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Production of Monoclonal Antibody Against *Listeria monocytogenes* and Its Application to Immunochromatography Strip Test

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Abstract An immunochromatography (ICG) strip test based on a monoclonal antibody for the rapid detection of *L. monocytogenes* in meat and processed-meat samples was developed in this study. A monoclonal antibody (MAb) specific to *L. monocytogenes* was produced from cloned hybridoma cells (FKLM-3B12-37) and used to develop an ICG strip test. The antibody showed a stronger binding to *L. monocytogenes* than other *Listeria* species, and a weak cross-reaction to *S. aureus* based on an ELISA. The detection limit of the ICG strip test was 10^5 cell/ml. In total, 116 meat and processed-meat samples were collected and analyzed using both the ICG strip test and a PCR. The ICG strip test and PCR indicated *L. monocytogenes* contamination in 34 and 27 meat samples, respectively. The 7 meat samples not identified as *L. monocytogenes* positive by the PCR were also tested using an API kit and found to be contaminated by *Listeria* species. In conclusion, the ICG strip test results agreed well with those obtained using the PCR and API kit. Thus, the developed ICG has potential use as a primary screening tool for *L. monocytogenes* in various foods and agricultural products, generating results within 20 min without complicated steps.

Keywords: *Listeria monocytogenes*, foodborne pathogen, immunochromatography strip test, monoclonal antibody, colloidal gold

Listeria is a Gram-positive, aerobic-to-facultatively anaerobic, foodborne bacterial pathogen. This microbe can grow over a wide range of temperatures (1 to 45°C), making it potentially hazardous, especially for refrigerated products [25]. Currently, there are six recognized species of *Listeria* (*L. innocua*, *L. ivanovii*, *L. monocytogenes*, *L. grayi*, *L. seeligeri*, *L. welshimeri*), among which *L. monocytogenes* is causing growing concern as a significant public health risk in terms of potential outbreaks of listeriosis [4, 23]. *L. monocytogenes* is a widespread bacterium that can be readily isolated from a number of sources, such as soil, water, vegetables, meat, ready-to-eat food, and even refrigerated foods [1, 17, 29]; thus a rapid, accurate, and easy-to-use method is urgently needed for the detection of *L. monocytogenes* [8, 19].

Although many culture methods and media for *L. monocytogenes* have already been developed that would seem to be reliable, such culture methods and media are laborious, time-consuming, require initial enrichment, involve complicated procedures, and are difficult to analyze when screening large numbers of samples [7, 14, 21, 29]. Moreover, despite improved culture methods and media, there is still no agreement among scientists.

Immunoassays based on antibodies [15, 20, 30] and various PCR methods using nucleic acid [16, 24, 31, 36] provide sensitive, specific, and reproducible detection of pathogenic bacteria. However, even though immunoassays reduce the assay time comparing to culture methods and are widely used in many laboratories, immunoassays still require a lot of equipment, long reaction time, skilled analysts, and multiple steps. Similarly, PCR methods also require a lot of equipment, multiple steps, and an understanding of molecular biology. However, a convenient

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and rapid test has been achieved using the novel concept of immunochromatography (ICG) that depends on the transportation of a reactant to its binding partner immobilized on a membrane surface.

Many papers have already reported on the use of ICG strips to detect hazardous factors, including mycotoxins [32, 34], pesticides [28, 35], antibiotics [33], and pathogenic bacteria [2, 18], in foods and agricultural products. An ICG strip test, or lateral-flow assay, is based on immunochromatographic procedures that utilize antigen and antibody properties for the rapid detection of an analyte, thereby combining several benefits, including a user-friendly format, short assay time, long-term stability over a wide range of climates, cost-effectiveness, and suitability for screening large numbers of samples by unskilled analysts [5, 28]. Accordingly, this study developed an ICG strip test using a colloidal gold-monoclonal antibody conjugate, and applied it to *L. monocytogenes* detection in various meat and processed-meat samples.

MATERIALS AND METHODS

Organism, Culture Conditions, and Materials

The *L. monocytogenes* (8 strains), *Listeria* species (5 strains), and non-*Listeria* species (7 strains) used in this study are listed in Table 1. The *L. monocytogenes* strains, including *L. monocytogenes* (ATCC 19111, serotype 1), *L. monocytogenes* (ATCC 19112, serotype 2), *L. monocytogenes* (ATCC 19113, serotype 3), *L. monocytogenes* (ATCC 19114, serotype 4a), *L. monocytogenes* (ATCC 19115, serotype 4b), *L. monocytogenes* (ATCC 19116, serotype 4c), *L. monocytogenes* (ATCC 19117, serotype 4d), and *L. monocytogenes* (ATCC 19118, serotype 4e), and the *Listeria* species, including *L. innocua* (ATCC 33090, serotype 6a), *L. ivanovii* (ATCC 19119), *L. grayi* (ATCC 19120), *L. welshimeri* (ATCC 35897, serotype 6b), and *L. seeligeri* (ATCC 35967), were cultured in a listeria enrichment broth base (LEB, Difco, MD, U.S.A.) with nalidixic acid (40 mg/ml), cycloheximide (50 mg/ml), and a listeria selective agar base (Oxford, Difco, MD, U.S.A.) at 37°C for 24 h.

The *Bacillus cereus* (ATCC 21366), *Escherichia coli* O157:H7 (ATCC 43888), *Staphylococcus aureus* (ATCC 25923), and *Salmonella typhimurium* (ATCC 13311) were cultured in a tryptic soy broth (TSB, Difco, MD, U.S.A.) at 37°C for 24 h, the *Vibrio parahaemolyticus* and *V. vulnificus* grown in TSB containing 3% NaCl at 37°C for 24 h, and the *Clostridium perfringens* cultured at 37°C in a cooked meat medium (Difco, MD, U.S.A.) under anaerobic conditions for 20 h, and then subcultured in a brain-heart infusion broth (Difco, MD, U.S.A.).

The complete and incomplete Freund's adjuvant, peroxidase-conjugated anti-mouse IgG, 2-2'-azinobis(3-ethylbenz-thiazoline)sulfonic acid (ABTS), horseradish peroxidase (HRP), tetrachloroauric acid, sodium citrate, and anti-mouse IgG were all purchased from Sigma (St. Louis, MO, U.S.A.). The P3-X63-Ag8.653 murine myeloma

Table 1. List of bacterial strains used in this study.

Strain	Strain No.	Origin	Serotype
L. monocytogenes	ATCC 19111	Poultry	1
L. monocytogenes	ATCC 19112	Spinal fluid of man	2
L. monocytogenes	ATCC 19113	Human	3
L. monocytogenes	ATCC 19114	Ruminant brain	4a
L. monocytogenes	ATCC 19115	Human	4b
L. monocytogenes	ATCC 19116	Chicken	4c
L. monocytogenes	ATCC 19117	Sheep	4d
L. monocytogenes	ATCC 19118	Chicken	4e
L. innocua	ATCC 33090	Cow brain	6a
L. ivanovii	ATCC 19119	Sheep	-
L. grayi	ATCC 19120	Chinchilla feces	-
L. welshimeri	ATCC 35897	Decaying plant material	6b
L. seeligeri	ATCC 35967	Soil	-
Bacillus cereus	ATCC 21366	Soil	-
Escherichia coli	ATCC 43888	Human feces	O157:H7
Staphylococcus aureus	ATCC 25923	Clinical isolate	-
Salmonella typhimurium	ATCC 13311	Human feces	-
Clostridium perfringens	ATCC 3624	-	-
Vibrio parahaemolyticus	ATCC 17802	Human	-
Vibrio vulnificus	ATCC 27562	Human blood	-

cell line was obtained from the Microbiology Laboratory, Medical College, Gyeongsang National University (Chinju, South Korea). The DMEM, RPMI, and fetal bovine serum (FBS) were obtained from Hyclone (Logan, UT, U.S.A.), and the protein G agarose purchased from Bioprogen (Daejeon, South Korea). The maxisorp polystyrene 96-microwell plates and removable strips were obtained from Nunc (Rockilde, Denmark), the nitrocellulose membranes, sample pads, conjugate pads, and absorbent pads obtained from Millipore (Bedford, MA, U.S.A.), and the semi-rigid polyethylene sheets purchased from a local market.

Production of Monoclonal Antibody

The preparation of immunogens for the production of a monoclonal antibody (MAb) against *L. monocytogenes* was performed as previously reported [29]. Two kinds of immunogen, formalin-killed cells (FKCs) and heat-killed cells (HKCs), were prepared and used to immunize mice. Briefly, the *L. monocytogenes* (ATCC 19115) was grown as described above, and the cells harvested by centrifugation at 5,000 rpm for 5 min and suspended in 0.1 M phosphate-buffered saline (PBS, pH 7.4). The cell concentration was adjusted to 2×10^8 cells/ml in the PBS. To prepare the FKCs, cells at the above-described concentration were collected using centrifugation, and then the pellet was suspended in an original volume of PBS containing 0.5% formaldehyde and incubated at room temperature for 24 h. After confirming the absence of any viable cells by cell counting on a listeria selective agar, the preparations were stored at -70°C. To prepare the HKCs, the adjusted cells were heated in a flask for 1 h at 95°C in a water bath, and the preparations stored at -70°C after confirming the absence of any live cells, as previously described. The MAb for *L. monocytogenes* was produced in the authors' laboratory using standard procedures [11]. Ten 5-week-old female BALB/c mice were separated into 2 groups, where one group was immunized with 2×10^8 FKCs and the other with 2×10^8 HKCs. The mice were all injected with the immunogens emulsified with an equal volume of Freund's complete adjuvant in 0.1 ml of sterilized PBS. Booster injections were then given 2, 4, and 6 weeks later. To develop the MAb, spleen cells obtained from the immunized mice showing a high titer in an indirect enzyme-linked immuoasorbent immunoassay (ELISA) were used for cell fusion with murine myeloma cells. The fused cells producing antibodies were screened by an indirect ELISA and cloned using the limiting dilution method. The cloned hybridoma cells (1.0×10^7 cell in PBS) were then intraperitoneally injected into BALB/c mice, pretreated with an intraperitoneal injection of 0.5 ml pristane, and the ascite fluids taken from the mice injected with the cloned hybridoma cells. The immunoglobulin fraction was prepared from the ascite fluids using precipitation with saturated ammonium sulfate, followed by affinity chromatography on a protein G column. The protein concentration of the purified MAb was determined using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA, U.S.A.).

Conjugation of Colloidal Gold and MAb

An ELISA normally uses the enzyme horseradish peroxidase (HRP) as the marker or tracer. However, for the ICG strip test, colloidal gold particles (diameter 40 nm) conjugated with an antibody or antigen were employed as the marker. The colloidal gold solutions were produced by a reduction method using sodium citrate, as described in a previous paper [10, 28], and then conjugated with the MAb using a previously described method [26, 27]. The colloidal gold-MAb conjugates were stored at 4°C before use.

Development of ICG Strip Test

The ICG strip test for *L. monocytogenes* was composed of three pads (sample, conjugate, and absorbent pads), plus one nitrocellulose membrane with the test and control lines (Fig. 1). The sample pads and absorbent pads were treated according to the method described in a previous paper by the current authors [28]. Five μl of the colloidal gold-MAb probe (absorbance at 540 nm was 1.5) was applied to an untreated glass-fiber membrane for use as the conjugate pad, and allowed to dry for 30 min at 37°C. The test and control lines on a nitrocellulose membrane were treated with FKLM-3B12-41 MAb (1.0 mg/ml in PBS) and goat anti-mouse IgG (1.0 mg/ml in PBS), and allowed to dry for 30 min at 37°C. The treated pads and membranes were all attached to a semi-rigid polyethylene sheet. When the samples were applied to the sample pads and allowed to migrate up the membrane, after 20 min, a positive test showed two red lines for the test and control lines, whereas a negative test only produced one red line in the control region. The strip test was incorrect if there was no red line for the control line (Fig. 1). To determine the sensitivity (detection limit) of the assay, a fresh culture of *L. monocytogenes* was washed and serially diluted from 10^8 to 10^1 cells in 1 ml of sterile PBS. Two-hundred μl of each

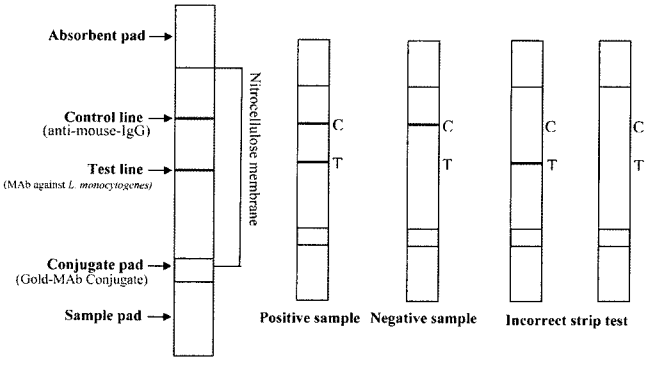

Fig. 1. Schematic diagram of immunochromatography strip test. C, control line; T, test line.

dilution ws then tested using the ICG strip test, and the results interpreted as described above within 20 min. To assess the specificity of the ICG strip test, 5 *Listeria* species and 7 non-*Listeria* species were cultured as described above. The fresh cultures were all serially diluted from 10^8 to 10^1 cells in 1 ml of sterile PBS and tested using the ICG strip test.

Evaluation of ICG Strip Test

To evaluate the ICG test strip, *L. monocytogenes* grown at 37°C for 24 h was adjusted to 10^8, 10^6, 10^4, and 10^2 cells in 100 µl of sterile PBS and then inoculated into 10 g of meat and processed-meat samples. The inoculated samples were left for 1 h at room temperature, suspended in 90 ml of LEB in stomacher bags, massaged by hand, and incubated at 37°C for 48 h.

Before incubation, 1-ml aliquots of the massaged samples were taken and transferred into Eppendorf tubes. The cells in the suspension were harvested by centrifugation and washing (three times). Finally, the pellets were resuspended in an original volume of PBS and 200 µl of each sample tested using the ICG strip test. One ml aliquots of each culture were also taken after enrichment for 24 h, transferred into Eppendorf tubes, treated as described above, and analyzed using the ICG strip test. Blank samples were prepared without any *L. monocytogenes* inoculation and treated as described above.

Sample Collection and Analysis

Pork (30 samples), beef (20 samples), chicken (26 samples), fish (20 samples), and processed-meat products (20 samples) were collected from supermarkets and traditional markets located in Chinju City (Gyeongnam Province, Korea), and 10 g of each sample aseptically transferred into a stomacher bag with a filter lining. The meat and processed-meat samples were suspended in 90 ml of LEB in the stomacher bag, massaged by hand, and incubated at 37°C for 24 h. One ml aliquots of the samples were then withdrawn and transferred into Eppendorf tubes. The cells in the enrichment samples were harvested by centrifugation and washing (three times). Finally, the pellets were resuspended in an original volume of sterile PBS and 200 µl of each sample tested using the ICG strip test.

The results of the ICG strip test were compared with those obtained from a PCR. For the PCR analysis, 100 µl aliquots of the enrichment samples were transferred and spread on an Oxford agar, and then the agar plates were incubated at 37°C for 24 h. Next, the black colonies on the Oxford plates were taken and inoculated into the enrichment broth, and then cultured at 37°C for 12 h. One ml aliquots of the cultures were withdrawn and transferred into Eppendorf tubes. The cells were harvested by centrifugation and washing with water containing 0.85% NaCl. Finally, the pellets were used for a PCR analysis. The DNA extraction and PCR analysis were performed as previously described by Ha *et al.* [12].

RESULTS AND DISCUSSION

Characterization of MAb

The antisera from the mice immunized with the FKCs showed a higher titration than the antisera from the mice immunized with the HKCs. Thus, it was anticipated that several hybridoma cell lines producing MAbs specific to *L. monocytogenes* would be developed if cells from the mice immunized with the FKCs were used in the cell fusion. As expected, five clones (3B12-17, 3B12-19, 3B12-21, 3B12-37, and 3B12-41) that produced MAbs with the ability to bind to intact *L. monocytogenes* were generated after cell fusion and cloning using spleen cells from the mice immunized with the FKCs. The isotype of the antibodies was determined using an isotyping kit (Roche Applied Science, Switzerland) and found to be an

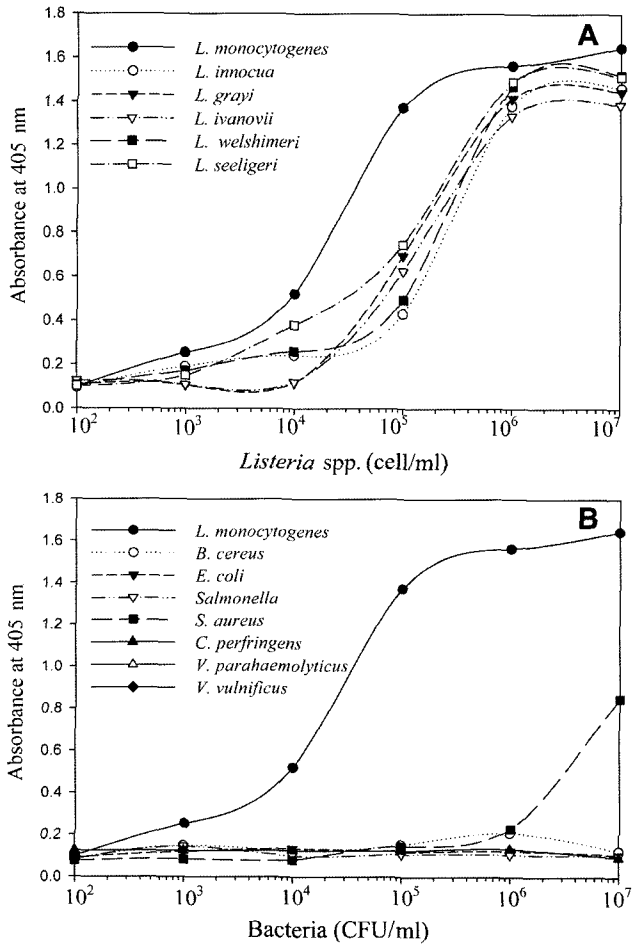

Fig. 2. Reactivities of MAb to *Listeria* species (**A**) and other pathogenic bacteria (**B**) using indirect ELISA.

IgG_1 subclass with a κ-type light chain. The reactivity towards *Listeria* species and other pathogenic bacteria was also determined with culture supernatants containing MAbs using an indirect ELISA. Four MAbs (3B12-17, 3B12-19, 3B12-21, and 3B12-41) showed a strong reaction to the *Listeria* species and *S. aureus* in the indirect ELISA (data not shown). The 3B12-37 MAb showed a stronger binding to *L. monocytogenes* than the *Listeria* species, and a weak cross-reaction to *S. aureus* in the indirect ELISA (Fig. 2). Thus, according to the above description, the 3B12-37 hybridoma cell line was selected and expanded for MAb mass production and purification. In previously reported papers [3, 29], a similar difficulty with a cross-reaction to *S. aureus* was also encountered, and the reason given was that protein A with a strong affinity to IgG is often expressed by *S. aureus* in its cell wall, and unfortunately, the MAb isotype developed in this study was found to be IgG_1.

Validation of Colloidal Gold-MAb Conjugate

Colloidal gold is often used as an immunospecific probe for immunocytochemistry and immunoblotting [9, 22], with further possible applications in immunoassays, biosensors, gene therapy, and DNA computations [6]. In this study, the colloidal gold was produced in the authors' laboratory using the method of Frens [10] and conjugation of the colloidal gold and the MAb performed according to the method of Roth [26, 27]. To determine the suitability of the colloidal gold-MAb conjugate for use in an ICG strip test, the colloidal gold without the MAb was blocked with bovine serum albumin (BSA) and used as a control to evaluate the colloidal gold-MAb. As shown in Fig. 3, the colloidal gold-BSA conjugate without the MAb did not bind to the test and control lines, as there were no red lines on the nitrocellulose membrane. However, different test line results were recorded when positive and negative samples of *L. monocytogenes* were applied to ICG strips treated with the colloidal gold-MAb conjugate on the conjugate pad. According to the above result, we certified that the colloidal gold-MAb conjugate could be used to develop the ICG strip test for the rapid detection of *L. monocytogenes*. In a previous paper, fluorescence spectrometry was used to confirm a colloidal gold-MAb conjugate [13]; however, this requires expensive equipment and complicated steps. Therefore, the confirmation method used in this study was simpler and equally effective.

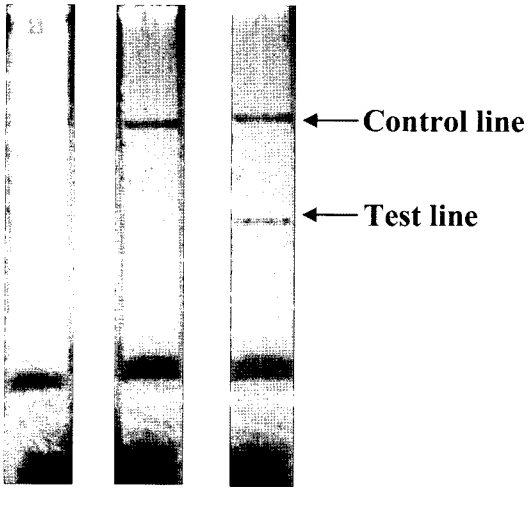

Fig. 3. Confirmation of colloidal gold-MAb conjugate.
To determine the nonspecific binding of the colloidal gold and immunoreagents in the test and control lines, the conjugated pad was treated with a colloidal gold-BSA conjugate (**a**). To confirm the colloidal gold-MAb conjugate, *L. monocytogenes* negative (**b**) and positive (**c**) solutions were applied to the ICG colloidal gold-MAb conjugate-treated conjugate pad.

ICG Strip Test

Since the main objective of the ICG strip was the qualitative detection of *L. monocytogenes*, it was important that the color intensity of the test line was strong enough to be seen and to enable a clear distinction between negative and positive samples. Thus, to develop a sensitive ICG strip, the optimal MAb concentration applied to the test line and optimal amount of colloidal gold-MAb sprayed onto the conjugate pad were determined, where a positive sample appeared clear within the shortest time and the color intensity between positive and negative samples could be easily distinguished by the naked eye. As such, the optimal conditions for the ICG strip were as follows: FKLM-3B12-37 MAb (0.5 μg/ml) was applied to the test line on the membrane and 5 μl of colloidal gold-MAb conjugate (absorbance at 540 nm was 1.5) was sprayed onto the conjugate pad.

The detection limit of the ICG strip test was defined using series-diluted *L. monocytogenes* (10^8–10^1 cells/ml) in PBS and the results determined within 20 min after starting

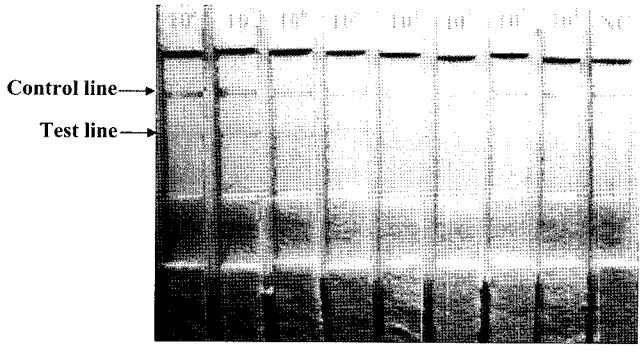

Fig. 4. Sensitivity of ICG strip test for detection of *L. monocytogenes*.
The tests were run four times at room temperature using PBS with series- diluted *L. monocytogenes*. The label (10^8–10^1) shows the *L. monocytogenes* count in 1 ml of PBS, and NC means no *L. monocytogenes*.

the reaction. Two red lines on the membrane indicated that the *L. monocytogenes* count was above the detection limit, whereas only one red line for the control line indicated that the *L. monocytogenes* count was below the detection limit. As a result, the detection limit of the ICG strip was 10^5 cells/ml, as a weak red line for the test line was produced with 10^4 cells/ml (Fig. 4).

The specificity of the ICG strip was evaluated with *Listeria* and non-*Listeria* species. As shown in Table 2, positive results were observed for the test line when *Listeria* species (>10^6 cells/ml) were applied to the ICG strip, and weak positive results obtained when applying *S. aureus* (>10^8 cells/ml). However, no cross-reaction was observed for other pathogenic bacteria. Although the ICG

Table 2. Specificity of ICG strip test for *L. monocytogenes*, *Listeria species*, and other pathogenic bacteria.

Strain	Result area	Result of ICG strip test for serial-diluted microorganisms (cells/ml)								
		10^8	10^7	10^6	10^5	10^4	10^3	10^2	10^1	NC[a]
L. monocytogenes (ATCC 19111)	Test line	+[b]	+	+	+	±[c]	–[d]	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19112)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19113)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19114)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19115)	Test line	+	+	+	+	±	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19116)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19117)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. monocytogenes (ATCC 19118)	Test line	+	+	+	+	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. innocua (ATCC 33090)	Test line	+	+	±	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. ivanovii (ATCC 19119)	Test line	+	+	+	±	±	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. grayi (ATCC 19120)	Test line	+	+	+	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. welshimeri (ATCC 35897)	Test line	+	+	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
L. seeligeri (ATCC 35967)	Test line	+	+	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
Bacillus cereus (ATCC 21366)	Test line	–	–	–	–	–	–	–	–	–
	Control line	±	±	+	+	±	+	+	±	+
Escherichia coli (ATCC 43888)	Test line	–	–	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
Staphylococcus aureus (ATCC 25923)	Test line	+	–	–	–	–	–	–	–	–
	Control line	±	±	±	+	+	+	+	+	+
Salmonella typhimurium (ATCC 13311)	Test line	–	–	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
Clostridium perfringens (ATCC 3624)	Test line	–	–	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
Vibrio parahaemolyticus (ATCC 17802)	Test line	–	–	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+
Vibrio vulnificus (ATCC 27562)	Test line	–	–	–	–	–	–	–	–	–
	Control line	+	+	+	+	+	+	+	+	+

[a]NC: negative control.
[b]+: obvious red band observed.
[c]±: faint band observed.
[d]–: no band observed.

strip test showed a weak positive result at 10^8 cells/ml of *S. aureus*, the ICG strip test exhibited a high specificity for *L. monocytogenes* and *Listeria* species. Therefore, the developed ICG strip test could be applied to screen large numbers of samples for *Listeria* species, with qualitative results within 20 min.

Screening of *L. monocytogenes* in Inoculated and Naturally Contaminated Samples

Meat and processed-meat samples inoculated with various counts of *L. monocytogenes* (10^8, 10^6, 10^4, and 10^2 cells/ml) with and without enrichment for 24 h were analyzed using the ICG strip test. As shown in Table 3, the inoculated samples without enrichment showed negative results, whereas the inoculated samples with enrichment showed positive results. Thus, the ICG strip test could not be effectively applied without sample enrichment because the microorganisms embedded in the meat and processed-meat and this condition cause difficulty of microorganism isolation from samples. Therefore, sample enrichment was needed before applying the ICG strip test.

Ninety-six meat samples, including beef (20 samples), pork (30 samples), chicken (26 samples), and fish (20 samples), plus 20 processed-meat samples were collected and grown as described above, and primarily analyzed using the ICG strip test. Thirty-four samples were found to be *Listeria* species positive according to the ICG strip test (Table 4). The positive samples, as revealed by the ICG strip, were then further tested for confirmation of *L. monocytogenes* contamination using a PCR. Twenty-seven of the positive samples were confirmed as contaminated with *L. monocyotogenes*. The 7 positive samples that were not found to be contaminated with *L. monocytogenes* by the PCR were also tested using an API kit (bioMeriux, France) according to provided instructions, resulting in confirmation of contamination with *Listeria* species, including *L. innocua* (5 samples) and *L. ivanovii* (2 samples). Therefore, even though the ICG strip test showed false positives for *L. monocytogenes*, this method can still be useful as a primary screening method for *L. monocytogenes* and *Listeria* species. As shown above, the positive results with the ICG strip test

Table 3. Analysis of *L. monocytogenes* in inoculated pork, beef, chicken, fish, and sausage samples using ICG strip test.

Sample	Inculated *L. monocytogenes* (cells/10 g)	Result of ICG strip test			
		Before enrichment		After enrichment	
		Test line	Control line	Test line	Control line
Pork (n=4)	10^8	–[b]	+[c]	+	+
	10^6	–	+	+	+
	10^4	–	+	+	+
	10^2	–	+	+	+
	NI[a]	–	+	–	+
Beef (n=4)	10^8	–	+	+	+
	10^6	–	+	+	+
	10^4	–	+	+	+
	10^2	–	+	+	+
	NI	–	+	–	+
Chicken (n=4)	10^8	–	+	+	+
	10^6	–	+	+	+
	10^4	–	+	+	+
	10^2	–	+	+	+
	NI	–	+	–	+
Fish (n=4)	10^8	–	+	+	+
	10^6	–	+	+	+
	10^4	–	+	+	+
	10^2	–	+	+	+
	NI	–	+	–	+
Sausage (n=4)	10^8	–	+	+	+
	10^6	–	+	+	+
	10^4	–	+	+	+
	10^2	–	+	+	+
	NI	–	+	–	+

[a]NI, no inoculation.
[b]–, no band observed.
[c]+, obvious red band observed.

Table 4. Results for *L. monocytogenes* screening in meat and processed-meat samples using ICG strip test and PCR.

Sample	Number of tested samples	Result of ICG strip test for *Listeria* spp.		Result of PCR for *L. monocytogenes*	
		Number of positives	Sample labels	Number of positives	Sample labels
Pork	30	10	PO-2, PO-4, PO-10, PO-15, PO-16, PO-19, PO-22, PO-23, PO-25, PO-29	9	PO-2, PO-4, PO-10, PO-15, PO-16, PO-19, PO-22, PO-23, PO-25,
Beef	20	7	BE-1, BE-5, BE-12, BE-13, BE-17, BE-19, BE-20	6	BE-5, BE-12, BE-13, BE-17, BE-19, BE-20
Chicken	26	14	CH-1, CH-2, CH-4, CH-5, CH-6, CH-9, CH-11, CH-14, CH-15, CH-16, CH-17, CH-21, CH-22, CH-25	10	CH-1, CH-2, CH-4, CH-5, CH-9, CH-14, CH-15, CH-16, CH-17, CH-21
Fish	20	3	FI-14, FI-17, FI-18	2	FI-14, FI-18
Processed meat	20	0	-	0	-
Total	116	34		27	

agreed well with the PCR results. It has been suggested that a PCR analysis is more exact than immunoassays for the detection of pathogenic bacteria. In this study, the final contamination ratio of *L. monocytogenes* in the meat and processed-meat samples was 23.3% (27/116) according to a PCR. In addition, the 82 meat and processed-meat samples that tested negative for *L. monocytogenes* and *Listeria* species with the ICG strip were also tested using a biochemical test and API kit, and none were found to be positive for *L. monocytogenes* but 2 chicken samples (CH-8 and CH-19), 1 pork sample (PO-9), and 1 fish sample (FI-3) were found to be *L. innocua* positive (data not shown).

In conclusion, although culture methods, biochemical tests, and PCR methods are reliable tools for the screening of *L. monocytogenes*, none of these methods are suitable for routine screening of large sample numbers, owing to the many time-consuming steps and expensive instruments involved. Thus, for the rapid screening of *L. monocytogenes* in meat and processed-meat samples, this study suggested an ICG strip test using a colloidal gold-MAb conjugate, as an ICG strip test does not require complicated steps and expensive instruments. The detection limit of the ICG strip developed in this study was 10^5 cells/ml, and the results for samples naturally contaminated with *L. monocytogenes* agreed well with those obtained using a PCR (Table 4). Moreover, the ICG strip test was easy to perform, and results were obtained within 20 min. Thus, for overall speed and simplicity, an ICG strip test is superior to other immunoassays, such as an ELISA.

The results in this present study confirmed that the proposed ICG strip test was sufficiently accurate to be useful in rapid screening for *L. monocytogenes* in various foods, vegetables, and agricultural products. Furthermore, the ICG strip was also able to detect *Listeria* species, as the MAb used in the ICG strip test had a high cross-reaction to *Listeria* species (Table 2). Finally, the developed ICG strip test has potential use as a rapid, cost-effective, on-site screening tool for food pathogen bacteria contamination in food samples and agricultural products, and could be applied as a primary screening method for the detection of pathogenic bacteria in various food samples.

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