Note

Evaluation of an Enzyme-Linked Fluorescent Assay for the Detection of *Listeria monocytogenes* from Food

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The VIDAS *Listeria monocytogenes* II (LMO2) method, which is an automated enzyme-linked fluorescent immunoassay (ELFA), was used for rapidly, specifically and sensitively detecting *L. monocytogenes* in food samples. All 31 *L. monocytogenes* strains examined gave positive results. The other bacterial species except *Salmonella* showed completely negative results, but it was suspected that *Salmonella* spp. had given a false-positive reaction to the assay. As the detectable limit of the assay was 10^6 cfu/ml in a food suspension, food samples were required to be enriched in order to increase the number of the bacteria to the detectable limit. However, the ELFA was reconfirmed to be applied satisfactorily as a rapid and precise method for the detection of *L. monocytogenes* in various food samples, even if the culture had to be enriched for 12 h prior to the assay.

Key words : *Listeria monocytogenes* / Enzyme-linked fluorescent assay / Food.

Listeria monocytogenes is widespread in nature, being associated with plants, soil, water, feed, and animals. It has also been recovered from the stool samples of an estimated 1 to 10% of people who are healthy (Farber and Peterkin, 1991). At the same time, *L. monocytogenes* is indicated as an important foodborne pathogen. Foodborne listeriosis has been caused by the ingestion of contaminated food such as cheese, meat, ice cream and fish (McLauchlin, 1997). In Japan, a food poisoning outbreak incriminating the ingestion of natural cheese in 2001 has been suspected as the only case of foodborne listeriosis (Kawamoto et al., 2004). The prevalence of this bacterium in numerous kinds of food ranges from 13% in raw meat to 3% in dairy products, 11% in fresh vegetables, and 3% in seafood (Luchansky et al., 2005). Also, the levels in ready-to-eat foods are known to be lower than those in various raw food materials. Farber and Peterkin (1991) summarized that the *L. monocytogenes* in various meat and dairy products was usually present at levels of 1.0 to 3.0 log cfu/g, and Gombas et al. (2003) reported a prevalence of 1.82% and levels of <0.3 most probable number (MPN)/g to 1.5×10^5 cfu/g in different types

of ready-to-eat food. To ensure food safety and maintain public health, it is necessary to monitor low levels of the bacteria accurately.

In this study, the VIDAS *Listeria monocytogenes* II (LMO2) method was used for rapidly, specifically and sensitively detecting *L. monocytogenes* in food samples. The VIDAS LMO2 assay is an automated enzyme-linked fluorescent immunoassay (ELFA) for the detection of the bacterium (Silbernagel et al., 2004; Oktay et al., 2006). Once the automated assay is complete, a positive or negative result can be decided within 70 min. In the present study, the presence and/or absence of *L. monocytogenes* in the test suspension was distinguished by using an automated fluorescent immunoassay instrument (miniVIDAS; bioMérieux) and the VIDAS LMO2 kit (bioMérieux) according to the protocol of the manufacturer. For the bacteriological examination, Brain Heart Infusion (BHI; Difco), UVM broth (Oxoid), Fraser broth (bioMérieux) and half-Fraser broth (bioMérieux) were used as the enrichment media, and PALCAM agar plates (Merck) were used for the determination of the *Listeria*s counts.

The specificity of the ELFA was evaluated on some *Listeria* spp. and *L. monocytogenes* strains including type cultures and known serovars from different

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sources, and other gram positive and negative bacteria with the bacterial suspensions at the levels of 10^7 - 10^8 cfu/ml of sterile saline. All 31 *L. monocytogenes* strains were found to give positive results, but other *Listeria* species strains except for *L. monocytogenes* showed negative results to the assay (Table 1). Ueda et al. (2006) reported that the *prfA* 1-2 primer

set reacted exclusively with different strains of *L. monocytogenes*, but other primer sets cross-reacted minutely or definitely with several species other than *L. monocytogenes*. In this study, the results from the ELFA were identical with those of the PCR assay with the *prfA* 1-2 primer. In this respect, the ELFA was found to be specific to *L. monocytogenes*.

TABLE 1. Specificity of ELFA and PCR to *Listeria* spp.^{a)}

<i>Listeria</i> s strain	serovar	response		
		ELFA assay		PCR assay ^{c)}
		TV ^{b)}	decision	
<i>L. grayi</i> ATCC25401		0	—	—
<i>L. innocua</i> ATCC33090		0	—	—
<i>L. innocua</i> SC11		0	—	—
<i>L. ivanovii</i> ATCC19119		0	—	—
<i>L. seeligeri</i> ATCC35897		0	—	—
<i>L. welshimeri</i> ATCC35897		0	—	—
<i>L. monocytogenes</i>				
ATCC7644		2.52	+	+
CH190	4b	2.65	+	+
isolates from food samples				
strain 1	1/2a	2.59	+	+
strain 2	1/2a	2.56	+	+
strain 3	1/2a	2.59	+	+
strain 4	1/2a	2.56	+	+
strain 5	1/2a	2.27	+	+
strain 6	1/2a	2.34	+	+
strain 7	1/2a	2.39	+	+
strain 8	1/2a	2.36	+	+
strain 9	1/2a	2.44	+	+
strain 10	1/2a	2.43	+	+
strain 11	1/2a	2.48	+	+
strain 12	1/2b	2.58	+	+
strain 13	1/2b	2.56	+	+
strain 14	1/2b	2.42	+	+
strain 15	1/2b	2.42	+	+
strain 16	1/2b	2.23	+	+
strain 17	1/2b	2.28	+	+
strain 18	1/2b	2.32	+	+
strain 19	1/2b	2.34	+	+
strain 20	1/2c	2.34	+	+
strain 21	1/2c	2.45	+	+
strain 22	1/2c	2.38	+	+
strain 23	4b	2.60	+	+
strain 24	4b	2.52	+	+
strain 25	4b	2.53	+	+
strain 26	4b	2.23	+	+
strain 27	4b	2.53	+	+
strain 28	4b	2.50	+	+
strain 29	4b	2.48	+	+

a) The bacterial levels in the tested suspension ranged from 10^7 to 10^8 cfu/ml.

b) TV : Test value (In the assay, the result is positive, when the test value is ≥ 0.05 .)

c) The PCR assay was carried out with the *prfA* 1-2 primer set (Ueda et al.;2006).

Furthermore, other gram negative and positive bacterial species were assayed as references. All species except *Salmonella* gave completely negative results to the ELFA (Table 2). However, it was suspected that *Salmonella* spp. gave false-positive results. If the test values calculated from the relative fluorescence values in the ELFA are less than 0.05, the results of the assays are negative, and if the test values are over 0.05, they are positive results. As the test values were 0.06 or 0.12, slightly higher than 0.05 for *Salmonella*, the results were tentatively decided as positive. On the other hand, the values for *L. monocytogenes* were 2.23-2.65 (Table 1, 2). Because the values were distinctly different between *Salmonella* and *L. monocytogenes*, the results of *Salmonella* were thought to be false-positive. Since the selective enrichment for *L. monocytogenes* was practically required prior to the ELFA, the assay

seemed to be as useful as the magnetic immuno-PCR assay (Ueda et al., 2006).

The sensitivity of the assay system to *L. monocytogenes* 4b was examined with different enrichment broth media and various food samples. The enrichment media used were UVM broth, half-Fraser broth and Fraser broth. Food samples used were eggs and egg dishes, milk and dairy products, meat and meat dishes, vegetables, vegetable salads, and fruits and fruit juices. A 10% suspension of each food sample was prepared with sterile saline solution, except in the case of milk and juices, and these fluid samples were inoculated with *L. monocytogenes* 4b at 10^3 - 10^6 cfu/ml (Table 3). The results of the ELFA were shown to be positive in almost all fluid samples inoculated with 10^5 cfu/ml, but 10^6 cfu/ml was required for the detection of the bacteria in such acidic samples as yoghurt and fruit juices.

TABLE 2. Response of various bacterial species to ELFA for *Listeria monocytogenes*

Bacterial species/serovar/strain/toxigenicity	TV ^{a)}	decision
<i>Escherichia coli</i> O127:H21 (EPEC ^{a)})	0.02	—
<i>Escherichia coli</i> O142:NNM (EIEC ^{b)})	0	—
<i>Escherichia coli</i> ST and LT producer (ETEC ^{c)})	0.03	—
<i>Escherichia coli</i> O157:H7 (VT1 and VT2 producer) (EHEC ^{d)})	0	—
<i>Escherichia coli</i> O157:H7 (VT2 producer) (EHEC)	0	—
<i>Escherichia coli</i> O157:H7 (non-VT producer) ATTC43888	0	—
<i>Escherichia coli</i> O111:HNM (VT1 and VT2 producer) (EHEC)	0	—
<i>Escherichia coli</i> O26:HNM (VT1 and VT2 producer) (EHEC)	0	—
<i>Escherichia coli</i> from food sample	0.01	—
<i>Escherichia coli</i> V517	0	—
<i>Escherichia coli</i> IFO3301	0	—
<i>Klebsiella aerogenes</i> from sewage sample	0	—
<i>Salmonella</i> Enteritidis	0.12	(+) ^{f)}
<i>Salmonella</i> Typhimurium	0.06	(+) ^{f)}
<i>Yersinia enterocolitica</i>	0	—
<i>Vibrio parahaemolyticus</i>	0	—
<i>Staphylococcus aureus</i> enterotoxin A producer	0	—
<i>Staphylococcus aureus</i> enterotoxin B producer	0	—
<i>Staphylococcus aureus</i> enterotoxin C producer	0	—
<i>Staphylococcus aureus</i> enterotoxin D producer	0	—
<i>Staphylococcus aureus</i> enterotoxin E producer	0	—
<i>Staphylococcus aureus</i> enterotoxin H producer	0	—
<i>Staphylococcus aureus</i> enterotoxin TSST-1 producer	0	—
<i>Bacillus cereus</i> : emetic type strain	0	—
<i>Bacillus cereus</i> : diarrheal type strain	0	—
<i>Bacillus cereus</i> IFO13484	0	—
<i>Bacillus subtilis</i> from air	0	—
<i>Bacillus licheniformis</i> from air	0	—
<i>Bacillus licheniformis</i> ATTC43888	0	—
<i>Bacillus pumilus</i> from air	0	—
<i>Clostridium botulinum</i> E type toxin producer	0	—
<i>Clostridium perfringens</i> Hobbs A type	0	—

a) Enteropathogenic *E. coli* ; b) Enteroinvasive *E. coli*

c) Enterotoxigenic *E. coli* ; d) Enterohemorrhagic *E. coli*

e) TV : Test value f) In the assay, the result is positive, when the test value is ≥ 0.05 .

TABLE 3. Detection results of *L. monocytogenes* 4b by ELFA from various food suspensions inoculated with different levels of the bacteria.

Food suspension ^{a)}	Levels (cfu/ml) of inocula :				
	Blank	10 ³	10 ⁴	10 ⁵	10 ⁶
Selective enrichment broth					
UVM broth	—	—	+	+	+
Half Fraser broth	—	—	+	+	+
Fraser broth	—	—	+	+	+
Eggs and their dishes					
Liquid whole egg	—	—	+	+	+
Thick omelet	—	—	+	+	+
Milk and dairy products					
Milk	—	—	+	+	+
Yoghurt	—	—	—	—	+
Cheese	—	—	+	+	+
Meat and their dishes					
Hamburger steak	—	—	—	+	+
Roastbeef	—	—	+	+	+
Raw beef	—	—	—	+	+
Raw pork	—	—	+	+	+
Raw chicken	—	—	+	+	+
Raw swine liver	—	+	+	+	+
Vegetables					
Parsley	—	—	+	+	+
Cabbage	—	—	—	+	+
Beansprouts	—	—	—	+	+
Leek	—	—	—	+	+
Welsh onion	—	—	+	+	+
Carrot	—	—	+	+	+
Tomato	—	—	—	+	+
Vegetable salads					
Pumpkin	—	—	+	+	+
Okara	—	—	+	+	+
Potato	—	—	+	+	+
Seafood	—	—	+	+	+
Fruits and their juices					
Water melon	—	—	+	+	+
Melon	—	—	—	+	+
Apple juice	—	—	—	—	+
Orange juice	—	—	—	—	+

a) All food samples, except milk and juices, were used as a 10 fold-diluted suspension with a sterile saline.

Silbernagel et al. (2004) said that the VIDAS LM02 assay could be completed within 70 min, when the culture sample contained a concentration of *L. monocytogenes* above 10⁵ cfu/ml. Food samples must be enriched in order to increase the number of bacteria to the detectable limit of the assay for a certain time, as it is normal to find bacteria at lower levels than 10² cfu/g in many kinds of food including milk, meat, fruits and vegetables. The growth of *L. monocytogenes* and the ELFA assay were examined in UVM and half-Fraser broth suspended with several food materials including egg, cheese, raw beef and cabbage. 10² cfu/ml of *L. monocytogenes* 4b was

inoculated into the suspension consisting of 1g of food material and 9 ml of selective enrichment broth, and incubated at 35°C. An aliquot of the culture was taken for the ELFA and the determination of the bacterial counts at appropriate intervals during the 12 h incubation. The bacterial counts increased to 10⁶-10⁷ cfu/ml during the 10-12 h incubation in all suspensions, and the results of the ELFA became positive in the same time (Table 4).

Sakai et al. (2005) showed that the ELFA assay was useful for the detection of *L. monocytogenes* in natural cheese. Also, in the present study, the ELFA was found to be applied satisfactorily as a rapid and

TABLE 4. Growth of *L. monocytogenes* 4b and ELFA results in selective enrichment media with several food materials added^{a)}

Food materials inoculated into selective broth	Culture time (h)	UVM broth		Half Fraser broth	
		Bacterial counts (log cfu/ml)	Result	Bacterial counts (log cfu/ml)	Result
Broth alone	0	2.8	—	2.3	—
	8	4.0	—	3.4	—
	10	5.6	—	4.3	—
	12	6.0	+	6.0	+
Liquid whole egg	0	2.8	—	2.7	—
	8	4.5	—	4.8	—
	10	5.5	—	6.3	+
	12	6.0	+	NT	
Cheese	0	2.7	—	2.1	—
	8	4.3	—	4.7	—
	10	5.1	—	6.6	+
	12	5.7	+	NT	
Raw beef	0	2.4	—	1.0	—
	8	4.1	—	4.8	—
	10	NT		6.1	+
	12	6.0	+	NT	
Cabbage	0	2.8	—	2.3	—
	8	4.5	—	4.7	—
	10	7.2	+	NT	
	12	NT		5.6	+

a) After 1 g of food material was added into 10 ml of selective enrichment broth, *L. monocytogenes* 4b was inoculated at the level of 10^2 - 10^3 cfu/ml.

precise method for the detection of *L. monocytogenes* in various food samples, even if the culture had to be enriched for 12 h prior to the assay.

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