Original Article

Quantitative Method for Detecting *Listeria* Species using the Bio-Theta DOX™ System

Shouichi Tanno[1] Naoki Fukui[1] Yukihiro Utaka[1] Saburo Ohkawa[2]

Yukio Morita [2] Sumalee Boonmar[2*]

Abstract

We developed a quantitative method for detecting *Listeria* species in food using the DOX™ system. The system could detect low concentrations (10 cfu/ml) of *Listeria monocytogenes*, *L. innocua*, and *L. welshimeri*, and could not detect medium concentrations (10^3 cfu/ml) of the 29 non-*Listeria* organisms examined in this study. The relationship between detection time and bacterial count of the 3 *Listeria* species had a good linear calibration curve. We carried out a recovery examination by inoculating 15 food samples with *L. monocytogenes*, and each sample was examined 5 times. *L. monocytogenes* was detected in 68 of the 75 samples inoculated with an ultra-low concentration of bacteria (<1 log cfu/ml), and the detection time of the positive samples was 1,052–1,870 min (average, 21.9 h). The DOX system provides rapid results (usually within 1 day), and requires no special techniques for measurement. The DOX system may be a useful tool for determining the presence of *Listeria* spp. in food and environmental samples at food processing companies, though more validation studies and field studies are needed.

Keywords: bacteria detecting system, DOX ™ system, total *Listeria* species counts

[1]*Daikin Industry, Ltd., 1-1, Nishi-Hitotsuya Settsu, Osaka 566-8585, Japan*
[2]*Tokyo Kasei University, 1-18-1 Kaga, Itabashi-ku, Tokyo 173-8602, Japan*
****Correspondence**: sumaleeka12@gmail.com*

Introduction

According to Bergey's Manual of Systematic Bacteriology, the genus *Listeria* contains 6 species and includes Gram-positive, rod-shaped, and aerobic bacteria (Mclauchlin and Rees, 2011). Recently, ten new species of *Listeria* such as *L. marthii, L. rocourtiae, L. weihenstephanensis, L. floridensis, L. aquatica, L. cornellensis, L. riparia, L. grandensis, L. booriae* and *L. newyorkensis* were reported (Graves et al, 2010; Leclercq et al, 2010; den Bakker et al, 2014; Weller et al, 2015), but these species of *Listeria* have not been associated with human and animal disease at this time.

L. monocytogenes is a major pathogenic species in humans and animals; however *L. monocytogenes* and *L. ivanovii* are zoonotic organisms (Mclauchlin and Rees, 2011). Variability in the growth limits of *L. monocytogenes* is wide, and the organism is able to grow at a wide pH range(pH 5.5–9.6), high bile concentration(40%), and high salinity(13.9% NaCl) (Wetzler et al, 1968; Schmid et al, 2005; Shabala et al, 2008; Mclauchlin and Rees, 2011). In addition, *L. monocytogenes* can grow in temperatures ranging from 0 °C to 45 °C (Mclauchlin and Rees, 2011) .

Listeriosis caused by *L. monocytogenes* has been recognized as an important food hygiene problem worldwide (Tasara and Stephan, 2006; Mclauchlin and Rees, 2011). This disease affects primarily pregnant women, newborns, and adults with weakened immune systems (Mclauchlin and Rees, 2011; Allerberger and Huhulescu, 2015). Reservoirs of *L. monocytogenes* are present in the environment and in human and animal populations (Mclauchlin and Rees, 2011), and are also commonly found in soil and water. Animals can carry the bacterium without appearing ill and can contaminate foodstuffs of animal origin, such as meat and dairy products. Uncooked meat, vegetables, unpasteurized milk and derived products, and ready-to-eat food are major sources of this pathogen for humans, and many countries have regulations regarding the safe limits of *L. monocytogenes* in foodstuffs. The CODEX Alimentarius contains "CAC/GL 61-2007, Guidelines on the Application of General Principles of Food Hygiene to the control of *Listeria monocytogenes* in Ready-to-Eat Foods." In one of the guidelines, the microbiological criteria for ready-to-eat foods state that the growth of *L. monocytogenes* should not be detected in 5 samples (sample volume of 25 g and microbiological limit < 0.04 cfu/g) and the organism should not grow in 5 food samples (microbiological limit: 100 cfu/g). In Japan, in 2014, the Ministry of Health, Labor, and Welfare published their regulations for the growth of *L. monocytogenes* in unheated processed meat products, soft cheese, and semi-hard cheese, and for the approved detection method. The standard limit in food was set at <100 cfu/g.

The DOX™ system (Bio-Theta, Osaka, Japan) provides a quantitative method for estimating bacterial counts based on respiration rate (Fig 1a) (Amano et al, 1999; Katayama, 2000). An oxygen electrode measures the level of dissolved oxygen in a sample diluted with media. Over time, a sample with a high bacterial load will cause the level of dissolved oxygen to decrease to a given threshold value faster than will a sample with a low bacterial load. The time required to reach the threshold level correlates with the amount of bacteria in the sample. Then, by creating a standard curve for each food matrix, the level of bacterial contamination can be estimated in unknown samples. In addition, the DOX system has a special feature that enables organisms to be isolated on agar gel by using the reagents in the DOX cassette (Fig 1b) once the organism has been detected by the DOX system. The DOX system provides rapid results and requires no special techniques for measurement. At present, Daikin Industries Ltd. staff have developed and supplied a total viable count (TVC) test kit, a quantitative coliforms and *Escherichia coli* detection test kit, a *Staphylococcus aureus* detection kit, a *Salmonella* detection kit, and a *Vibrio* spp. detection kit(Amano et al, 2001; Kawasaki et al, 2003; Tanno et al, 2014). Furthermore, the Association of Analytical Communities International Research Institute has recognized the TVC test (Certificate No. 040801) and quantitative coliforms and *E. coli* test (Certificate No. 120801) in the DOX system.

(a)

(b)

Figure 1 (a) Detection unit of the Bio-Theta DOX™ system. (b) The DOX cassette

Conventional quantitative culture procedures, such as ISO11290-1 and IDF143A, take approximately 2 days to perform, but the DOX system only requires 1 day. When an inspector gets a quick result, they will be able to give a quick response, so time is of the essence. Here, we developed a novel quantitative method for detecting 3 species of *Listeria* including *L. monocytogenes* using the DOX system.

Materials and Methods

Isolates: A total of 8 strains of 3 *Listeria* species (4 strains of *L. monocytogenes*, 2 strains of *L. innocua*, and 2 strains of *L. welshimeri*) and 29 strains of non-*Listeria* species were studied (Table 1). The serotypes of the 4 *L. monocytogenes* strains were 1/2a (strain no. 2 and no.4), 4a (strain no. 1), and 4b (strain no. 3). Twenty-two strains were supplied by the American Type Culture Collection (ATCC), 3 strains by the Japan Collection of Microorganisms (JCM), RIKEN BioResource Center, Japan, 1 strain by the Biological Resource Center, National Institute of Technology and Evaluation, Japan, and 1 strain by the Research Institute for Microbial Diseases (RIMD), Osaka University, Japan. The other 10 strains were isolated from meat (4 strains), non-meat food (3 strains), seawater (3 strains), and a patient (1 strain).

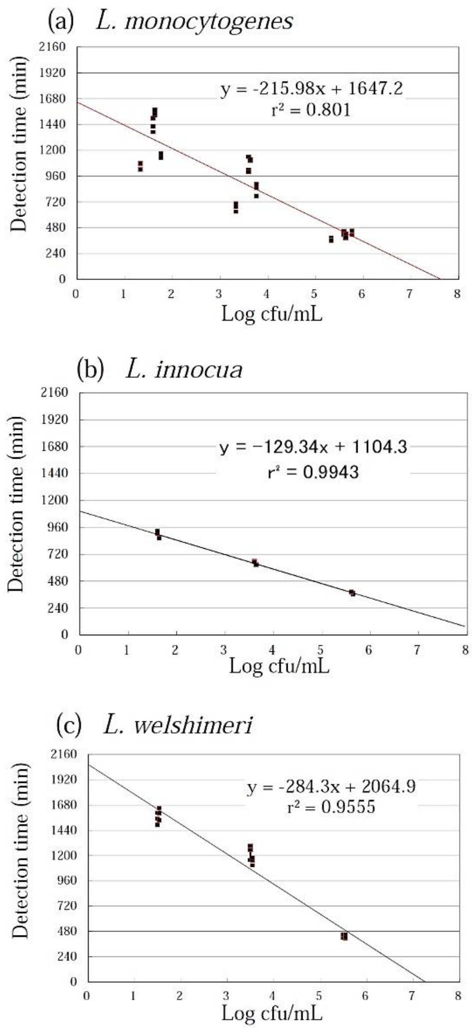

Figure 2 The relationship between detection time and bacterial count. (a) 4 strains of *L. monocytogenes*, (b) 2 strains of *L. innocua*, and (c) 2 strains of *L. welshimeri*.

DOX system examination of the isolates: The *Listeria* strains were streaked on trypticase soy agar (Nissui, Tokyo, Japan) with 0.6% yeast extract (Oxoid, Hampshire, UK) and the non-*Listeria* strains were streaked on trypticase soy agar alone and incubated at 35 °C for 16–20 h under aerobic conditions. The growing *Listeria* strains were adjusted to approximately 10^5 cfu/ml (high concentration), 10^3 cfu/ml (medium concentration), and 10^1 cfu/ml (low concentration) in a physiological salt solution (PSS; pH 7.2). The non-*Listeria* strains were adjusted to approximately 10^6 cfu/ml (high concentration) and 10^3 cfu/ml (medium concentration) in PSS. Beforehand, the concentrations of the bacteria were measured using the optical density (OD) value at 660 nm. Then, 1 ml custom diluted solution containing the test organisms and 1 ml DOX *Listeria* media were inoculated in the DOX coliform cassette and shaken by hand for 30 s. The cassette was placed in the sample port of the DOX system and left for 36 h. When the DOX system revealed a positive result, we recorded the total measurement time. All samples were examined in triplicate.

***Recovery examination of food inoculated with* L. monocytogenes:** *L. monocytogenes* JCM 7674 was streaked on trypticase soy agar with 0.6% yeast extract, and incubated at 35 °C for 16–20 h under aerobic conditions. The growing strain was adjusted to approximately 10^3 cfu/ml and 10^2 cfu/ml in PSS. We collected 15 food samples consisting of 5 ground pork samples, 2 processed cheese samples, 2 king salmon (*Oncorhynchus tshawytscha*) samples, a masu salmon (*O. masou masou*) sample, a bigeye tuna (*Thunnus obesus*) sample, a giant tiger prawn (*Penaeus monodon*) sample, a Japanese flying squid (*Todarodes pacificus*) sample, a candlefish (*Anoplopoma fimbria*) sample, and a parmesan cheese sample from markets in Japan. Before adding *L. monocytogenes*, we performed a *Listeria* detection examination by using the ISO11290-2 method. One ground pork sample (sample no. 4) was positive (50 cfu/g) and the isolated bacterium was identified as *L. welshimeri*. For the detection of *L. monocytogenes*, each 25-g food sample, excluding the masu salmon sample, was placed in 225 ml PSS and mixed thoroughly for 1 min. The masu salmon sample (25 g) was placed in 725 ml PSS because masu salmon contains a high concentration of astaxanthin, which consumes the dissolved oxygen in the sample and detection solution (Ambati et al, 2014). Then, 1 ml custom diluted food solution (×30 for the masu salmon solution and ×10 for the other sample solutions), 10 µl diluted approximately 10^3 cfu/ml or 10^2 cfu/ml *Listeria* solution, and 1 ml DOX *Listeria* media were inoculated in the DOX coliform cassette and shaken by hand for 30 s. The cassette was placed in the sample port of the DOX system and left for 36 h. When the DOX system revealed a positive result, we recorded the total measurement time. All samples were examined 5 times.

Results

Qualitative analysis of* Listeria *strains: The results for the high-, medium-, and low-concentration *Listeria* samples detected by the DOX system are shown in Table 2. The mean positive rate of the high- (10^5 cfu/ml), medium- (10^3 cfu/ml), and low-concentration (10 cfu/ml) *Listeria* samples was all 100%. The detection time of the *Listeria* samples was 367.7–437.3 min for the high-concentration samples, 625.7–1234.3

min for the medium-concentration samples, and 867.0–1565.3 min for the low-concentration samples. The rate of change of *Listeria* samples during the detection period was 0.7–4.7% for the high-concentration samples, 0.2–7.4% for the medium-concentration samples, and 0.5–4.6% for the low-concentration samples. A linear calibration curve between the detection time and bacterial count was observed for all *Listeria* strains examined in this study. The correlation coefficient (r) of the calibration curve was lowest for *L. welshimeri* (sample no. 7; 0.9658), followed by *L. monocytogenes* (sample no. 2; 0.9835), *L. welshimeri* (sample no. 8; 0.9890), and *L. monocytogenes* (sample no. 4; 0.9897). The correlation coefficient for all other *Listeria* strains was >0.99.

Qualitative analysis of* non-Listeria *strains: For the 29 non-*Listeria* samples, the mean negative rate for the high-concentration (10^6 cfu/ml) samples was 86.2% (25/29) and that for the medium-concentration (10^3 cfu/ml) samples was 100% (29/29). The results for the 4 positive non-*Listeria* samples detected by the DOX system are shown in Table 3. For the high-concentration samples, *Acinetobacter baumannii* ATCC 19606 (strain no. 9), *Providencia rettgeri* ATCC 29944 (strain no.24), and *Vibrio cholerae* non-O:1 RIMD 2203259 (strain no. 36), *Candida albicans* ATCC 10231 (strain no. 37), were identified with detection times of 161.7 min, 303.3 min, 96.0 min, and 34.0 min, respectively.

Table 1 *Listeria* and Non-*Listeria* strains examined in this study.

Strain No.	Species	Strain or source	Serotype of *L.monocytogenes*
1	*L. monocytogenes*	JCM[a] 7674	4a
2	*L. monocytogenes*	Food 1	1/2a
3	*L. monocytogenes*	Food 2	4b
4	*L. monocytogenes*	Ground chicken	1/2a
5	*L. innocua*	Ground pork	
6	*L. innocua*	Food	
7	*L. welshimeri*	Ground pork 1	
8	*L. welshimeri*	Ground pork 2	
9	*Acinetobacter baumannii*	ATCC[b] 19606	
10	*Aeromonas hydrophila*	JCM 1027	
11	*Bacillus cereus*	NBRC[c] 3457	
12	*Bacillus subtilis*	ATCC 6633	
13	*Citrobacter freundii*	ATCC 8090	
14	*Edwardsiella tarda*	ATCC 15947	
15	*Enterobacter cloacae*	ATCC 13047	
16	*Enterococcus faecalis*	ATCC 29212	
17	*Enterococcus faecium*	ATCC 35667	
18	*Escherichia coli*	ATCC 25922	
19	*Hafnia alvei*	ATCC 13337	
20	*Klebsiella pneumoniae*	ATCC 13883	
21	*Kocuria rhizophila*	ATCC 9341	
22	*Leclercia adecarboxylata*	ATCC 23216	
23	*Proteus mirabilis*	ATCC 29906	
24	*Providencia rettgeri*	ATCC 29944	
25	*Pseudomonas aeruginosa*	ATCC 27853	
26	*Pseudomonas oleovorans*	Seawater	
27	*Salmonella typhimurium*	ATCC 14028	
28	*Serratia marcescens*	ATCC 13880	
29	*Staphylococcus aureus*	ATCC 25923	
30	*Staphylococcus epidermidis*	ATCC 12228	
31	*Staphylococcus pasteuri*	Sea water	
32	*Staphylococcus saprophyticus*	Clinical	
33	*Staphylococcus xylosus*	ATCC 29971	
34	*Staphylococcus intermedius*	JCM 2422	
35	*Streptococcus pyogenes*	ATCC 19615	
36	*Vibrio cholerae* non-O:1	RIMD[d] 2203259	
37	*Candida albicans*	ATCC 10231	

a) JCM: Japan Collection of Microorganisms
b) ATCC: American Type Culture Collection
c) NBRC: Biological Resource Center, NITE
d) RIMD: Research Institute for Microbial Diseases, Osaka University, Japan

Table 2 Results for high-, medium-, and low- concentration *Listeria* samples analyzed by the DOX system.

Strain No.	Species	High concentration	Medium concentration	Low concentration	Correlation coefficient (r)
1	*L. monocytogenes*	5.33 [a)] 370.7 (4.1%) [b)]	3.33 670.0 (5.7%)	1.33 1060.0 (3.1%)	y = -172.33x + 1274.1 0.9933
2	*L. monocytogenes*	5.59 429.7 (3.4%)	3.59 1052.0 (7.4%)	1.59 1429.7 (4.6%)	y = -250x + 1867.9 0.9835
3	*L. monocytogenes*	5.76 436.7 (4.2%)	3.76 853.7 (6.8%)	1.76 1149.3 (1.7%)	y = -178.17x + 1477.1 0.9925
4	*L. monocytogenes*	5.63 401.7(4.7%)	3.63 1113.3(0.7%)	1.63 1546.3(1.8%)	y = -286.17x + 2059.1 0.9897
5	*L. innocua*	5.64 367.7(1.5%)	3.64 625.7(0.2%)	1.64 867.0(0.5%)	y = -124.83x + 1074.8 0.9997
6	*L. innocua*	5.61 382.3(0.7%)	3.61 654.0(0.7%)	1.61 917.0(1.1%)	y = -133.67x + 1133 0.9996
7	*L. welshimeri*	5.49 437.3(2.9%)	3.49 1234.3(5.7%)	1.49 1548.3(3.7%)	y = -277.75x + 2043.1 0.9658
8	*L. welshimeri*	5.54 432.0(3.9%)	3.54 1147.0(3.1%)	1.54 1565.3(3.7%)	y = -290.83x + 2087 0.9890

[a] Log cfu/mL of sample.
[b] Mean detection period (min.) and rate of change (%) of triplicate examinations per sample.

Table 3 Results for positive non-*Listeria* samples by the DOX system.

Strain No.	Species	High concentration			Medium concentration		
		1st	2nd	3rd	1st	2nd	3rd
			Mean (Rate of change)			Mean (Rate of change)	
9	*Acinetobacter baumannii*		6.68 [a)]			3.68	
		150 [b)]	165	170	ND [c)]	ND	ND
			161.7 (6.4%)				
24	*Providencia rettgeri*		6.77			3.77	
		288	312	301	ND	ND	ND
			303.3 (4.0%)				
36	*Vibrio cholerae* non-O:1		6.88			3.88	
		88	100	100	ND	ND	ND
			96.0 (7.2%)				
37	*Candida albicans*		6.64			3.64	
		35	39	28	ND	ND	ND
			34.0 (16.4%)				

[a] log cfu/mL
[b] Detection time (min) of the 1st examination for the high concentration samples.
[c] No detection.

Table 4 Results for food samples inoculated with *L. monocytogenes* by the DOX system.

Sample No.	Foodstuff	Ultra-low concentration	1st	2nd	3rd	4th	5th	Mean (Rate of change)	Low concentration	1st	2nd	3rd	4th	5th	Mean (Rate of change)
1	Ground pork	0.49[a]	1138[b]	1275	1187	1183	1188	1194.2 (4.2%)	1.49	993	997	996	1017	1016	1003.8 (1.2%)
2	Ground pork	0.49	1225	1183	1279	1141	1131	1191.8 (5.1%)	1.49	982	1064	1073	1050	1010	1035.8 (3.7%)
3	Ground pork	0.77	1325	ND[c]	1228	1183	1218	1238.5 (4.9%)	1.77	1163	1184	1183	1169	1193	1178.4 (1.0%)
4	Ground pork[d]	1.04	1208	1224	1261	1475	1213	1276.2 (8.9%)	1.81	1133	1159	1045	1166	1187	1138.0 (4.9%)
5	Ground pork	0.77	1260	1318	ND	1330	1191	1274.8 (5.0%)	1.77	1072	1154	1216	1108	1155	1141.0 (4.8%)
6	Masu salmon (*Oncorhynchus masou masou*)	0.68	1082	1169	1131	1137	1162	1136.2 (3.0%)	1.68	1044	1037	1007	961	1019	1013.6 (3.2%)
7	King salmon (*Oncorhynchus tshawytscha*)	0.65	1568	1262	1529	1154	1402	1383.0 (12.7%)	1.65	1237	1178	1125	1036	1236	1162.4 (7.3%)
8	King salmon (*Oncorhynchus tshawytscha*)	0.77	ND	1629	1690	1870	1692	1720.3 (6.0%)	1.77	1242	1201	1274	1146	1298	1232.2 (4.9%)
9	Bigeye tuna (*Thunnus obesus*)	0.65	1354	ND	1859	1442	ND	1551.7 (17.4%)	1.65	1218	1162	1138	1317	1202	1207.4 (5.7%)
10	Giant tiger prawn (*Penaeus monodon*)	0.49	1117	1143	ND	ND	1166	1142.0 (2.1%)	1.49	899	914	952	997	982	948.8 (4.4%)
11	Japanese flying squid (*Todarodes pacificus*)	0.49	1142	1217	1190	1182	1175	1181.2 (2.3%)	1.49	981	993	992	952	961	975.8 (1.9%)
12	Candlefish (*Anoplopoma fimbria*)	0.49	1122	1052	1200	1179	1112	1133.0 (5.2%)	1.49	927	878	944	897	913	911.8 (2.8%)
13	Cheese (processed)	0.49	1495	1519	1290	1384	1500	1437.6 (6.8%)	1.49	1143	1074	1146	1112	1146	1124.2 (2.8%)
14	Cheese (processed)	0.49	1398	1227	1314	1220	1305	1292.8 (5.6%)	1.49	851	855	876	913	928	884.6 (3.9%)
15	Cheese (parmesan)	0.77	1786	1624	1653	1577	1680	1664.0 (4.7%)	1.77	1588	1735	1588	1403	1270	1516.8 (12.0%)

a) Log cfu/mL
b) Detection time (min) of the 1st examination for the ultra-low concentration samples
c) No detection.
d)The sample was naturally-contaminated with *L. welshimeri*, and the volume was 50 cfu/g.

Relationship between detection time and bacterial count in 3* Listeria *species: The relationship between detection time and bacterial count is shown in Fig 2. Of the multiple strains examined for a single species, the multiple correlation coefficient (r^2) was 0.801 for *L. monocytogenes* (4 strains; Fig 2a), 0.9943 for *L. innocua* (2 strains; Fig 2b), and 0.9555 for *L. welshimeri* (2 strains; Fig. 2c), showing a high coefficient of determination for multiple strains.

Recovery examination of food inoculated with* L. monocytogenes*: The results for the recovery examination of food inoculated with *L. monocytogenes* JCM 7674 are shown in Table 4. All 15 samples inoculated with a low concentration (1–2 log cfu/ml) of *L. monocytogenes* and 11 of the 15 samples inoculated with an ultra-low concentration (<1 log cfu/ml) of *L. monocytogenes* were positive in all 5 examinations. However, 1 of 5 examinations of 2 samples (ground pork sample no. 5 and king salmon sample no. 8) and 2 of 5 examinations of 2 samples (bigeye tuna sample no. 9 and giant tiger prawn sample no. 10) were negative. A ground pork sample that was naturally contaminated with *L. welshimeri* (50 cfu/g) was positive. The detection time for the positive samples inoculated with *Listeria* was 851–1,735 min (average 18.3 h for 75 positive examinations) for the low-concentration samples and 1,052–1,870 min (average 21.9 h for 68 positive examinations) for the ultra-low concentration samples.

Discussion

Listeriosis is a serious infection usually caused by eating food contaminated with *L. monocytogenes*, and the disease is an important public health problem worldwide (Schmid et al, 2005; Tasara and Stephan, 2006; Mclauchlin and Rees, 2011; Allerberger and Huhulescu, 2015). Now a day 16 *Listeria* species are listed, but most important species is *L. monocytogenes*. Many countries, in addition to the Codex Alimentarius, have designated regulations for the safe levels of *L. monocytogenes* in ready-to-eat food and/or natural cheese.

We developed a method for quantitatively detecting 3 species *Listeria* including *L. monocytogenes* in food using the DOX system. This system could detect all low-concentration (10 cfu/ml) *Listeria* samples, and could not detect any of the medium-concentration (10^3 cfu/ml) non-*Listeria* samples examined in this study. The relationship between detection time and bacterial count of the 3 *Listeria* species showed a good linear calibration curve. In addition, we could detect inoculated ultra-low concentration (less than 1 log cfu/ml) *Listeria* samples. The principle of the DOX system is the detection of oxygen dissolved in samples and detecting solution. In this study, many samples were diluted 10-fold in PSS, but the masu salmon sample needed to be diluted 30-fold. Ambati et al.(2014) showed that astaxanthin consumes dissolved oxygen. Usually fishes and crustaceans have a high concentration of astaxanthin; however we could detect *Listeria* in fish and crustacean samples such as king salmon, bigeye tuna, and giant tiger prawn. Preliminary examinations are needed before using the DOX system method for fish and crustacean samples.

On the basis of the results of the present laboratory analysis, the DOX system provides rapid results (usually within 1 day) and requires no special techniques for measurement. When the DOX system gave a positive result, we could isolate *Listeria* on conventional *Listeria* isolation agars by using the reagents in the DOX cassette. The DOX system may be a useful tool for proving the absence of *Listeria* spp. in food and environmental samples at food processing companies, although further consideration about a validation study between the DOX system and the many available official methods such as the Ministry of Health, Labor and Welfare, Japan, ISO, and Codex Alimentarius is needed.

References

Allerberger F and Huhulescu S 2015. Pregnancy related listeriosis: treatment and control. Expert Rev Anti Infect Ther. 13(3):395-403.

Amano Y, Arai J, Yamanaka S and Isshiki K 2001. Rapid and convenient estimation of bacterial cell count in food using oxygen electrode sensor. Nippon Shokuhin Kagaku Kogaku Kaishi. 48(2): 94-98(in Japanese).

Amano Y, Okumura C, Yoshida M, Katayama H, Unten S, Arai J, Tagawa T, Hoshina S, Hashimoto H and Ishikawa H 1999. Measuring respiration of cultured cell with oxygen electrode as a metabolic indicator for drug screening. Hum Cell. 12(1): 3-10.

Ambati RR, Phang SM, Ravi S and Aswathanarayana RG 2014. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications-a review. Mar Drugs 12(1): 128-152.

den Bakker HC, Warchocki S, Wright EM, Allred AF, Ahlstrom C, Manuel CS, Stasiewicz MJ, Burrell A, Roof S, Strawn LK, Fortes E, Nightingale KK, Kephart D and Wiedmann M 2014. *Listeria floridensis* sp. nov., *Listeria aquatica* sp. nov., *Listeria cornellensis* sp. nov., *Listeria riparia* sp. nov. and *Listeria grandensis* sp. nov., from agricultural and natural environments. Int J Syst Evol Microbiol.64:1882-1889.

Graves LM, Helsel LO, Steigerwalt AG, Morey RE, Daneshvar MI, Roof SE, Orsi RH, Fortes ED, Milillo SR, den Bakker HC, Wiedmann M, Swaminathan B and Sauders BD 2010. *Listeria marthii* sp. nov., isolated from the natural environment, Finger Lakes National Forest. Int J Syst Evol Microbiol. 60:1280-1288.

Katayama, H 2000. New method of bacterial count by dissolved oxygen measurement. Chemical Sensors. 16: 38-46. (in Japanese).

Kawasaki S, Nazuka E, Bari ML, Amano Y, Yoshida M and Isshiki K 2003. Comparison of traditional culture method with DOX System for detecting coliform and *Escherichia coli* from vegetables. Food Sci Technol Res. 9(3): 304-308.

Leclercq A, Clermont D, Bizet C, Grimont PA, Le Flèche-Matéos A, Roche SM, Buchrieser C, Cadet-Daniel V, Le Monnier A, Lecuit M and Allerberger F 2010. *Listeria rocourtiae* sp. nov. Int J Syst Evol Microbiol. 60:2210-2214.

Mclauchlin J and Rees EDC 2011. Genus I. *Listeria*. In: Bergey's Manual of Systematic Bacteriology, 2nd ed Volume three, PD Vos, GM Garrity, D Jones, NR Krieg, W Ludwig, FA Rainey, KH Schleifer and WB Whitman (eds) Berlin: Springer Science+Business Media 244-257.

Schmid MW, Ng EY, Lampidis R, Emmerth M, Walcher M, Kreft J, Goebel W, Wagner M and Schleifer KH 2005. Evolutionary history of the genus *Listeria* and its virulence genes. Syst Appl Microbiol. 28(1): 1-18.

Shabala L, Lee SH, Cannesson P and Ross T 2008. Acid and NaCl limits to growth of *Listeria monocytogenes* and influence of sequence of inimical acid and NaCl levels on inactivation kinetics. J Food Prot. 71(6): 1169-1177.

Tanno S, Fukui N, Utaka Y, Ohkawa S, Morita Y and Boonmar S 2014. Quantitative method for detecting *Vibrio* species using Bio-Theta DOX™ system. Thai J Vet Med. 44(4): 541-546.

Tasara T and Stephan R 2006. Cold Stress Tolerance of *Listeria monocytogenes*: A Review of Molecular Adaptive Mechanisms and Food Safety Implications. J Food Prot. 69(6): 1473-1484.

Weller D, Andrus A, Wiedmann M and den Bakker HC 2015. *Listeria booriae* sp. nov. and *Listeria newyorkensis* sp. nov., from food processing environments in the USA. Int J Syst Evol Microbiol. 65:286-292.

Wetzler TF, Freeman NR, French ML, Renkowski LA, Eveland WC and Carver OJ 1968. Biological characterization of *Listeria monocytogenes*. Health Lab Sci. 5(1): 46-62.

บทคัดย่อ

การตรวจหาปริมาณเชื้อลิสทีเรียโดยใช้ระบบ Bio-Theta DOX™

***Shouichi Tanno*[1] *Naoki Fukui*[1] *Yukihiro Utaka*[1] *Saburo Ohkawa*[2] *Yukio Morita*[2] *Sumalee Boonmar*[2*]**

ได้มีการพัฒนาวิธีตรวจหาปริมาณของเชื้อลิสทีเรียในอาหารโดยใช้ระบบ *DOX ™* . ระบบนี้สามารถตรวจหาปริมาณต่ำสุดคือ *10 cfu/ml* ของเชื้อลิสทีเรีย *3* สปีชีส์ คือ *Listeria monocytogenes Listeria innocua Listeria welshimeri* และไม่สามารถตรวจพบปริมาณระดับกลาง 10^3 *cfu/ml* ของเชื้อที่ไม่ใช่ลิสทีเรียจำนวน *29* ตัว ความสัมพันธุ์ของเวลาที่ใช้ในการตรวจพบและจำนวนแบคทีเรียของเชื้อทั้งสามสปีชีส์เป็น สัดส่วนที่ดีคือ เป็น *linear calibration curve.* เมื่อทดลองใส่เชื้อ *L. monocytogenes* ลงในอาหารต่างๆ จำนวน *15* ตัวอย่าง และทำการทดลองตัวอย่างละ *5* ครั้ง พบว่าสามารถตรวจหาเชื้อ *L. monocytogenes* ได้ในตัวอย่างอาหารจำนวน *68* ตัวอย่างจาก *75* ตัวอย่าง ที่ใส่เชื้อในปริมาณต่ำมากคือ *< 1 log cfu/ml* และใช้เวลาในการตรวจพบระหว่าง *1,052-1,870* นาที *(*เฉลี่ย *21.9* ชั่วโมง*).* ระบบ *DOX ™* นี้ใช้เวลาเพียง *1* วันในการรู้ผลและไม่ต้องอาศัยเครื่องมือพิเศษอะไร. ระบบนี้เป็นเครื่องมือในการหาเชื้อลิสทีรียในตัวอย่างอาหารและตัวอย่างสิงแวดล้อมในขั้นตอนของการผลิตอาหารอย่างไรก็ดียังคงต้องทำการศึกษาต่อไปในภาคสนาม

คำสำคัญ: ระบบการหาปริมาณเชื้อแบคทีเรีย ระบบ *DOX™* จำนวนเชื้อลิสทีเรีย

[1]*Daikin Industry, Ltd., 1-1, Nishi-Hitotsuya Settsu, Osaka 566-8585, Japan*
[2]*Tokyo Kasei University, 1-18-1 Kaga, Itabashi-ku, Tokyo 173-8602, Japan*
***ผู้รับผิดชอบบทความ** *E-mail: sumaleeka12@gmail.com*