-use dry media sheets comprising culture medium and a cold-soluble gelling agent, rehydrated by inoculating 1 ml diluted sample into the centre of the self-diffusible medium. The Compact Dry X-SA medium is described by the manufacturer as a "ready-to-use, chromogenic plate for detection of *Staphylococcus aureus*. The CD X-SA contains chromogenic medium and selective agents for the detection and enumeration of *S. aureus*, which according to the manufacturer's instructions appear as light blue/blue colonies. This method is an alternative method to the standard method enabling determination *S. aureus* counts in foods after 24 ± 2h incubation.
This study compared the performance of the Compact Dry X-SA medium against standard method BS EN ISO 6888-1:1999 which is described as a method for the enumeration of coagulase-positive staphylococci. The medium used in the standard method (Baird-Parker medium) was originally developed as a selective diagnostic medium for the isolation and enumeration of *S. aureus* in foods. Furthermore, although *S. aureus* is the most common species associated with coagulase activity, it is recognised that other coagulase staphylococci exist, notably *S. delphini*, *S. hyicus* and *S. intermedius*.
No confirmation procedure is currently described or recommended by the manufacturer for the Compact Dry X-SA medium.
Note: In this study a selection of typical colonies on X-SA plates from each sample were also subjected to the coagulase test. Additionally, typical colonies from X-SA plates isolated from a selection of naturally and artificially contaminated samples were identified as *S. aureus* using an appropriate biochemical identification method, e.g. API Staph or VITEK GP card; bioMérieux and/or latex agglutination test.

DISCUSSION OF THE VALIDATION STUDY (1)
The results from the one way ANOVA showed that there were no statistically significant evidence of differences between the Compact Dry X-SA method and reference method for the food types tested and the individual contamination levels.
It is concluded that the Compact Dry X-SA method is able to provide a rapid (24h), quick and convenient method for the enumeration of *S. aureus* in foods. The results of this study showed good agreement between the Compact Dry X-SA method and the standard conventional culture method (ISO 6888-1; 1999) for the enumeration of *S. aureus*.

Table 2: *Staphylococcus aureus* strains used to determine the inclusivity of the Compact Dry X-SA method (1)

Number	Strain code	Source	Enterotoxin
1.	1197	Chicken	C
2.	1208	Smoked fish	C
3.	1210	Smoked fish	C
4.	1211	Shellfish	C and D
5.	1213	Chicken	ND
6.	1214	Cooked beef	ND
7.	1215	Cheese	C
8.	1216	NCTC 10655, ATCC 19095	C
9.	1217	Cooked beef	ND
10.	1219	Raw beef	C
11.	1223	Chicken	A and E
12.	1224	Margarine	D
13.	1225	Cooked chicken	C and D
14.	1227	Frozen cooked peeled prawns	B
15.	1228	Frozen shrimp	A and B
16.	1230	Shellfish	C and D
17.	1231	Food poisoning outbreak	A
18.	1232	Prawns	C
19.	1234	Food poisoning outbreak	E
20.	1239	Raw pork	ND
21.	1242	Food poisoning outbreak	A
22.	1244	Cheese	C
23.	1246	Pork sausage	ND
24.	1446	Dairy product	A and D
25.	1992	Raw chicken	ND
26.	1993	Raw chicken	ND
27.	1994	Beefburger	C
28.	2078	Milk powder	A and D
29.	3097	Pasta	A
30.	3098	Rice salad	ND
31.	4105	NCIMB 12702, ATCC 25923	A
32.	16482	ATCC 27734 (coagulase negative strain)	A and D

ND = not determined

Table 3: Strains used to determine the exclusivity of the Compact Dry X-SA method (1)

Number	Organism	CCFRA code	Source	Origin
1.	*Bacillus cereus*	1761	Dairy product	Campden BRI
2.	*Bacillus cereus*	4110	ATCC 10876 NCTC 7464	ATCC
3.	*Bacillus subtilis*	4112	ATCC 6633 NCTC 10400	ATCC
4.	*Brochothrix thermospacta*	16019	NCTC 10822	NCTC
5.	*Enterococcus faecalis*	4113	NCTC 775	NCTC
6.	*Enterococcus faecalis*	16049	NCIMB 13280 ATCC 29212	NCIMB
7.	*Listeria monocytogenes*	1104	Soft cheese	HPA
8.	*Pediococcus pentosaceus*	16030	Brine	Campden BRI
9.	*Staphylococcus caprae*	265	Goat	Campden BRI
10.	*Staphylococcus carnosus*	284	Fermented sausage	Campden BRI
11.	*Staphylococcus cohnii*	16604	Human skin NCTC 11041	NCTC
12.	*Staphylococcus epidemidis*	271	Human skin	Campden BRI
13.	*Staphylococcus hominis*	1527	Dried milk powder	Campden BRI
14.	*Staphylococcus hyicus*	281	Pig skin	Campden BRI
15.	*Staphylococcus intermedius*	7298	unknown	Campden BRI
16.	*Staphylococcus piscifermentans*	5929	unknown	Campden BRI
17.	*Staphylococcus sciuri*	6690	unknown	Campden BRI
18.	*Staphylococcus simulans*	244	Human skin	NCTC 11046
19.	*Staphylococcus warneri*	262	German salami	Campden BRI
20.	*Staphylococcus xylosus*	266	Mettwurst sausage	Campden BRI
21.	*Micrococcus luteus*	16258	NCTC 2665	NCTC
22.	*Pseudomonas aeruginosa*	8299	NCIMB 10753	NCIMB
23.	*Escherichia coli*	16041	Raw ground beef	Campden BRI

NCIMB = National Collection of Industrial, Marine and Food Bacteria, Aberdeen, Scotland, United Kingdom.
NCTC = National Collection of Type Cultures, Colindale, London, United Kingdom. ATCC = American Type Culture Collection, Manassas, USA.
Campden BRI = Campden BRI Microbiology Department

Table 8: Summary of results obtained using GMFR (including abnormal results) for *S. aureus* count by Compact Dry X-SA method against ISO 6888-1 (1999) (1)

Food Type (Category)	Pearson Correlation Coefficient (r)		ISO Correlation Coefficient (r)	Intercept (a)	Slope (b)	Residual standard deviation (S) y:x	Standard deviation of intercept (S) a	P {a-0}	Standard deviation of slope b (S)b	P [b=1}
	Between Data	Between Level mean								
(16140; 2003 ref.)	See Display 2		3.2	3.3	3.3	3.4	3.5	3.5	3.6	
Cooked ham (meat products)	0.993	1.000	0.733	-0.552	1.085	376	0.345	251	0.065	318
			0.				0.		0.	
Prawns (fish and seafood)	0.987	0.998	1.195	-0.103	0.990	228	0.192	646	0.050	863
			0.				0.		0.	
Milk (dairy products)	0.993	1.000	1.252	129	0.955	045	0.048	112	0.010	047
Cake (bakery products)	0.994	0.999	1.132	-0.310	1.039	185	0.164	198	0.035	378
			0.				0.		0.	
Pasta (other)	0.986	0.996	1.020	-0.723	1.112	296	0.430	235	0.085	317
All Foods (global)	0.992	0.997	0.827	-0.331	1.044	228	0.089	002	0.018	024

REFERENCES CITED

1. Baylis, C., Evaluation of the Hyserve Compact Dry X-SA Method, AOAC *Performance Tested Methods*[SM] certification number 081001.
2. BS EN ISO 6888-1:1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) – Part 1: Technique using Baird-Parker agar medium.