Evaluation of Immunochromatography for the Rapid and Specific Identification of *Listeria monocytogenes* from Food

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To rapidly, simply and specifically detect and identify *Listeria monocytogenes* from food samples, an immunochromatographic assay, based on gold-labeled monoclonal antibodies directed against an antigen common to all serovars of *L. monocytogenes*, was used. All strains of *L. monocytogenes* serovars showed a positive reaction to the assay, but all other gram-positive and negative bacteria did not. The detection limit of the assay was in the order of $10^6$ cfu/ml in fluid medium. The assay could simply and rapidly identify *L. monocytogenes* within 30 min by a pure culture without special instruments. Even if the selective enrichment cultivation was employed for the isolation and growth of bacteria from food materials, the application of the assay system could detect and identify *L. monocytogenes* precisely in various food materials within 2 to 3 days.

Key words: *Listeria monocytogenes* / Immunochromatographic assay / Food.

*Listeria monocytogenes* is a gram-positive bacterium that is ubiquitously present in the environment. It is physiologically resistant to various environmental stresses, and is able to grow at a wide temperature range of 0 to 45°C and continue to exist within food processing plants and retail and distribution outlets for long periods of time (Swaminathan and Gerner-Smidt, 2007; Freitag et al., 2009). It can also cause severe foodborne illness (listerosis). The outbreaks of listeriosis are mostly associated with a multiple range of ready-to-eat meats, dairy products, vegetables, and fish (Uyttendaele et al., 2009; Gormley, et al., 2010; Pertuzelli et al., 2010). In Europe and the United States, listeriosis is reported to be the second most frequent cause of foodborne infection-related deaths, following salmonellosis (Allerberger and Wagner, 2010). Although 13 serovars of *L. monocytogenes* have been recognized, all serovars do not have the equal capacity to cause disease, and more than 90% of human listeriosis cases involve strains of three specific serovars, 1/2a, 1/2b, and 4b (McLauchlin, 1990; Gasanov et al., 2005; Swaminathan and Gerner-Smidt, 2007). In Japan, a food poisoning outbreak incriminating the consumption of natural cheese in 2001 has been suspected as the only case of foodborne listeriosis (Kawamoto et al., 2004), and *L. monocytogenes* has been detected often from various food materials and the environmental samples in food processing plants and market places at low levels (Okutani et al., 2004).

Because the detection and identification of *L. monocytogenes* by conventional methods requires the extensive labor and time, a number of rapid and highly sophisticated real-time PCR-based techniques have been developed (O’Grady et al., 2009; Aparecida et al., 2010, Clayton et al., 2011). Immunochromatographic assays, using monoclonal antibodies prepared against a specific antigen, have been available for rapidly, simply and sensitively detecting other pathogenic bacteria such as *Salmonella* (Bautista et al., 2002), *Legionella pneumophila* (Munoz et al., 2009), *Vibrio parahaemolyticus* (Kawatsu et al., 2006) and multi-drug resistant *Pseudomonas aeruginosa* (Kitao-Ando et al., 2010).

To ensure food safety and maintain public health, it is necessary to simply, rapidly and accurately monitor *L. monocytogenes* even at too levels. The objective...
of the study was to evaluate a commercially available immunochromatographic assay as a rapid detection tool for the bacteria in food materials. The assay was carried out by using an immunochromatographic device (Singlepath™ L’mono, Merck) based on gold-labeled monoclonal antibodies directed against an antigen common to all serovars of L. monocytogenes according to the protocol of the manufacturer. In the assay, 200 μl of the bacterial culture or suspension was added into the bottom of the microwell of the strip, and a diagnostic band appeared if the strain was L. monocytogenes, in parallel to a control band.

The specificity of the assay was evaluated using pure cultures of 27 strains of known L. monocytogenes serovars, 6 of other Listeria spp. and 34 of non-Listeria bacteria (9 of gram negative bacteria containing Escherichia coli and Salmonella, and 15 of gram positive bacteria containing Staphylococcus aureus and Bacillus spp.). All bacterial cultures were grown in Brain Heart Infusion broth (BHI) for 18 – 24 h at 37°C to obtain more than $10^8$ cfu/ml. Among Listeria spp., only L. monocytogenes was found to give the positive result, but all other Listeria spp. showed negative results. Furthermore, although all 27 L. monocytogenes strains containing different serovars gave positive readings in the assay, all 34 non-Listeria bacteria tested showed negative results (Table 1). In this respect, the assay could simply and selectively identify the L.

### Table 1. Specificity of the immunochromatographic assay for the detection of *Listeria monocytogenes*.

| Bacterial species strain/serovar | Detection bands | Bacterial species strain/serovar | Detection bands |
|----------------------------------|-----------------|----------------------------------|-----------------|
|                                  | Control         | Diagnostic                       | Control         | Diagnostic |
| Control                          | +               |                                  | +               |          |
| *Listeria* spp.                  |                 |                                  |                 |          |
| L. grayi ATCC 25401              | +               |                                  |                |
| L. innocua ATCC 3390             | +               |                                  |                |
| L. innocua SC11                  | +               |                                  |                |
| L. ivavovii ATCC 19119           | +               |                                  |                |
| L. seelingeri ATCC 35967         | +               |                                  |                |
| L. welshimari ATCC 35897         | +               |                                  |                |
| L. monocytogenes ATCC 7644       | +               |                                  |                |
| **L. monocytogenes from different sources** |     |                                  |                |
| strain 1: 1/2a                   | +               |                                  |                |
| strain 2: 1/2a                   | +               |                                  |                |
| strain 3: 1/2a                   | +               |                                  |                |
| strain 4: 1/2a                   | +               |                                  |                |
| strain 5: 1/2a                   | +               |                                  |                |
| strain 6: 1/2a                   | +               |                                  |                |
| strain 7: 1/2a                   | +               |                                  |                |
| strain 8: 1/2a                   | +               |                                  |                |
| strain 9: 1/2a                   | +               |                                  |                |
| strain 10: 1/2a                  | +               |                                  |                |
| strain 11: 1/2a                  | +               |                                  |                |
| strain 12: 1/2b                  | +               |                                  |                |
| strain 13: 1/2b                  | +               |                                  |                |
| strain 14: 1/2b                  | +               |                                  |                |
| strain 15: 1/2b                  | +               |                                  |                |
| strain 16: 1/2b                  | +               |                                  |                |
| strain 17: 1/2b                  | +               |                                  |                |
| strain 18: 1/2b                  | +               |                                  |                |
| strain 19: 1/2b                  | +               |                                  |                |
| strain 20: 1/2c                  | +               |                                  |                |
| strain 21: 1/2c                  | +               |                                  |                |
| strain 22: 1/2c                  | +               |                                  |                |
| strain 23: 4b                    | +               |                                  |                |
| strain 24: 4b                    | +               |                                  |                |
| strain 25: 4b                    | +               |                                  |                |
| strain 26: 4b                    | +               |                                  |                |
| strain 27: 4b                    | +               |                                  |                |
| **Escherichia coli**             |                 |                                  |                |
| E. coli IFO3301                  | +               |                                  |                |
| E. coli V517                     | +               |                                  |                |
| E. coli O127:H21 (EPEC)          | +               |                                  |                |
| E. coli O124:HNM (EIEC)          | +               |                                  |                |
| E. coli ST and LT producer (ETEC) | +               |                                  |                |
| E. coli O157:H7 (VT 1, 2 producer) (EHEC) | +       |                                  |                |
| E. coli O157:H7 (VT 2 producer) (EHEC) | +       |                                  |                |
| E. coli O157:H7 ATCC43888        | +               |                                  |                |
| E. coli O1111:HNM (VT 1, 2 producer) (EHEC) | +       |                                  |                |
| E. coli O126:H (-) (VT 1 producer) (EHEC) | +       |                                  |                |
| **Klebsiella aerogenes**          |                 |                                  |                |
| **Citrobacter freundii**         |                 |                                  |                |
| **Salmonella Enteritidis phage4** |                 |                                  |                |
| **Salmonella Typhimurium**       |                 |                                  |                |
| **Campylobacter jejuni**         |                 |                                  |                |
| **Vibrio alginolyticus**         |                 |                                  |                |
| **Vibrio parahemolyticus**       |                 |                                  |                |
| **Aeromonas hydrophila 011**     |                 |                                  |                |
| **Yersinia enterocolitica 03**   |                 |                                  |                |
| **Staphylococcus aureus**        |                 |                                  |                |
| S. aureus: enterotoxin A producer | +               |                                  |                |
| S. aureus: enterotoxin B producer | +               |                                  |                |
| S. aureus: enterotoxin C producer | +               |                                  |                |
| S. aureus: enterotoxin D producer | +               |                                  |                |
| S. aureus: enterotoxin E producer | +               |                                  |                |
| S. aureus: enterotoxin H producer | +               |                                  |                |
| **Bacillus spp.**                |                 |                                  |                |
| B. cereus: emetic strain         |                 |                                  |                |
| B. cereus: diarrheal strain      |                 |                                  |                |
| B. cereus IFO13484               |                 |                                  |                |
| B. subtilis                      |                 |                                  |                |
| B. licheniformis                 |                 |                                  |                |
| B. pumilus                       |                 |                                  |                |
| **Clostridium botulinum: A type toxin producer** | + |                                 |                |
| **Clostridium perfringens: Hobbs type 13** | + |                                 |                |

1) Enteropathogenic *E. coli*, 2) Enteroinvasive *E. coli*, 3) Enterotoxigenic *E. coli*, 4) Enterohemorrhagic *E. coli*. 
**Table 2.** Growth of *L. monocytogenes* strain 23 cultivated in various enrichment media and results of the immunochromatographic assay.

| Enrichment broth | Incubation time (h) | Listeria counts (log of cfu/ml) | Detection bands |
|------------------|---------------------|--------------------------------|-----------------|
| BHI              | 0                   | 2.3                            | +               |
|                  | 4                   | 3.4                            | +               |
|                  | 6                   | 3.8                            | +               |
|                  | 8                   | 4.3                            | +               |
|                  | 24                  | 9.5                            | + +             |
|                  | 48                  | 9.1                            | + +             |
| Trypticase soy broth | 0         | 2.4                            | +               |
|                  | 4                   | 3.2                            | +               |
|                  | 6                   | 3.8                            | +               |
|                  | 8                   | 4.5                            | +               |
|                  | 24                  | 9.4                            | + +             |
|                  | 48                  | 9.1                            | + +             |
| Half Fraser broth | 0                   | 2.7                            | +               |
|                  | 4                   | 3.2                            | +               |
|                  | 6                   | 3.5                            | +               |
|                  | 8                   | 4.0                            | +               |
|                  | 24                  | 8.4                            | + +             |
|                  | 48                  | 8.9                            | + +             |

**Table 3.** Sensitivity of the assay to *L. monocytogenes* enriched in BHI broth.

| Levels of inocula$^{1)}$ | Detection bands |
|---------------------------|-----------------|
| Order | log of cfu/ml | Control | Diagnostic |
| 10$^2$ | 2.9          | +       | -          |
| 10$^3$ | 3.9          | +       | -          |
| 10$^4$ | 4.8          | +       | -          |
| 10$^5$ | 5.8          | +       | -          |
| 10$^6$ | 6.9          | +       | +          |
| 10$^7$ | 7.8          | +       | +          |
| 10$^8$ | 8.9          | +       | +          |

1) *L. monocytogenes* strain 23 (serovar 4b) was used.

*L. monocytogenes* strains from various sources.

Since 4b was found to be the predominant serovar of *L. monocytogenes* from many clinical materials in Japan (Okutani et al., 2004), strain 23 (Table 1) was used as a representative of serovar 4b to further evaluate the sensitivity of the assay in the following way. After *L. monocytogenes* strain 23 was inoculated into BHI broth (Difco), Trypticase soy broth (BBL), Half-Fraser broth (Merck) at levels of 10$^6$ cfu/ml and incubated at 37°C, the enumeration of the bacterial counts and the assays of the cultures were carried out at the incubation time of 4, 6, 8, 24 and 48 h. The count of each culture amounted to the order of 10$^5$ cfu/ml after the 8 h-incubation, and the assay showed negative results from all cultures. However, after 24 h-incubation, the bacterial counts of 3 enrichment broth cultures increased to the order of 10$^8$–10$^9$ cfu/ml and the results of the assays were positive (Table 2). Thus, the detection limit of the assay was suspected to be in the range of 10$^5$ to 10$^6$ cfu/ml. When using 10-fold dilutions of the 24 h-BHI culture of *L. monocytogenes* strain 23, the sensitivity of the assay was observed to be in the order of 10$^5$ cfu/ml (Table 3).

Food samples contaminated artificially with *L. monocytogenes* strain 23 were also used to evaluate the sensitivity of the assay. Food samples used were cheese, beef, hot-dog, salmon, cabbage and vegetable salad, and cow milk. A 10 % suspension of each sample was prepared with sterile saline solution, except milk, and these fluid samples were inoculated with *L. monocytogenes* at the level of 10$^3$, 10$^4$, 10$^5$, or 10$^6$ cfu/ml. In consequence, the assay was shown to be capable of detecting *L. monocytogenes* at the level of 10$^5$ cfu/ml, similar to that in the enrichment broth (Table 4). However, the assay cannot be applied directly to the detection of *L. monocytogenes* in food suspensions, because the bacterial contents of food materials are commonly estimated to less than 10$^5$ cfu/g (Ueda et al., 2006, 2010). Therefore, the selective enrichment culture prior to the assay is important to the detection of *L. monocytogenes* from suspected food materials. After 1 ml/g of food material (milk, cheese or salmon) was added into 10 ml of Half-Fraser broth, *L. monocytogenes* strain 23 (serovar 4b) was inoculated at the level of 10$^5$ cfu/ml and incubated at 37°C to 48 h. The count of each culture amounted to the order of 10$^4$–10$^5$ cfu/ml after the 8 h-incubation and the assays showed negative results from all cultures. After the 24 h-incubation, the bacterial counts of enrichment broth cultures increased to the order of 10$^8$–10$^9$ cfu/ml and the results of the assays were positive (Table 5).

In conclusion, the immunochromatographic assay could simply, rapidly and specifically detect *L. monocytogenes* within 30 min by using the pure culture without special techniques and instruments. Even if the enrichment procedure is introduced prior to the assay, the application of the assay system may detect and identify *L. monocytogenes* precisely in various food materials within 2 to 3 days. Thus, the present assay is considered to be available as an alternative method for the identification of the bacteria. Similarly, Bautista et al. (2002), Munoz et al. (2009), Kawatsu et al. (2006) and Kitao-Ando et al. (2010) reported that the particular pathogen targeted by their assays was identified in a shorter time by the immunochromatographic assay in comparison with the conventional method, after clinical specimens containing the pathogenic bacteria were incubated in an enrichment broth.
TABLE 4. Results from food samples contaminated artificially with different levels of L. monocytogenes strain 23 cells in the immunochromatographic assay.

| Materials | Levels of inocula | Detection bands |
|-----------|------------------|----------------|
|           | Order | log of cfu/ml | Control | Diagnostic |
| Milk      | $10^2$ | 3.0 | + | - |
|           | $10^3$ | 4.5 | + | - |
|           | $10^4$ | 5.4 | + | - |
|           | $10^5$ | 6.5 | + | + |
|           | $10^6$ | 7.6 | + | + |
| Cheese    | $10^2$ | 3.0 | + | - |
|           | $10^3$ | 4.0 | + | - |
|           | $10^4$ | 5.1 | + | - |
|           | $10^5$ | 6.9 | + | + |
| Beaf      | $10^2$ | 3.0 | + | + |
|           | $10^3$ | 3.9 | + | + |
|           | $10^4$ | 4.9 | + | + |
|           | $10^5$ | 5.8 | + | + |
|           | $10^6$ | 6.9 | + | + |
| Hot dog   | $10^2$ | 3.5 | + | + |
|           | $10^3$ | 4.3 | + | + |
|           | $10^4$ | 5.3 | + | + |
|           | $10^5$ | 6.3 | + | + |
| Salmon    | $10^2$ | 3.6 | + | + |
|           | $10^3$ | 4.6 | + | + |
|           | $10^4$ | 5.7 | + | + |
|           | $10^5$ | 6.5 | + | + |
|           | $10^6$ | 7.5 | + | + |
| Cabbage   | $10^2$ | 2.9 | + | + |
|           | $10^3$ | 3.9 | + | + |
|           | $10^4$ | 4.9 | + | + |
|           | $10^5$ | 5.8 | + | + |
|           | $10^6$ | 7.1 | + | + |
| Vegetable salad | $10^2$ | 3.6 | + | + |
|           | $10^3$ | 4.4 | + | + |
|           | $10^4$ | 5.4 | + | + |
|           | $10^5$ | 6.4 | + | + |
|           | $10^6$ | 7.3 | + | + |

1) L. monocytogenes 4b was inoculated into 10% suspension of materials except milk and BHI broth.

TABLE 5. Growth of L. monocytogenes cultivated in enrichment media to which various food materials were added and results of the immunochromatographic assay.

| Enrichment broth | Food material | Incubation time (h) | Listeria counts (log of cfu/ml) | Detection bands |
|------------------|---------------|---------------------|--------------------------------|----------------|
|                  |               |                     | Control | Diagnostic |
| Milk             |               |                     | 0      | 3.5          | + | - |
|                  |               |                     | 4      | 4.4          | + | - |
|                  |               |                     | 6      | 5.1          | + | - |
|                  |               |                     | 8      | 5.7          | + | - |
|                  |               |                     | 24     | 9.3          | + | + |
|                  |               |                     | 48     | 8.6          | + | + |
| BHI broth Cheese |               |                     | 0      | 3.4          | + | - |
|                  |               |                     | 4      | 4.4          | + | - |
|                  |               |                     | 6      | 4.9          | + | - |
|                  |               |                     | 8      | 5.8          | + | - |
|                  |               |                     | 24     | 8.8          | + | + |
|                  |               |                     | 48     | 8.8          | + | + |
| Hot dog          |               |                     | 0      | 3.3          | + | - |
|                  |               |                     | 4      | 4.1          | + | - |
|                  |               |                     | 6      | 4.5          | + | - |
|                  |               |                     | 8      | 5.0          | + | - |
|                  |               |                     | 24     | 8.2          | + | + |
|                  |               |                     | 48     | 8.6          | + | + |
| Salmon           |               |                     | 0      | 3.4          | + | - |
|                  |               |                     | 4      | 4.2          | + | - |
|                  |               |                     | 6      | 4.5          | + | - |
|                  |               |                     | 8      | 5.1          | + | - |
|                  |               |                     | 24     | 9.0          | + | + |
|                  |               |                     | 48     | 9.1          | + | + |
| Half Fraser broth| Cheese        |                     | 0      | 3.4          | + | - |
|                  |               |                     | 4      | 4.0          | + | - |
|                  |               |                     | 6      | 4.4          | + | - |
|                  | Salmon        |                     | 0      | 3.4          | + | - |
|                  |               |                     | 8      | 4.9          | + | - |
|                  |               |                     | 24     | 8.7          | + | + |
|                  |               |                     | 48     | 8.9          | + | + |

1) After 1g of food material was added into 10 ml of enrichment broth, L. monocytogenes strain 23 (serovar 4b) was inoculated with the level of $10^3$ cfu/ml.

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