

Full Length Research Paper

Evaluation of the dry sheet medium (Compact Dry LS) for screening of total *Listeria* count in food samples

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The Compact Dry LS (CD-LS), ready-to-use dry sheet selective medium for *Listeria* species, was evaluated for inclusivity and exclusivity by using 107 strains including 20 *Listeria* species strains. All tested *Listeria* species strains other than *Listeria seeligeri* that grew as blue colored colony on CD-LS. CD-LS were compared with those on Oxford agar and ALOA agar as conventional methods using *Listeria* species inoculated from 100 food samples. The correlation coefficients between CD-LS and Oxford agar, and CD-LS and ALOA agar were 0.983 and 0.978, respectively. Our results suggested CD-LS was a suitable alternative medium for screening of *Listeria* species.

Key words: Chromogenic selective medium, ready-to-use medium, *Listeria*, compact dry.

INTRODUCTION

Listeria monocytogenes is known as one of major food-borne pathogen worldwide which causes septicemia, meningitis and encephalitis through the consumption of contaminated foods (Barancelli et al., 2011; Inoue et al., 2000; Makino et al., 2005; Swaminathan et al., 2007). In addition *L. monocytogenes* can grow in cold condition (Carpentier and Cerf, 2011). Hence, the control of contamination by *L. monocytogenes* is a very important issue for food safety (Becker et al., 2006; Midelet-Bourdin et al., 2007; Vermeulen et al., 2011).

Both Oxford agar and PALCAM agar have been used for detection of *Listeria* species for a long time (Beumer and Curtis, 2003; Reissbrodt, 2004). Recently, several chromogenic agar media such as ALOA agar have been developed (Jantzen et al., 2006; Vlaemynck et al., 2000). These chromogenic media can not only detect more quickly than Oxford agar and PALCAM agar, but also differentiate *L. monocytogenes* from other *Listeria* species strains. However, these agar media have high cost for daily screening tests and short shelf life of prepared plate since these media contain several antibiotics as selective agents. What is more, long shelf

life and cost effective medium is needed for screening tests in food processing facilities.

In consideration of these points, Compact Dry LS method (CD-LS; Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) has been developed based on the unique Compact Dry system (Mizuochi and Kodaka, 2000) as a more cost effective screening dry sheet medium for *Listeria* species. 1 ml amount can be inoculated onto CD-LS after 1 h of pre-incubation at 20°C by Buffered Peptone Water (BPW; Nissui Pharmaceutical Co., Ltd.). *Listeria* species strains grow as blue colored colonies on CD-LS after 24 h of incubation at 37°C. This method eliminates the disadvantages of conventional methods since CD-LS is a cost effective pre-sterilized ready-to-use medium with long shelf life. CD-LS was developed primarily for selective medium for *Listeria*. CD-LS system consists of a unique dish, self diffusible fabric sheet, peptone, salts, chromogenic substrate and two kinds of antibiotic. The chromogenic substrate for β – glucosidase can differentiate *Listeria* species strains from other bacteria which may grow on CD-LS plate. The two kinds of antibiotic inhibit the growth of bacteria other than *Listeria* strains.

The purpose of this study was to evaluate the performance of CD-LS method for the detection and enumeration of *Listeria* species strains in comparison to conventional methods.

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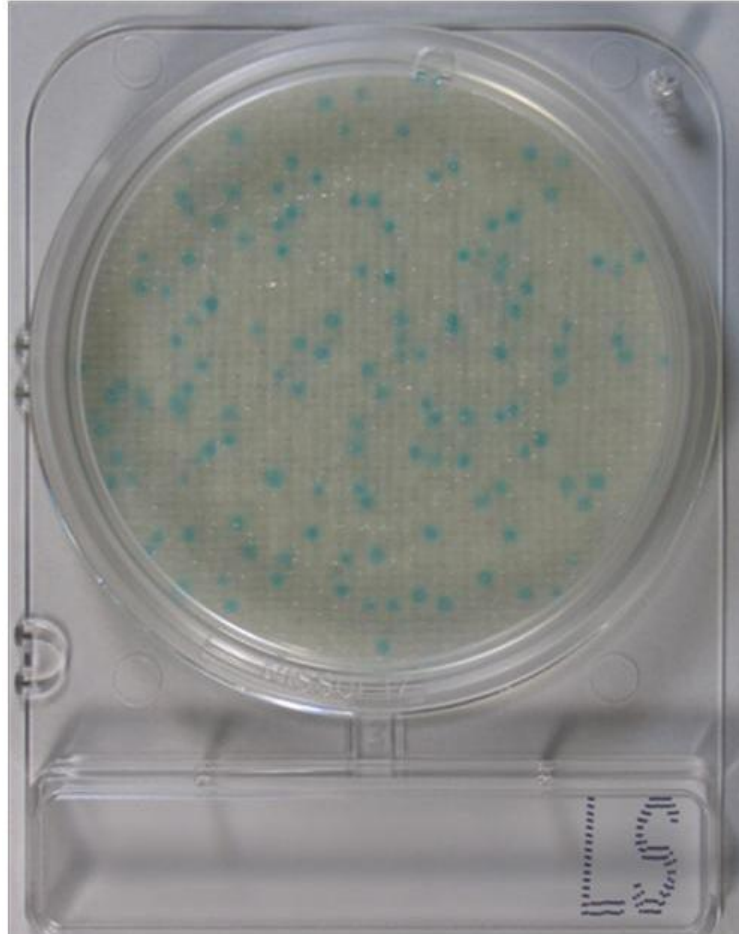


Figure 1. Typical appearance of *L. monocytogenes* JCM 7680 on a CD-LS plate.

MATERIALS AND METHODS

Samples

For comparison studies, 38 processed meat products, 15 processed fish products, 34 cheese products, 6 ready-to-eat vegetables and 6 delicatessen products were purchased from retail stores in Japan.

Inclusivity and exclusivity studies

20 strains of *Listeria* spp. were used for the inclusivity study. For the exclusivity study, 41 Gram-positive bacterial strains other than *Listeria* spp., 43 Gram-negative bacterial strains and 3 yeast strains were inoculated, respectively. Bacterial strains were prepared in Tryptic Soy Broth (Difco, Becton Dickinson, Detroit, MI, USA) at 37°C for 24 h, and yeast strains were cultured in Sabouraud Dextrose Broth (Difco) at 25°C for 72 h. Each culture was diluted serially by saline (0.85% NaCl). 1 ml of each suspension was inoculated onto CD-LS. After 24 h of incubation at 37°C, the blue colored colonies (Figure. 1) were read as *Listeria* species. For the two other methods, Oxford agar (Difco) and ALOA agar (Merck Ltd. Japan, Tokyo) were inoculated 0.1 ml of each suspension onto the surface of each medium with sterilized plastic bacterial spreader

(Nissui Pharmaceutical Co., Ltd.). After 24 h of incubation at 37°C, the characteristics and the number of colonies were examined. Inoculated bacterial number was confirmed by Tryptic Soy Agar (TSA; Difco) under the same conditions.

Method comparison study

The CD-LS method was compared with Oxford agar and ALOA agar methods using artificially contaminated food samples. 100 samples (38 processed meat products, 15 processed fish products, 34 cheese products, 6 ready-to-eat vegetables and 6 delicatessen products) were purchased from retail stores in Japan. For confirmation that these samples were negative for *Listeria* spp., after 25 g of each sample was homogenized with 9-fold volume of half Fraser Broth (Difco) for 1 min by a homogenizer (Pro-media SH-001, ELMEX LIMITED, Tokyo, Japan), homogenized samples were incubated for 24 h at 30°C. Subsequently, 0.1 ml of each sample was transferred into Fraser Broth (Difco). After 48 h of incubation at 37°C, each sample was streaked onto Oxford agar and ALOA agar and incubated for 24 h at 37°C.

For the comparison experiments, three strains of *L. monocytogenes* (JCM 7680, NS 5197, NS 5116, JCM; Japan Collection of Microorganisms, Saitama, Japan, NS; isolated strains of clinical specimens) were used randomly for inoculation. Each 25

Table 1a. Gram positive bacteria^{ab} strains tested for color and growth using CD-LS.

Bacteria	CD-LS ^c		Oxford agar		ALOA agar		TSA
	Color	logCFU/ml	Color	logCFU/ml	Color	logCFU/ml	logCFU/ml
Listeria spp.							
<i>L. grayi</i> ATCC 19120	blue	9.23	black	9.20	blue	9.11	9.18
<i>L. grayi</i> NS 5165	blue	7.92	black	6.48	light blue	7.61	7.88
<i>L. innocua</i> ATCC 33090	blue	9.43	black	9.36	blue	9.36	9.35
<i>L. innocua</i> NS 5166	blue	9.47	black	9.40	blue	9.42	9.41
<i>L. ivanovii</i> JCM 7681	light blue	9.19	black	8.91	blue + halo	9.23	9.42
<i>L. ivanovii</i> NS 5167	blue	9.01	black	8.80	blue + halo	9.26	9.39
<i>L. monocytogenes</i> ATCC 15313	blue	8.62	black	8.66	blue + halo	9.00	8.16
<i>L. monocytogenes</i> JCM 7671	blue	9.22	black	9.40	blue + halo	9.36	9.41
<i>L. monocytogenes</i> JCM 7679	blue	9.02	black	9.20	blue + halo	9.58	9.55
<i>L. monocytogenes</i> JCM 7680	blue	9.50	black	9.53	blue + halo	9.65	9.47
<i>L. monocytogenes</i> NS 5057	blue	8.43	black	8.53	blue + halo	8.74	8.01
<i>L. monocytogenes</i> NS 5168	blue	9.60	black	9.36	blue + halo	9.56	9.55
<i>L. monocytogenes</i> NS 5169	blue	9.37	black	9.18	blue + halo	9.22	9.30
<i>L. monocytogenes</i> NS 5170	blue	9.51	black	9.31	blue + halo	9.33	9.37
<i>L. monocytogenes</i> NS 5197	blue	9.14	black	9.19	blue + halo	9.14	8.91
<i>L. monocytogenes</i> NS 5199	blue	8.89	black	9.03	blue + halo	9.04	8.66
<i>L. seeligeri</i> ATCC 35967	not grown	—	not grown	—	not grown	—	8.96
<i>L. seeligeri</i> NS 5171	not grown	—	not grown	—	not grown	—	9.06
<i>L. welshimeri</i> ATCC 35897	blue	9.01	black	8.91	blue	9.19	9.12
<i>L. welshimeri</i> NS 5172	blue	9.24	black	9.23	blue	9.27	9.22
Non-Listeria spp. bacteria							
<i>Bacillus cereus</i> ATCC 19637	not grown	—	not grown	—	not grown	—	5.74
<i>B. licheniformis</i> ATCC 14580	not grown	—	not grown	—	not grown	—	7.06
<i>B. subtilis</i> ATCC 6633	not grown	—	not grown	—	not grown	—	6.56
<i>Corynebacterium renale</i> ATCC 19412	not grown	—	not grown	—	not grown	—	7.73
<i>C. minutissimum</i> ATCC 23348	not grown	—	not grown	—	not grown	—	7.06
<i>C. xerosis</i> ATCC 373	not grown	—	not grown	—	not grown	—	7.51
<i>Enterococcus avium</i> ATCC 14025	not grown	—	not grown	—	not grown	—	7.52
<i>E. durans</i> ATCC 19432	not grown	—	not grown	—	not grown	—	7.42
<i>E. casseliflavus</i> ATCC 51328	not grown	—	not grown	—	not grown	—	7.49
<i>E. faecalis</i> ATCC 19433	not grown	—	not grown	—	not grown	—	8.53

Table 1. Contd.

<i>E. faecalis</i> ATCC 29212	not grown	—	not grown	—	not grown	—	8.56
<i>E. faecium</i> ATCC 19434	not grown	—	not grown	—	not grown	—	8.48
<i>E. gallinarum</i> ATCC 49608	not grown	—	not grown	—	not grown	—	6.82
<i>E. hirae</i> ATCC 8043	not grown	—	not grown	—	not grown	—	7.69
<i>E. mundtii</i> ATCC 43186	not grown	—	not grown	—	not grown	—	7.89
<i>E. raffinosus</i> ATCC 49427	not grown	—	not grown	—	not grown	—	7.05
<i>Lactobacillus lactis</i> ATCC 12315	not grown	—	not grown	—	not grown	—	6.73
<i>Leuconostoc citreum</i> JCM 9698	not grown	—	not grown	—	not grown	—	8.05
<i>L. mesenteroides</i> ATCC 27258	not grown	—	not grown	—	not grown	—	7.99
<i>Micrococcus luteus</i> ATCC 9341	not grown	—	not grown	—	not grown	—	7.51
<i>Pediococcus acidilactici</i> JCM 5885	not grown	—	not grown	—	not grown	—	7.88
<i>Staphylococcus aureus</i> ATCC 12600	not grown	—	not grown	—	not grown	—	7.81
<i>S. aureus</i> ATCC 25923	not grown	—	not grown	—	not grown	—	8.16
<i>S. aureus</i> ATCC 29213	not grown	—	not grown	—	not grown	—	7.72
<i>S. aureus</i> ATCC 6538	not grown	—	not grown	—	not grown	—	8.11
<i>S. aureus</i> ATCC 6538P	not grown	—	not grown	—	not grown	—	7.83
<i>S. aureus</i> MRSA NS 7167	not grown	—	not grown	—	not grown	—	8.04
<i>S. auricularis</i> ATCC 33753	not grown	—	not grown	—	not grown	—	6.56
<i>S. capitis</i> ATCC 27840	not grown	—	not grown	—	not grown	—	8.21
<i>S. epidermidis</i> ATCC 12228	not grown	—	not grown	—	not grown	—	7.33
<i>S. epidermidis</i> ATCC 14990	not grown	—	not grown	—	not grown	—	7.44
<i>S. haemolyticus</i> ATCC 29970	not grown	—	not grown	—	not grown	—	6.52
<i>S. intermedius</i> ATCC 29663	not grown	—	not grown	—	not grown	—	7.51
<i>S. lentus</i> ATCC 29070	not grown	—	not grown	—	not grown	—	7.28
<i>S. saprophyticus</i> ATCC 15305	not grown	—	not grown	—	not grown	—	7.44
<i>S. sciuri</i> ATCC 29062	not grown	—	not grown	—	not grown	—	7.48
<i>S. simulans</i> ATCC 27848	not grown	—	not grown	—	not grown	—	7.84
<i>S. warneri</i> ATCC 27836	not grown	—	not grown	—	not grown	—	7.51
<i>S. xylosus</i> ATCC 29971	not grown	—	not grown	—	not grown	—	6.59
<i>S. thermophilus</i> ATCC 14485	not grown	—	not grown	—	not grown	—	7.56

g sample was randomly inoculated at the following levels: 2 to 3, 3 to 4, 4 to 5 and 5 to 6 log CFU/g. After 3 days of preservation at 4°C, each artificially contaminated sample was added to 9-fold volume of BPW and was homogenized for 1 min with a homogenizer. After 1

h of incubation at 20°C for resuscitation, each homogenized sample was subjected to 10-fold serial dilution by BPW. Dual measurements were then carried out for each method. 1 ml of each dilution was inoculated onto the CD-LS plate. For the two other methods, Oxford

Table 1b. Gram negative bacteria^{ab} strains tested for color and growth using CD-LS.

Gram negative bacteria	CD-LS		Oxford agar		ALOA agar		TSA
	Color	logCFU/ml	Color	logCFU/ml	Color	logCFU/ml	logCFU/ml
<i>Aeromonas hydrophila</i> JCM 3976	not grown	—	not grown	—	not grown	—	8.51
<i>Alcaligenes denitrificans</i> JCM 5490	not grown	—	not grown	—	not grown	—	7.06
<i>A. xylosoxidans</i> JCM 9659	not grown	—	not grown	—	not grown	—	6.56
<i>A. faecalis</i> JCM 1474	not grown	—	not grown	—	not grown	—	7.73
<i>Citrobacter amalonaticus</i> ATCC 25405	not grown	—	not grown	—	not grown	—	7.06
<i>C. freundii</i> ATCC 8090	not grown	—	not grown	—	not grown	—	7.51
<i>C. koseri</i> ATCC 25408	not grown	—	not grown	—	not grown	—	7.52
<i>Enterobacter aerogenes</i> ATCC 13048	not grown	—	not grown	—	not grown	—	7.42
<i>E. amnigenus</i> ATCC 33072	not grown	—	not grown	—	not grown	—	7.49
<i>E. cloacae</i> ATCC 13047	not grown	—	not grown	—	not grown	—	8.53
<i>E. intermedius</i> ATCC 33423	not grown	—	not grown	—	not grown	—	8.56
<i>E. intermedius</i> ATCC 33110	not grown	—	not grown	—	not grown	—	8.48
<i>E. sakazakii</i> ATCC 29544	not grown	—	not grown	—	not grown	—	6.82
<i>Escherichia blattae</i> JCM 1650	not grown	—	not grown	—	not grown	—	7.69
<i>E. coli</i> ATCC 11775	not grown	—	not grown	—	not grown	—	7.89
<i>E. coli</i> ATCC 25922	not grown	—	not grown	—	not grown	—	7.05
<i>E. coli</i> ATCC 8739	not grown	—	not grown	—	not grown	—	6.73
<i>E. coli</i> ATCC 9637	not grown	—	not grown	—	not grown	—	8.05
<i>E. coli</i> Serotype O157 ATCC 35150	not grown	—	not grown	—	not grown	—	7.99
<i>E. coli</i> Serotype O157 ATCC 43888	not grown	—	not grown	—	not grown	—	7.51
<i>E. fergusonii</i> JCM 5897	not grown	—	not grown	—	not grown	—	7.88
<i>E. fergusonii</i> JCM 5899	not grown	—	not grown	—	not grown	—	7.81
<i>E. Hermannii</i> JCM 1473	not grown	—	not grown	—	not grown	—	8.16
<i>Hafnia alvei</i> ATCC 13337	not grown	—	not grown	—	not grown	—	7.72
<i>Klebsiella oxytoca</i> ATCC 13182	not grown	—	not grown	—	not grown	—	8.11
<i>K. ozaenae</i> ATCC 11296	not grown	—	not grown	—	not grown	—	7.83
<i>K. pneumoniae</i> ATCC 13883	not grown	—	not grown	—	not grown	—	8.04
<i>Kluyvera ascorbata</i> ATCC 33433	not grown	—	not grown	—	not grown	—	6.56
<i>K. cryocrescens</i> ATCC 33435	not grown	—	not grown	—	not grown	—	8.21
<i>Morganella morganii</i> ATCC 25830	not grown	—	not grown	—	not grown	—	7.33
<i>Proteus mirabilis</i> ATCC 29906	not grown	—	not grown	—	not grown	—	7.44

Table 1B. Cont.

<i>P. vulgaris</i> ATCC 13315	not grown	—	not grown	—	not grown	—	6.52
<i>S. hominis</i> ATCC 27844	not grown	—	not grown	—	not grown	—	7.32
<i>Pseudomonas aeruginosa</i> ATCC 10145	not grown	—	not grown	—	not grown	—	7.51
<i>P. aeruginosa</i> ATCC 9721	not grown	—	not grown	—	not grown	—	7.28
<i>P. aeruginosa</i> ATCC 27853	not grown	—	not grown	—	not grown	—	7.44
<i>P. aeruginosa</i> ATCC 9027	not grown	—	not grown	—	not grown	—	7.48
<i>P. putida</i> ATCC 12633	not grown	—	not grown	—	not grown	—	7.84
<i>Rahnella aquatilis</i> ATCC 33071	not grown	—	not grown	—	not grown	—	7.51
<i>Salmonella</i> Typhimurium ATCC 13311	not grown	—	not grown	—	not grown	—	6.59
<i>Serratia fonticola</i> ATCC 29844	not grown	—	not grown	—	not grown	—	7.56
<i>S. liquefaciens</i> ATCC 29844	not grown	—	not grown	—	not grown	—	7.56
<i>S. marcescens</i> ATCC 13880	not grown	—	not grown	—	not grown	—	7.56
<i>S. marcescens</i> ATCC 8100	not grown	—	not grown	—	not grown	—	7.56
Yeasts							
<i>Candida albicans</i> ATCC 2091	not grown	—	not grown	—	not grown	—	7.56
<i>C. albicans</i> ATCC 10231	not grown	—	not grown	—	not grown	—	7.56
<i>Saccharomyces cerevisiae</i> ATCC 9080	not grown	—	not grown	—	not grown	—	7.56

^aStandard strains were derived from ATCC (American Type Culture Collection) and JCM (Japan Collection of Microorganisms) and NBRC (NITE Biological Resource Center, Japan).

^bNS strains were isolated from clinical specimens. ^cRecovered bacterial number is represented as logCFU/ml.

agar and ALOA agar were inoculated onto the surface of each medium with sterilized plastic bacterial spreader, blue colonies on CD-LS and ALOA agar, and black colonies on Oxford agar were read as *Listeria* strains, respectively.

Statistical analysis

Results from method comparison study were converted into log CFU of *Listeria* per gram of each tested food. All statistical analyses were carried out with the Microsoft Excel 2000 at the significance level of $P = 0.05$. The linear correlation coefficients (r), slopes, intercepts between CD-LS and Oxford agar, and CD-LS and ALOA agar were calculated, respectively. A one-way analysis of variance (ANOVA) was performed to determine differences between CD-LS and both methods.

RESULTS

Results from both inclusivity and exclusivity studies are shown in Table 1. Of 20 *Listeria* species strains, 18 strains (90%) grew as blue colored colony on CD-LS. *L. seeligeri* strains used in this study were not grown on CD-LS, Oxford agar and ALOA agar. 18 *Listeria* species strains other than *L. seeligeri* also grew as typical colony on Oxford agar and ALOA agar, respectively. Especially only ALOA agar differentiated *L. monocytogenes* from other *Listeria* species strains. A total of 41 Gram-positive bacteria other than *Listeria* species, 43 Gram-negative bacteria and 3 yeasts failed to grow on all tested selective

media.

Figure 2 shows the liner regression lines, correlation coefficients (r), slopes, intercepts between CD-LS and Oxford agar, and CD-LS and ALOA agar from method comparison study. The r between CD-LS and Oxford agar, and CD-LS and ALOA agar, were 0.983 and 0.978, respectively. No significant difference was shown between CD-LS and both methods by one-way ANOVA ($P > 0.05$).

DISCUSSION

For the control of *L. monocytogenes* and *Listeria*

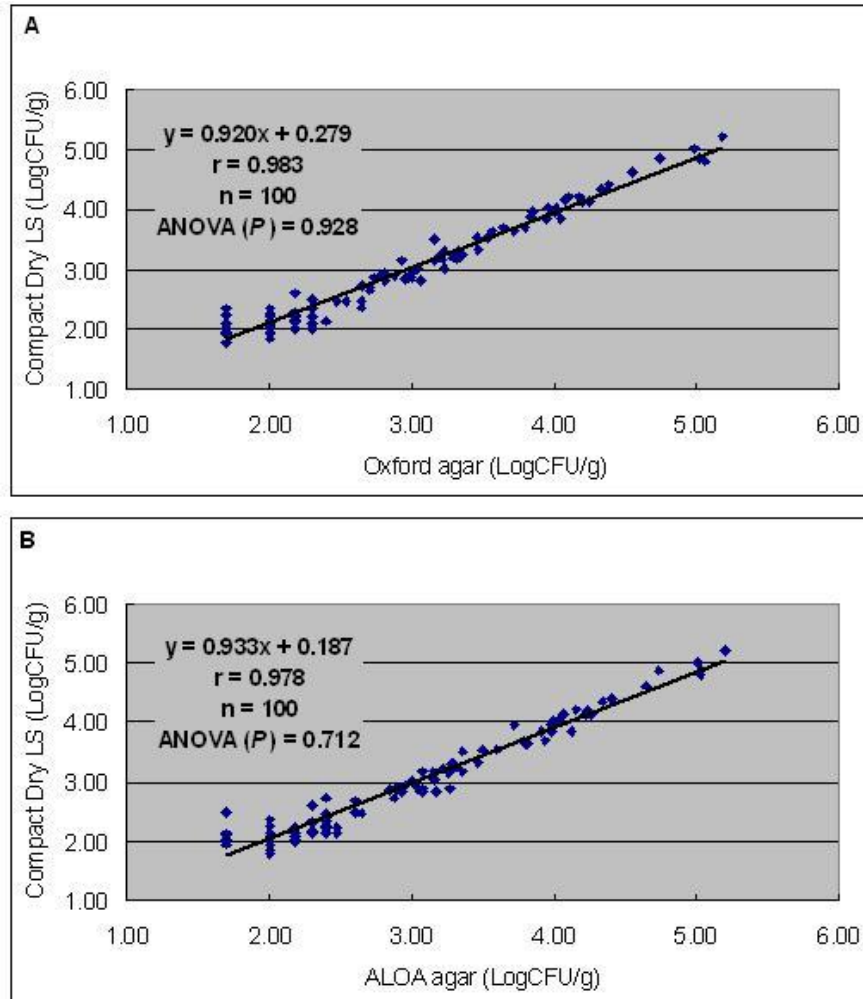


Figure 2. Regression line for data from CD-LS method plotted against Oxford agar method (A) and ALOA agar method (B) for determining population of *L. monocytogenes* in 100 artificially contaminated food samples. ANOVA (one way analysis of variance) was performed at a significance level of 0.05. $P < 0.05$ is a statistically significant difference between two methods.

species, PCR-based methods (Mafu et al., 2009; Traunsek et al., 2011; Wallace et al., 2011) and chromogenic selective culture methods (Greenwood et al., 2005; Hegde et al., 2007; Willis et al., 2006) have been developed. These molecular methods such as PCR-based methods can differentiate *L. monocytogenes* from other *Listeria* species rapidly but they need specific devices, skills and have high costs for daily hygiene control. The chromogenic selective culture methods can also detect *L. monocytogenes* and other *Listeria* species separately. However these methods need preparation of medium and have short shelf life and relatively high cost. CD-LS can not differentiate *L. monocytogenes* from other *Listeria* species. However CD-LS makes operations more cost-effective, and comparatively detect the existence of *Listeria* species easily and rapidly, since CD-LS is ready-to-use chromogenic selective medium and has long shelf

life of 1 year at room temperature. Moreover CD-LS has both high selectivity and a good correlation with conventional methods. Therefore our results suggest that CD-LS is a suitable alternative medium for the screening of *Listeria* species in daily hygiene control.

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