



# CERTIFICATION

**AOAC<sup>®</sup> Performance Tested<sup>SM</sup>**

Certificate No.

**081001**

The AOAC Research Institute hereby certifies the test kit 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<sup>®</sup> *Performance Tested Methods*<sup>SM</sup> Program and found to perform as stated by the manufacturer contingent to the comments contained in the manuscript. This certificate means that an AOAC<sup>®</sup> Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC *Performance Tested*<sup>SM</sup> 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 27, 2019 – December 31, 2020). Renewal may be granted at the end of one year under the rules stated in the licensing agreement.

A handwritten signature in black ink that reads "Scott Coates".

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Scott Coates, Senior Director  
Signature for AOAC Research Institute

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December 27, 2019

Date

<b>METHOD AUTHORS</b> Christopher L. Baylis	<b>SUBMITTING COMPANY</b> HyServe GmbH & Co. KG Hechenrainer Straße 24 D-82449 Uffing	<b>CURRENT COMPANY</b> NISSUI Pharmaceutical Co., Ltd. 3-24-6, Ueno Taito-ku, Tokyo Japan 110-8736
<b>KIT NAME(S)</b> Compact Dry X-SA	<b>CATALOG NUMBERS</b> 06746, 06747	
<b>INDEPENDENT LABORATORY</b> Campden BRI Chipping Campden Gloucestershire GL55 6LD UK	<b>AOAC EXPERTS AND PEER REVIEWERS</b> Yi Chen <sup>1</sup> , Jo Klaessens <sup>2</sup> , Henk Stegeman <sup>3</sup> , Michael Brodsky <sup>4,5</sup> <sup>1</sup> USDA FDA CFSAN, College Park, MD, USA <sup>2</sup> Consultant, The Netherlands <sup>3</sup> Consultant, The Netherlands <sup>4</sup> Brodsky Consultants, Ontario, Canada <sup>5</sup> Modification February 2019	
<b>APPLICABILITY OF METHOD</b> Target organism – <i>Staphylococcus aureus</i>  Matrices – 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 <i>S. aureus</i> counts in foods after 24 ± 2h incubation.	<b>REFERENCE METHOD</b> BS EN ISO 6888-1:1999 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coagulase-positive staphylococci ( <i>Staphylococcus aureus</i> and other species) – Part 1: Technique using Baird-Parker agar medium. (2)	
<b>ORIGINAL CERTIFICATION DATE</b> August 13, 2010	<b>CERTIFICATION RENEWAL RECORD</b> Renewed annually through December 2020	
<b>METHOD MODIFICATION RECORD</b> 1. February 2019 Level 2	<b>SUMMARY OF MODIFICATION</b> 1. Shelf life extension to 21 months and corporate address change.	
Under this AOAC® Performance Tested <sup>SM</sup> License Number, 081001 this method is distributed by: NONE	Under this AOAC® Performance Tested <sup>SM</sup> License Number, 081001 this method is distributed as: NONE	

#### 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.	<i>Bacillus cereus</i>	1761	Dairy product	Campden BRI
2.	<i>Bacillus cereus</i>	4110	ATCC 10876 NCTC 7464	ATCC
3.	<i>Bacillus subtilis</i>	4112	ATCC 6633 NCTC 10400	ATCC
4.	<i>Brochothrix thermospacta</i>	16019	NCTC 10822	NCTC
5.	<i>Enterococcus faecalis</i>	4113	NCTC 775	NCTC
6.	<i>Enterococcus faecalis</i>	16049	NCIMB 13280 ATCC 29212	NCIMB
7.	<i>Listeria monocytogenes</i>	1104	Soft cheese	HPA
8.	<i>Pediococcus pentosaceus</i>	16030	Brine	Campden BRI
9.	<i>Staphylococcus caprae</i>	265	Goat	Campden BRI
10.	<i>Staphylococcus carnosus</i>	284	Fermented sausage	Campden BRI
11.	<i>Staphylococcus cohnii</i>	16604	Human skin NCTC 11041	NCTC
12.	<i>Staphylococcus epidermidis</i>	271	Human skin	Campden BRI
13.	<i>Staphylococcus hominis</i>	1527	Dried milk powder	Campden BRI
14.	<i>Staphylococcus hyicus</i>	281	Pig skin	Campden BRI
15.	<i>Staphylococcus intermedius</i>	7298	unknown	Campden BRI
16.	<i>Staphylococcus piscifermentans</i>	5929	unknown	Campden BRI
17.	<i>Staphylococcus sciuri</i>	6690	unknown	Campden BRI
18.	<i>Staphylococcus simulans</i>	244	Human skin	NCTC 11046
19.	<i>Staphylococcus warneri</i>	262	German salami	Campden BRI
20.	<i>Staphylococcus xylosum</i>	266	Mettwurst sausage	Campden BRI
21.	<i>Micrococcus luteus</i>	16258	NCTC 2665	NCTC
22.	<i>Pseudomonas aeruginosa</i>	8299	NCIMB 10753	NCIMB
23.	<i>Escherichia coli</i>	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
Prawns (fish and seafood)	0.987	0.998	1.195	-0.103	0.990	228	0.192	646	0.050	863
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
Pasta (other)	0.986	0.996	1.020	-0.723	1.112	296	0.430	235	0.085	317
<b>All Foods (global)</b>	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<sup>SM</sup>* 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.