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Note

Evaluation of the Compact Dry X-SA Method for Enumerating *Staphylococcus aureus* in Artificially Contaminated Food Samples

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Compact Dry X-SA (CD-XSA), a ready-to-use and self-diffusible dry medium sheet culture system for the detection and enumeration of *Staphylococcus aureus*, was evaluated. A total of 50 *S. aureus* strains, which were studied for the inclusivity study, grew as blue-colored colonies on the CD-XSA. When 114 bacteria other than *S. aureus* and 3 yeasts were inoculated for the exclusivity study, 37 strains produced white colonies, and 4 strains produced blue colonies, and 3 strains produced magenta colonies, while 73 other strains failed to grow.

The CD-XSA method was compared with the mannitol salt agar with egg yolk (MSEY) method, the Baird-Parker agar (BP) method and the 3M Petrifilm™ STX (3M-STX) method in 105 artificially contaminated food samples. The correlation coefficients between CD-XSA and MSEY, CD-XSA and BP, and CD-XSA and 3M-STX were 0.945, 0.960 and 0.977, respectively.

Key words : *Staphylococcus aureus*/Compact Dry X-SA/Enumerating *S. aureus*/Ready-to-use medium.

Staphylococcus aureus is a major foodborne pathogen which produces several kinds of heat-stable enterotoxins. Thus it causes health problems worldwide, and patients experience nausea, vomiting, diarrhea and abdominal pains after the consumption of contaminated foods (Balaban and Rasooly, 2000, Jablonski and Bohach, 1997, Nema et al., 2007). A large-scale outbreak of *S. aureus* food poisoning through the consumption of milk and milk products occurred around Osaka, Japan in 2000 (Asao et al., 2003, Yamashita et al., 2003, Ministry of Health and Welfare and Osaka City, 2001). The control of contamination by *S. aureus* is a very important issue for manufacturers in food processing and distribution facilities.

Both mannitol salt agar with egg yolk (MSEY) and Baird-Parker agar (PB) are used for the detection of *S. aureus* (Baird, 1995, Baird-Parker, 1962, Gunn et al., 1972). The U.S. Food and Drug Administration *Bacteriological Analytical Manual* recommends 45-48h of incubation with BP for enumeration of *S. aureus*. Similarly, EN ISO 6888-1 for enumeration of *S. aureus* with BP recommends not only 24h of incubation but also 48h (De Buyser et al., 2003). However these methods require a lot of time for preparing the media and thereby raises labor costs. Additionally, spreading samples onto these media is troublesome.

The 3M Petrifilm STX™ (3M-STX; 3M Microbiology Products, St. Paul, MN, USA) method is accepted as a ready-to-use medium. This method not only eliminates these disadvantages but also has been evaluated as a suitable alternative (Fedio et al., 2008, Ingham et al., 2003).

The Compact Dry X-SA method (CD-XSA; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) is a novel method based on the unique Compact Dry system (Mizuochi and Kodaka, 2000). The CD-XSA system consists of a unique dish, nonwoven fabric, peptone, mineral salts, mannitol, EDTA (ethylenediaminetetraacetic acid), chromogenic

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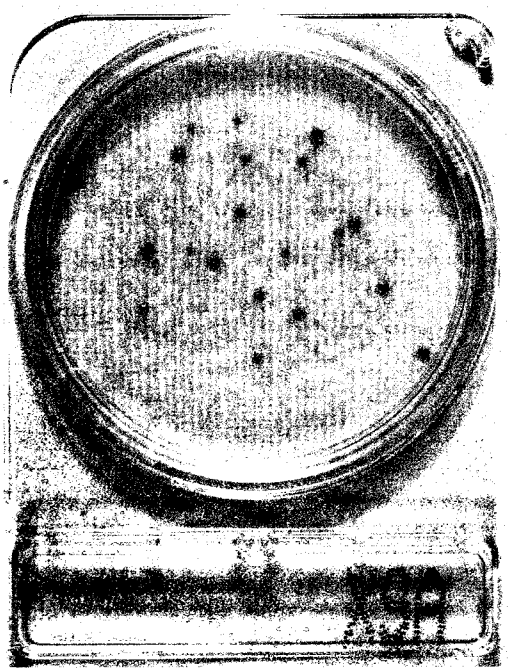

FIG. 1. CD-XSA. *S. aureus* ATCC 12600 grew as blue colored colonies on CD-XSA after 24h of incubation at 35℃.

substrates, antibiotics and a gelling agent. After 1ml samples are inoculated and incubated at 35℃ for 24 ± 2h, *S. aureus* grows as blue colonies on the plate. (Fig.1) This method also eliminates the disadvantages of conventional methods since CD-XSA is a pre-sterilized ready-to-use medium. EDTA and antibiotics inhibit the growth of bacteria, yeasts and molds other than *S. aureus*. Mannitol and two kinds of chromogenic substrates for acid phosphatase and β-glucosidase can differentiate *S. aureus* from other bacteria which can grow on the CD-XSA plate.

In this study, the CD-XSA method was compared with conventional methods and its performance in the detection and enumeration of *S. aureus* was evaluated.

Fifty strains of *S. aureus* were inoculated for the inclusivity study. For the exclusivity study, 40 Staphylococcus sp. strains other than *S. aureus*, 37 gram-positive strains excluding *Staphylococcus* spp., 37 gram-negative strains and 3 yeasts were inoculated. After bacteria and yeasts were cultured in Tryptic Soy Broth (Difco, Becton Dickinson, Detroit, MI, USA) at 35℃ for 24h and Sabouraud Dextrose Broth (Difco) at 25℃ for 72h respectively, each culture was diluted serially by saline (0.85% NaCl). One milliliter of each suspension was inoculated onto the CD-XSA. After inoculation, the CD-XSA plate were incubated at 35℃ for 24h.

Results from both the inclusivity and the exclusivity studies are shown in Table 1. A total of 50 *S. aureus* strains grew as blue-colored colonies on CD-XSA. Of 40 *Staphylococcus* sp. strains other than *S. aureus*, 35 strains grew as white colonies, whereas 5 strains did not grow on CD-XSA. Of 37 gram-positive bacteria other than *Staphylococcus* spp., 8 *Bacillus* spp. and 1 *Corynebacterium* sp. grew on CD-XSA. Of 8 *Bacillus* spp., 3 strains formed magenta-colored colonies, 4 strains formed blue-colored colonies and 1 strain formed white-colored colonies. The colonies of 4 blue-colony-forming *Bacillus* spp. were spread and had a frosted glass appearance. As a result, these colonies could be distinguished from *S. aureus*. None of the 37 gram-negative strains and the 3 yeasts grew on CD-XSA.

The CD-XSA method was compared with the MSEY, BP and the 3M-STX methods using artificially contaminated food samples. One hundred and five samples (30 meat, 30 milk and dairy, and 45 confectionery samples) were purchased from retail stores. For confirmation that these samples were negative for *S. aureus*, after being homogenized with saline for 2min by a homogenizer (Pro-media SH-001, ELMEX LIMITED, Tokyo, Japan), samples were inoculated onto two plates of MSEY and BP, and incubated for 48h at 35℃. For the comparison experiments, three strains of *S. aureus* (ATCC 6538, ATCC 12600, ATCC 13565, American Type Culture Collection, Manassas, VA,) were used randomly for inoculation. Each 10g sample was inoculated at the following levels: low (2-3 log CFU/g), medium (3-4 log CFU/g) and high (4-5 log CFU/g). Each artificially contaminated sample was added to a 9-fold volume of saline and was homogenized for 2min. Subsequently, each homogenated sample was subjected to 10-fold serial dilution by saline. Dual measurements were then carried out for each method. One milliliter of each dilution was inoculated onto the CD-XSA plate and onto 3M-STX. After 24h of incubation at 35℃, the blue colonies on CD-XSA and the purple colonies on 3M-STX were determined to be *S. aureus* respectively. For the two other methods, MSEY and BP were inoculated with 0.1ml of the sample. Samples were then spread onto the surface of each medium with sterilized rods. After 48h of incubation at 35℃, typical colonies were counted.

Results were calculated as a log CFU of *S. aureus* per gram of the tested food. Statistical analyses were carried out with Microsoft Excel 2000 at the significance level of $P = 0.05$.

The correlation coefficients (r), slopes, intercepts and 95% confidence limits between the CD-XSA and MSEY methods, the CD-XSA and BP methods, and the CD-XSA and 3M-STX methods are shown in

TABLE 1-1. Strains tested for growth and color on CD-XSA

Name of organism	No. of tested strains			No. of strains grown[c]	
	Standard[a]	Isolate[b]	Total		
Staphylococcus spp.					
S. aureus	25A, 1J	24	50	50	(B)
S. auricularis	1A	0	1	0	
S. capitis	1A	1	2	2	(W)
S. caprae	0	2	2	2	(W)
S. caseolyticus	1A	0	1	1	(W)
S. cohnii	0	3	3	3	(W)
S. delphini	0	1	1	1	(W)
S. epidermidis	2A	3	5	5	(W)
S. haemolyticus	1A	2	3	2	(W)
S. hominis	1A	2	3	1	(W)
S. hyicus	0	2	2	2	(W)
S. intermedius	1A	1	2	2	(W)
S. lentus	1A	0	1	1	(W)
S. lugdunensis	0	2	2	2	(W)
S. saprophyticus	1A	1	2	2	(W)
S. schleiferi	0	2	2	2	(W)
S. sciuri	1A	1	2	2	(W)
S. simulans	1A	1	2	2	(W)
S. warneri	1A	1	2	1	(W)
S. xylosus	1A	1	2	2	(W)
Subtotal	40	50	90	85	
Gram positive bacteria except for *Staphylococci*					
Bacillus cereus	1A	4	5	3, 1, 1	(LB, M, W)
B. coagulans	1A	0	1	0	
B. licheniformis	1A	0	1	1	(M)
B. pumilus	1A	0	1	0	
B. sphaericus	1A	0	1	0	
B. subtilis	1A	0	1	1	(M)
B. thuringiensis	2N	0	2	1	(LB)
Corynebacterium renale	1A	0	1	0	
C. xerosis	1A	0	1	1	(W)
C. minutissimum	1A	0	1	0	
Enterococcus avium	1A	0	1	0	
E. durans	1A	0	1	0	
E. casseliflavus	1A	0	1	0	
E. faecalis	6A	0	6	0	
E. faecium	3A	0	3	0	
E. gallinarum	1A	0	1	0	
E. hirae	1A	0	1	0	
E. mundtii	1A	0	1	0	
E. raffinosus	1A	0	1	0	
Lactobacillus lactis	1A	0	1	0	
Leuconostoc citreum	1A	0	1	0	
L. mesenteroides	1A	0	1	0	
Micrococcus luteus	1A	0	1	0	
Pediococcus acidilactici	1A	0	1	0	
Streptococcus thermophilus	1A	0	1	0	
Subtotal	33	4	37	9	

[a]Standard strains were from A; ATCC, J; JCM (Japan Collection of Microorganisms) and N; NBRC (NITE Biological Resource Center, Japan).
[b]Isolated strains were derived from clinical specimens.
[c]Parentheses indicate colony color: B, blue; LB, light blue; W, white; M, magenta.

Table 1 represents results of inclusivity and exclusivity studies for CD-XSA.
Each strain was cultured on CD-XSA for 24h at 35℃.

TABLE 1-2. Strains tested for growth and color on CD-XSA

Name of organism	No. of tested strains			No. of strains grown[c]
	Standard[a]	Isolate[b]	Total	
Gram negative bacteria				
Aeromonas hydrophila	1J	0	1	0
Citrobacter amalonaticus	1A	0	1	0
C. freundii	1A	0	1	0
C. koseri	1A	0	1	0
Enterobacter aerogenes	1A	0	1	0
E. amnigenus	1A	0	1	0
E. cloacae	1A	0	1	0
E. intermedius	2A	0	2	0
E. sakazakii	1A	0	1	0
Escherichia coli	4A	0	4	0
E. coli O-157	2A	0	2	0
E. hermanii	1J	0	1	0
Hafnia alvei	1A	0	1	0
Klebsiella ozytoca	1A	0	1	0
K. ozaenae	1A	0	1	0
K. pneumoniae	1A	0	1	0
Kluyvera ascorbata	1A	0	1	0
K. cryocrescens	1A	0	1	0
Morganella morganii	1A	0	1	0
Proteus mirabilis	1A	0	1	0
P. vulgaris	1A	0	1	0
Pseudomonas aeruginosa	3A	0	3	0
P. putida	1A	0	1	0
Rahnella aquatilis	1A	0	1	0
Salmonella Choleraesuis	1A	0	1	0
S. Typhimurium	1A	0	1	0
Serratia fonticola	1A	0	1	0
S. liquefaciens	1A	0	1	0
S. marcescens	2A	0	2	0
Subtotal	37	0	37	0
Yeasts				
Candida albicans	2A	0	2	0
Saccharomyces cerevisiae	1A	0	1	0
Subtotal	3	0	3	0
Total	113	54	167	94

[a]Standard strains were from A; ATCC, J; JCM (Japan Collection of Microorganisms) and N; NBRC (NITE Biological Resource Center, Japan).
[b]Isolated strains were derived from clinical specimens.
[c]Parentheses indicate colony color: B, blue; LB, light blue; W, white; M, magenta.

Table 1 represents results of inclusivity and exclusivity studies for CD-XSA.
Each strain was cultured on CD-XSA for 24h at 35℃.

Table 2. The *r* values between CD-XSA and MSEY, CD-XSA and BP, and CD-XSA and 3M-STX, were 0.945, 0.960, and 0.977 respectively. The slopes and intercepts of analyzed regression lines for all comparisons were close to 1.00 and 0.00, respectively.

For the monitoring of *S. aureus*, PCR-based methods (Chiang et al., 2007) and chromogenic culture methods (Gaillot et al., 2000, Perry et al., 2003, Ritter et al., 2009) have been developed. These molecular methods such as PCR-based methods can detect *S. aureus* rapidly but they also need specific devices, skills and have high costs. The chromogenic culture methods, including the CD-XSA and the 3M-STX, make operations more cost-effective, and comparatively detect *S. aureus* easily and rapidly. Moreover the CD-XSA method has both high selectivity and a good correlation with conventional methods. Therefore the simplicity of usage of CD-XSA and

TABLE 2. Concurrence among three methods for enumerating *S. aureus* in artificially contaminated food samples[a,b]

Parameter	CD-XSA vs. MSEY	CD-XSA vs. BP	CD-XSA vs. 3M-STX
No. of samples	101[d]	105	105
Correlation coefficient	0.945	0.960	0.977
Slope	0.973	0.964	0.952
Intercept	0.002	-0.076	0.130
95% confidence limits	±0.16	±0.17	±0.17
ANOVA (p)[c]	0.56	0.15	0.94

[a]Artificially contaminated food samples were inoculated with *S. aureus* ATCC6538, ATCC12600 or ATCC13565 randomly.
[b]Each strain was inoculated at high (4-5 logCFU/g), medium (3-4 logCFU/g) or low (2-3 logCFU/g) bacterial levels randomly.
[c]ANOVA was performed at a significance level of 0.05. P<0.05 is a statistically significant difference between two methods.
[d]MSEY failed to recover *S. aureus* from 4 samples.

Table 2 represents the concurrence of CD-XSA and each method in the examination of artificially contaminated food. After the counts (CFU/g) from artificially contaminated food samples were converted to logarithm (LogCFU/g) values, linear regression equations for CD-XSA with respect to each method were calculated.
AVOVA was performed for each method.

these results suggest the CD-XSA method is a suitable alternative method for the detection and enumeration of *S. aureus* in daily food hygiene control.

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