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ORIGINAL PAPER

Identification and quantification of eight *Listeria monocytogene* serotypes from *Listeria spp.* using a gold nanoparticle-based lateral flow assay

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Abstract A lateral flow assay for rapid, simple and efficient determination of *L. monocytogenes* is presented. A monoclonal antibody (mAb) 1C1 against the peptide from P60 protein of *L. monocytogenes* was prepared and labeled with gold nanoparticles (AuNPs). The mAb 1C1 was paired with the mAb 10E7 against the P60 protein of all the Listeria spp. and used as a capture bioligand in a lateral flow assay. The AuNP-based strip test can detect the supernatant of eight common *L. monocytogenes* serotypes including 1/2a, 1/2b, and 4b with an equivalent detection limit of 3.7×10^6 CFU·mL^{-1} but does not detect four other Listeria spp. (*L. ivanovii, L. innocua, L. welshimeri,* and *L. grayi*). There was no cross-reactivity with six other Gram-negative and Gram-positive bacteria. The method was applied to the quantification of L. monocytogenes species in spiked milk samples within 13 h.

Keywords Detection · Immunoassay · Monoclonal antibody · Foodborne pathogen · Listeriosis · *L. ivanovii* · Transmission electron microscopy · Gold nanoparticles · Milk analysis

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Introduction

Listeria monocytogenes is a Gram-positive bacterial foodborne pathogen [1–4] that belongs to the genus *Listeria* along with *L. grayi, L. innocua, L. welshimeri, L. seeligeri, L. ivanovii* and contains thirteen serotypes (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and 7) [5, 6]. *L. monocytogenes* causes listeriosis in humans, and *L. ivanovii* mainly infects ruminants [7–9]. The prevalent serotypes of *L. monocytogenes* are 1/2a, 1/2b, and 4b, which account for >95% of *Listeria* spp. isolated from humans [10]. *L. monocytogenes,* which can grow at low temperatures (4 °C), has been detected in dairy products, raw meat, and seafood [11, 12]. The standard detection method of *L. monocytogenes* is culture-based and requires chromogenic and biochemical confirmation, which are time-consuming, labor-intensive, and complex [5, 11]. More rapid and effective detection methods are required in the food industry and medical field.

Immunoassays and biosensors based on antibody-antigen reactions represent rapid and powerful tools for the analyses of pathogens and related toxins [12–16]. Compared with PCR, immunoassays are cost-effective and do not rely on sophisticated instrument or trained technicians [17–20]. The enzyme linked immunosorbent assay (ELISA) for *L. monocytogenes* detection is time-consuming (3–4 h) and limited to the laboratory [21, 22]. In contrast, the lateral flow immunochromatographic (ICG) strip assay is fast (10 min), simple, and portable [23, 24]. The ICG strip developed by Shim and co-authors was based on monoclonal antibodies (mAbs) that had stronger affinity towards *L. monocytogenes* than towards other *Listeria* spp., but cross-reacted with *L. innocua* and *L. ivanovii* at higher concentrations [25]. Kim and co-authors developed an ICG strip based on commercial mAbs and improved sensitivity using immuno-

magnetic separation (IMS) [26]; however, the cross-reactivity of this strip was not clear because only *L. monocytogenes* was evaluated. Several biosensors have greatly improved the sensitivity of *L. monocytogenes* detection [3, 26–32]. The majority of mAbs are either specific to a certain serotype or cross-react with non-virulent *Listeria* spp. [33–35], which limit the analyses of unknown samples. MAbs against all *L. monocytogenes* serotypes have been prepared; however, it is challenging to obtain paired mAbs, which are essential for immunoassays and biosensors. Therefore, the mAbs for detection of *L. monocytogenes* are still limited.

We prepared mAb 1C1, which specifically recognized all *L. monocytogenes* serotypes, using bovine serum albumin (BSA) conjugated with peptide PepD as the immunogen. MAb 1C1 was paired with mAb 10E7, which was prepared against P60 protein present in all *Listeria* spp. including *L. monocytogenes*. Based on the signals obtained with gold-labeled nanoparticles (AuNPs), an ICG strip assay was successfully established for the rapid and accurate detection of *L. monocytogenes* without cross reactivity with other bacteria including *Listeria* spp.

Table 1 The bacterial strains in this work

Species	Strain	Serotype
L. monocytogenes	ATCC 19111	1/2a
L. monocytogenes	ATCC 19115	4b
L. monocytogenes	ATCC 19118	4e
L. monocytogenes	CMCC 54002	1/2c
L. monocytogenes	CMCC 54003	1/2a
L. monocytogenes	CMCC 54004	unknown
L. monocytogenes	CMCC 54007	unknown
L. monocytogenes	From frozen beef sample	unknown
L. grayi	ATCC 25400	unknown
L. ivanovii	ATCC 19119	5
L. welshimeri	ATCC 35897	6b
L. innocua	ATCC 33090	6a
E.coli	ATCC 25922	
E. coli O157: H7	CICC 21530	
Cronobacter sakazakii	ATCC 29544	
Salmonella enteritidis	ATCC 13076	
Staphylococcus aureus	ATCC 29213	
Campylobacter jejuni	ATCC 49443	

Material and methods

Reagents and instruments

Peptides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China, www.sangon.com). 4-(N-Maleimidomethyl) cyclohexanecarboxylic acid N-hydroxysuccinimide ester (SMCC), BSA, goat anti-mouse IgG antibody, horse radish peroxidase (HRP), Freund's adjuvant, chloroauric acid ($HAuCl_4$), and sodium citrate were obtained from Sigma-Aldrich Co., LLC (St. Louis, MO, USA, www.sigmaaldrich.com). All the other chemicals (analytical grade) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China, www.sinoreagent.com). The High-binding 96-well microplate was obtained from GuoSheng Bio-Engineering Co., Ltd. (Wu Xi, China, www.gsbio.cn), and the strip reader was obtained from Huaan Magnech Bio-Tech Company Corp (Beijing, China, www.magnech.com). The UV-Vis spectrophotometer (Evolution 60S) was supplied by Thermo Fisher Scientific (Miami, USA, www.thermofisher.com). A transmission electron microscope (TEM; JEM-2100, JEOL, Japan, www.jeol.co.jp/en/) was used to visualize and characterize the AuNPs.

Bacteria and growth conditions

The bacterial strains are listed in Table 1. *Campilobacteri jejuni* was cultured at 37 °C for 2–3 d in a micro-aerobic environment (4% O_2, 10% CO_2 and 86% N_2) in a three-gas incubator (Binder CB210, Tuttlinger, Germany, www.binder-world.com). *L. monocytogenes* and other bacterial strains were cultured overnight at 37 °C in Brain-Heart Infusion broth (Oxoid, Basingstoke, UK, www.oxoid.com/UK/blue/).

Preparation of mAbs specific to *L. monocytogenes* and *Listeria* spp.

A peptide of the *L. monocytogenes* P60 protein (PepD; QQQTAPKAPTE) was synthesized with cysteine on the N-terminal end [36] and conjugated to BSA with SMCC. Briefly, 20 mg BSA was dissolved in 0.1 M phosphate buffered saline (PBS, 100 mM phosphate buffer containing 150 mM NaCl, pH 7.2). SMCC (6 mg) was dissolved in 100 μL N,N-dimethylformamide, added dropwise to the BSA solution, and allowed to react at room temperature for 1 h under constant stirring. Excess SMCC was removed by performing ultrafiltration twice (Milipore-Amicon, cut off 3000, www.merckmillipore.com) at 7000×*g* for 25 min and the SMCC activated BSA on the hyperfiltration membrane was dissolved in 0.1 M PBS. Subsequently, 12.88 mg and 6.44 mg of the BSA solution were respectively mixed with 500 μL peptide (10 mg·mL^{-1}, in 0.1 M PBS) and allowed to react for 12 h. The conjugates with different reaction ratios were dialyzed prior to protein electrophoresis (Bio-Rad, Shanghai, China, www.bio-rad.com) and both of them were respectively used as immunogens in BALB/c mice [37]. Following cell fusion, positive cell lines were selected against culture medium of different *Listeria* spp. (diluted 4 × for indirect ELISA) and confirmed with recombinant P60 protein of

L. monocytogenes (0.3 μg·mL^{-1} for coating). MAb 10E7 against the whole P60 protein was produced in our laboratory. The purified mAb was conjugated to HRP with oxidation method by sodium periodate [38].

AuNPs and development of the ICG strip

The synthesis of 15 nm AuNPs was performed using a citrate reduction method [39]. $HAuCl_4$ solution (0.0 1%, 100 mL) was boiled in a conical flask for 10 min under constant stirring. Trisodium citrate (10%, 200 μL) was quickly added, and the solution was boiled for another 15 min. Once the solution developed a red wine color, it was allowed to cool at room temperature and stored at 4 °C. The diameter of AuNPs was determined by TEM.

MAb 1C1 against *L. monocytogenes* was conjugated to AuNPs [23]. Briefly, the pH of the AuNP solution (20 mL) was adjusted to 7.5 with 110 μL of 0.1 M K_2CO_3. Subsequently, 67 μL of mAb (3 mg·mL^{-1}) was added and allowed to react at room temperature for 2 h under gentle stirring. BSA (10% *w/v*, 1 mL) was added and incubated for 2 h under gentle stirring. The solution was washed twice with washing buffer (0.02 M PBS, 1% BSA, and 2% sucrose) and centrifuged at 6000×*g* for 30 min at 4 °C. AuNP-labeled mAbs were suspended in 200 μL of 0.02 M PBS containing 0.02% NaN_3 and stored at 4 °C.

The ICG strip was developed by attaching an absorption pad, a nitrocellulose membrane (NC membrane), and a sample pad (JieYi Biotechnology Co., Ltd., Shanghai, China, www.joey-bio.cn) to a polyvinylchloride sheet (Fig. 1). MAb 10E7 (4 mg·mL^{-1}) and goat anti-mouse IgG antibody (0.5 mg·mL^{-1}) were respectively sprayed onto the NC membrane as test line (T line) and control line (C line) using a BioJet Quanti3000 dispenser (Kinbio Tech Co., Ltd., Shanghai, China, www.kinbio.com). The distance between the T line and C line was 10 mm. The membrane was air-dried in an oven (37 °C, 2 h) and cut into individual strips (4 mm wide) using a CM4000 Guillotine Cutting Module (Kinbio Tech Co., Ltd., Shanghai, China, www.kinbio.com). The strips were stored with a desiccant at room temperature.

Evaluation of the performance of the ICG strip

To evaluate the sensitivity and cross-reactivity of the ICG strip, culture solution of eight *L. monocytogenes* serotypes, four *Listeria* spp., and six other common bacteria were centrifuged (5000×*g*, 10 min) and the supernatants were tested directly or diluted with 10 mM PBS (3×, 9×, or 27×) before detection. In addition, recombinant P60 protein of *L. monocytogenes* was diluted to 5, 10, 25, 50, 100, and 250 ng·mL^{-1} with PBS and analyzed with the ICG strip. PBS was used as a control.

For each single test, 7 μL of AuNP-labeled mAb 1C1 was mixed with 43 μL of suspension buffer (10 mM PBS, 2% BSA, 0.1% Tween, and 0.2% sucrose) and 100 μL of sample. Following incubation at 37 °C for 5 min, the solution was loaded onto the strip and allowed to react for 10 min. The results were visualized by bare eyes. Two red bands on both the T line and C line were indicative of a *L. monocytogenes*-positive sample, while one red band on the C line was indicative of a *L. monocytogenes*-negative sample.

Analysis of spiked milk samples using the ICG strip

To test the effectiveness of the ICG strip, real samples were analyzed. Pure milk was purchased from a local

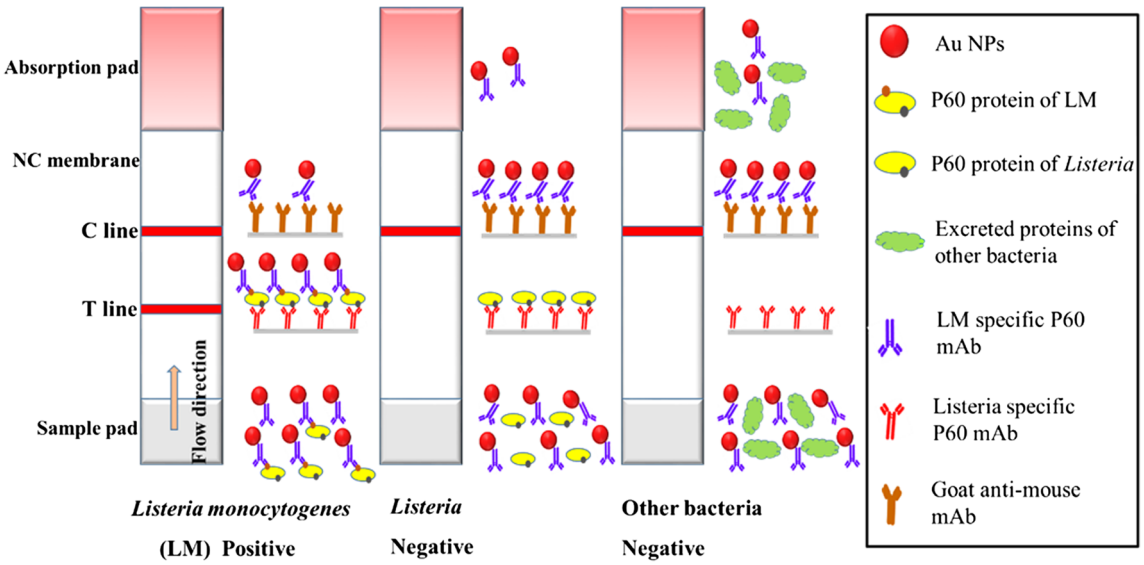


Fig. 1 Scheme of the gold nanoparticle-based paper assay for identification of *Listeria monocytogenes* from listeria spp.

market and confirmed to be free of *L. monocytogenes* and *Listeria* spp. by a culture-based method [40]. Different strains of *L. monocytogenes* (ATCC 19111, ATCC 19115, ATCC 19118, and CMCC 54003) were cultured and added to pure milk to simulate samples contaminated with *L. monocytogenes*. One to nine colony-forming unit·mL^{-1} (CFU·mL^{-1}) were added to the milk samples by serial dilution. Subsequently, 25 mL of the spiked samples were mixed with 225 mL of *L. monocytogenes* enrichment broth (Oxoid) and cultured at 37 °C. Samples (1 mL) of each culture solution were collected after 8 and 12 h and centrifuged (5000×*g*, 10 min). The supernatant of each sample was analyzed by the ICG strip.

Results and discussion

Principle of the ICG strip

The detection of *L. monocytogenes* was based on an antibody-antigen reaction on the NC membrane (Fig. 1). First, the sample was added to a tube with a pipette and reacted with the Au NPs labeled mAb of *L. monocytogenes* in the tube. The sample pad of the strip was then loaded into the sample solution. With the capillary force, AuNPs labeled mAb and the proteins in the sample flowed toward the absorption pad. The results can be quickly visualized as the color of the T and C lines change. MAb 10E7 against the P60 protein of all *Listeria* spp. (including *L. monocytogenes*) was coated on the T line, and mAb 1C1 against the P60 protein of *L. monocytogenes* was labelled with AuNPs and used as a detector probe. In *L. monocytogenes*-positive samples, P60 proteins first react with the detector probe and are captured by mAb 10E7 on the T line. Red bands on both T and C lines are indicative of positive samples. When *Listeria* spp. are present, P60 proteins cannot react with the detector probe. Even though P60 proteins are captured on the T line, a red band only appears on the C line (excess AuNP-labeled mAb captured by the goat anti-mouse antibody), which is indicative that the sample is *L. monocytogenes*-negative. Furthermore, negative results are obtained in samples containing bacterial strains devoid of P60 protein.

MAbs specific to *L. monocytogenes* pep D and *Listeria* spp. P60

L. monocytogenes-specific mAb was prepared against the peptide Pep D of *L. monocytogenes* P60 protein. Pep D was conjugated to BSA and used as an immunogen. The NH_2 group of BSA first reacted with the N-hydroxysuccinimide ester group of SMCC. Following the removal of excess SMCC, maleimidomethyl group-activated BSA was conjugated with the cysteine SH group on the N-terminal end of Pep D. Conjugation of the peptide to the BSA plays a key role in preparing an effective immunogen, therefore we optimized two reaction ratios between BSA and the peptide(MW: 1300) with 1: 20 and 1: 40. The SDS-PAGE image in Fig. 2a shows that the molecular weight of SMCC-activated BSA increased compared with that of BSA, and increased further following conjugation with Pep D, which confirmed the successful conjugation of Pep D with BSA. Immunization of mice with the two immunogens revealed the titer of antibodies against *L. monocytogenes* was higher with the conjugates having higher reaction ratio (1:40). After cell fusion, mAb 1C1 was obtained. MAb 1C1 had high binding affinity towards both the recombinant P60 protein and the supernatant of *L. monocytogenes*. While specific to P60 protein secreted by *L. monocytogenes*, mAb 1C1 did not react with P60 protein of other *Listeria* spp. or with surface-associated P60 protein on *L. monocytogenes*. Pep D is a specific peptide of all *L. monocytogenes* serotypes, and is not accessible when P60 proteins are embedded in the cell membrane [36, 41].

MAb 10E7, prepared with the whole P60 protein, was cross-reactive with both secreted and surface-associated P60 protein of all *Listeria* spp. Horseradish peroxidase-conjugated mAb 1C1 paired with mAb 10E7 had a detection limit (signal to ratio ≥ 2.1) of 1.2 ng·mL^{-1} P60 protein (Fig. S1) based on sandwich ELISA results. This ELISA was specific to the supernatant of *L. monocytogenes* culture solution but not to that of other *Listeria* spp. (Figure S2). The supernatant still can be detected after 1000 times dilution.

Identification of *L. monocytogenes* from *Listeria* spp. and other bacteria

The TEM image in Fig. S3a shows that AuNPs had a diameter of 15 nm ± 2.8 nm. The UV data in Fig. S3b reveals that the maximum absorption of AuNP-labeled mAb 1C1 and AuNPs was 524 and 521 nm, respectively. The red shift in the absorption band revealed the successful conjugation of mAb with AuNPs. Recombinant P60 protein was analyzed with the strip assay. Figure 2b shows that the visual detection limit of P60 protein was 25 ng·mL^{-1} in PBS.

The presence of other *Listeria* spp. and bacterial strains may interfere with the detection and identification of *L. monocytogenes*. Supernatant of eight *L. monocytogenes* strains including ATCC 19111 (serotype1/2a), CMCC 54007, ATCC 19115 (4b), CMCC 54003 (1/2a), CMCC 54004, CMCC 54002 (1/2c), ATCC 19118 (4e), and a wild strain isolated from frozen beef were all detected by the lateral

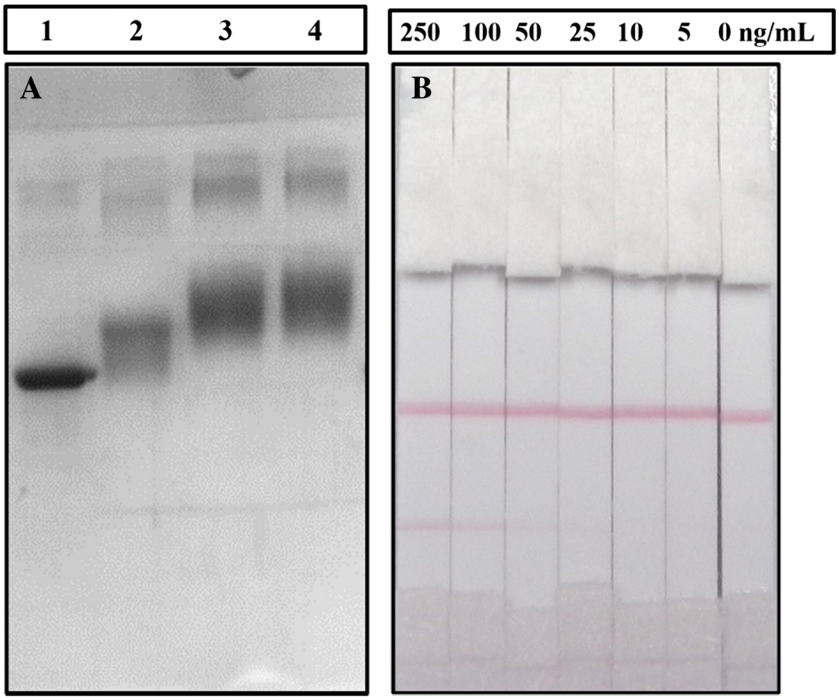


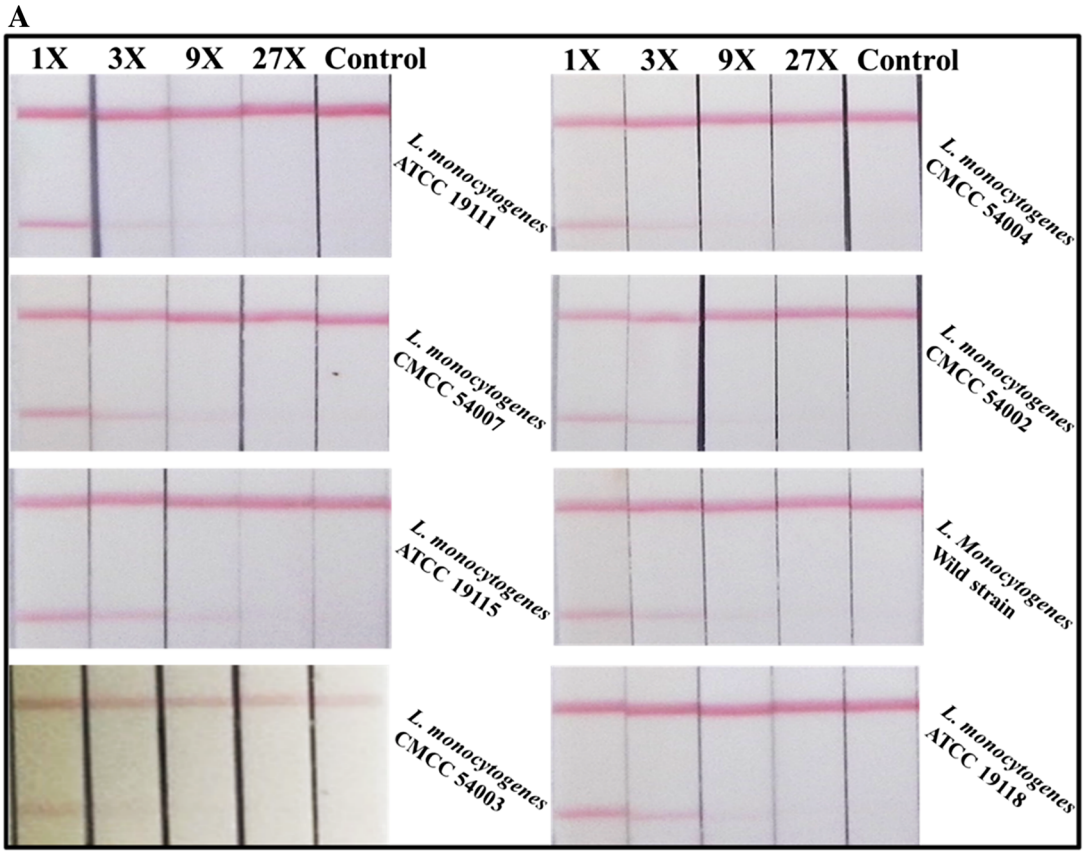
Fig. 2 **a** Characterization of the prepared immunogen. 1, BSA; 2, SMCC activated BSA with reaction ratio at 50:1; 3, PepD and BSA conjugate with reaction ratio at 20:1; 4, PepD and BSA conjugate with reaction ratio at 40:1. **b** Detection of P60 protein from *L. monocytogenes* with gold nanoparticle-based paper assay. From *left* to *right*, the concentrations of P60 protein were 250, 100, 50, 25, 10, 5, 0 ng·mL^{-1} in 10 mM PBS

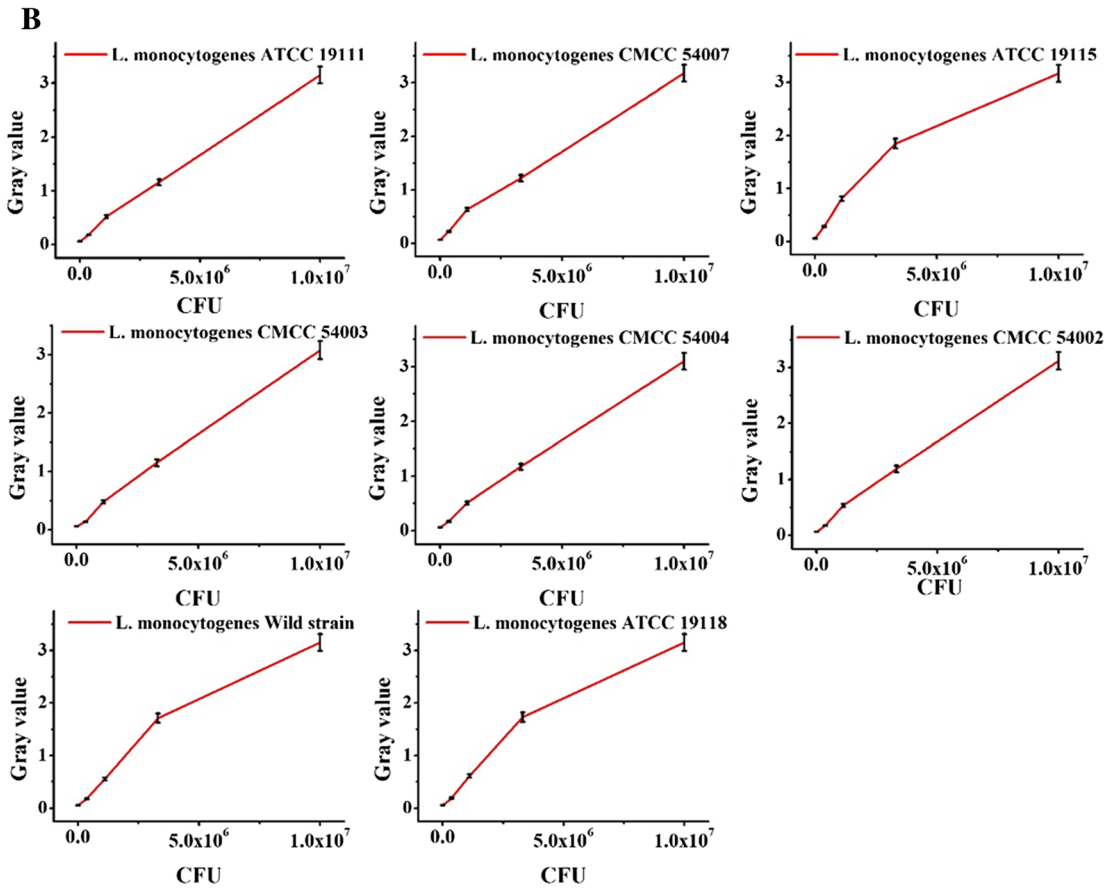
flow assay (Fig. 3a). As shown in Fig. 3b, the gray values decreased along with the decrease of the equivalent bacteria numbers. Based on the gray value, the practical detection limit ($P/N \geq 2.1$) was 27X of the supernatant, which is equivalent to 3.7×10^6 CFU·mL^{-1} of *L. monocytogenes* because the original concentration was 1×10^8 CFU·mL^{-1} after culturing overnight (12 h). Therefore, the assay had broad cross-reactivity among different *L. monocytogenes*. The detection limit was relatively high because only secreted P60 proteins can be detected (P60 proteins that were surface-associated were not accessible to mAb 1C1). The detection sensitivity was also limited to the affinity of mAb 1C1 due to the short incubation time of the ICG strip assay.

In spite of the presence of P60 protein in the supernatant, *L. ivanovii*, *L. innocua*, *L. welshimeri*, and *L. grayi* did not interfere with the detection of *L. monocytogenes* (Fig. 4a). When other Listeria spp. presented with *L. monocytogenes* together in the food and were tested with the strip after enrichment, the Au NPs labeled 1C1 selectively reacted with the P60 protein from the *L. monocytogenes* other than the listeria strains. When they moved to the test line, both the P60 protein from the L. monocytogenes and from listeria strains were equally captured by the mAb 10E7 but only P60 protein from the *L. monocytogenes* generated color on the test line. The equal competition slightly decreased the color on the test line, but the result was still positive. Additionally, there was no cross-reactivity with other bacterial strains such as *Escherichia coli*, *E. coli* O157:H7, *Cronobacter sakazakii*, *Salmonella enteritidis*, *Staphylococcus aureus*, or *C. jejuni* (Fig. 4b). The results confirmed the specificity of the method (Fig. 4c). MAbs 1C1 and 10E7 were prepared against the conserved peptide (Pep D) of *L. monocytogenes* P60 protein and whole P60 protein of *Listeria* spp., respectively. The combination of the two mAbs contributed to a very specific detection method. Of the 13 different serotypes in *L. monocytogenes*, 1/2a, 1/2b, 1/2c, and 4b account for 95% of human listeriosis cases [10]. Therefore, the ICG strip represents a portable and effective tool for the identification of *L. monocytogenes*.

Analysis of milk samples spiked with *L. monocytogenes*

Pure milk samples spiked with low levels (1 to 9 CFU·mL^{-1}) of four *L. monocytogenes* strains were analyzed by the ICG strip following a short enrichment period (8 and 12 h). Figure 5 shows that *L. monocytogenes* strains ATCC 19111, ATCC 19115, ATCC 19118, and CMCC 54003 were detected following a 12-h enrichment period. On the other hand, the 8-h enrichment period was too short to detect P60 proteins in the culture solution. The evaluation of the tested strains with popular serotypes (1/2a and 4b) demonstrated that our method was effective for the analysis of low concentrations of *L. monocytogenes* in foods. The results obtained by the ICG strip were consistent with those obtained by the culture-based method but more efficient and simple (Table S1). Nowadays, PCR assays are available and reliable, but need skilled training and sophisticated instrument. In contrast, immunoassays like lateral flow assay are very simple and portable but sometimes


A
1X 3X 9X 27X Control
1X 3X 9X 27X Control
L. monocytogenes ATCC 19111
L. monocytogenes CMCC 54004
L. monocytogenes CMCC 54007
L. monocytogenes CMCC 54002
L. monocytogenes ATCC 19115
L. Monocytogenes Wild strain
L. monocytogenes CMCC 54003
L. monocytogenes ATCC 19118


B
L. monocytogenes ATCC 19111
L. monocytogenes CMCC 54007
L. monocytogenes ATCC 19115
L. monocytogenes CMCC 54003
L. monocytogenes CMCC 54004
L. monocytogenes CMCC 54002
L. monocytogenes Wild strain
L. monocytogenes ATCC 19118
Gray value
CFU
0.0
5.0x10^6
1.0x10^7

◀ **Fig. 3** **a** Gold nanoparticle-based paper assay for detection of eight strains of *L. monocytogenes.* The supernatants of culture solution were tested directly or after diluted at 3 times, 9 times, 27 times with PBS, control check was the 10 mM PBS. **b** The plot of grey value on the test line versus the equivalent CFU values, Error bar was the standard deviation and the gray value was the average of six replicates of measuring the same bacterial sample

not accurate due to poor quality of the mAbs. Therefore, it is necessary to develop qualified mAbs against the pathogens and make the immunoassays also very dependable. Furthermore, once obtained, these antibodies can be produced in a large scale with homogenous quality. The Blazkova and coauthors developed an effective nucleic acid lateral flow immunoassay for listeria spp. and *L. monocytogenes*, which was also portable and simple [42]. However, the nucleic acid needs further enrichment by the PCR before lateral flow assay. This lowered the simplicity of the method and increased the cost. In our work, the sample was analyzed without PCR after enrichment by culture broth, which was more timesaving and use-friendly.

L. monocytogenes mAbs against different surface antigens such as autolysin IspC [35], internalin A [21, 34], and P60

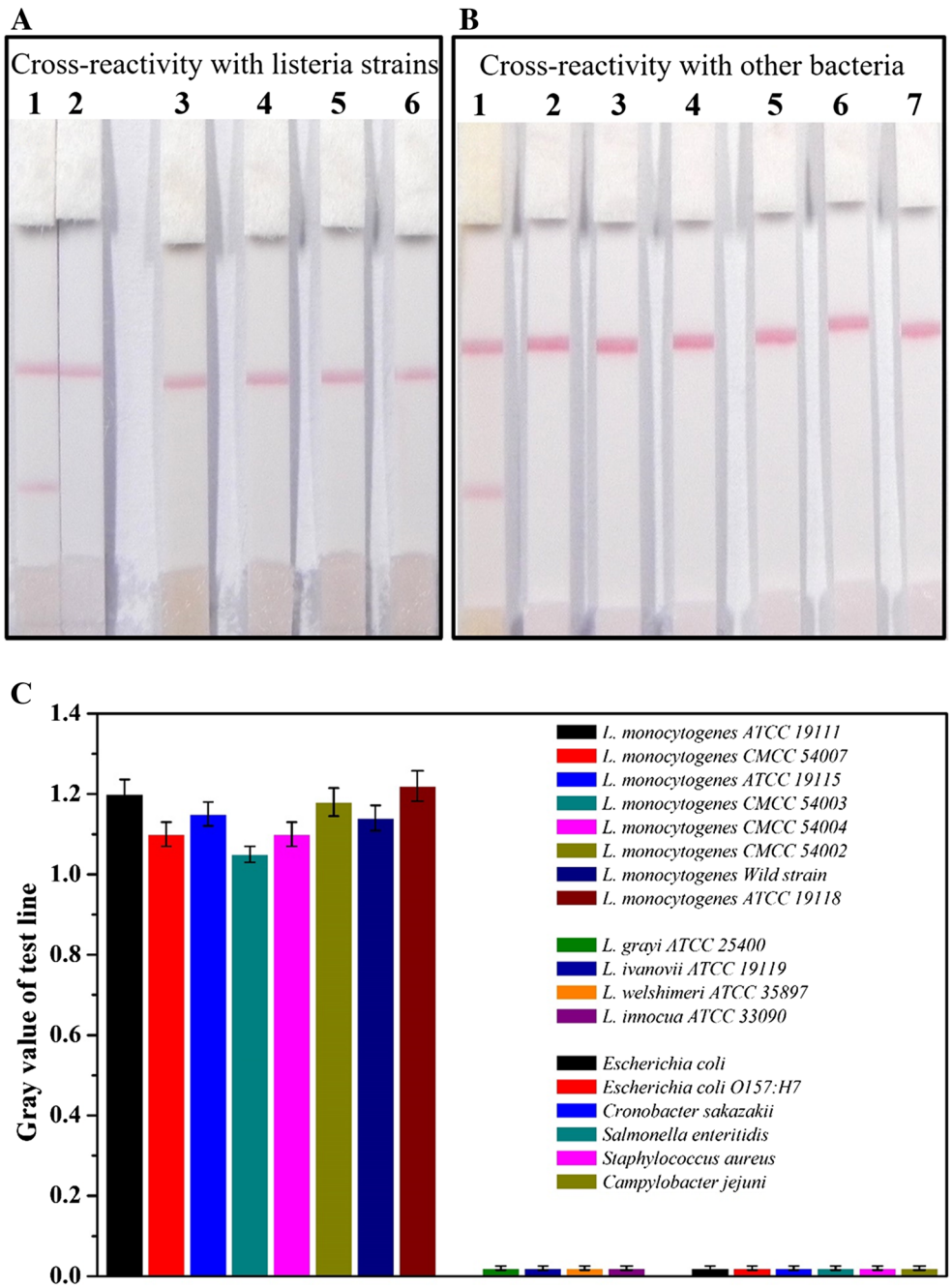


Fig. 4 Cross-reactivity of the gold nanoparticle-based paper assay: **a** with four *Listeria* strains, 1, *L. monocytogenes*; 2, Uncultured medium as control check; 3, *L. grayi*; 4, *L. ivanovii*; 5, *L. welshimeri*; 6, *L. innocua*. **b** with other bacteria, 1, *L. monocytogenes; 2, E.coli*; 3, *E. coli* O157:H7; 4, *Cronobacter sakazakii*; 5, *Salmonella enteritidis*; 6, Staphylococcus aureus; 7, *Campylobacter jejuni*. Strains were cultured overnight and tested without dilution after centrifugation. **c** The overall performance of the gold nanoparticle-based paper assay. Error bar was the standard deviation and the gray value was the average of six replicates of measuring the same bacterial sample

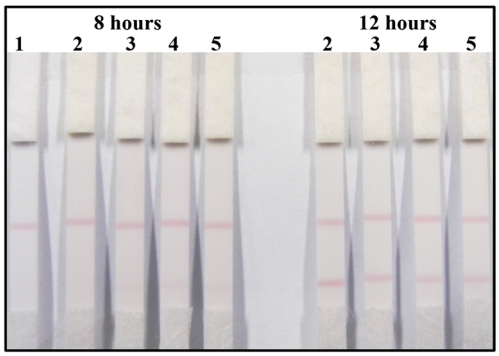


Fig. 5 Analyzing low level of *L. monocytogenes* in Milk sample with gold nanoparticle-based paper assay after 8 h and 12 h enrichment: 1 PBS as control check, 2 *L. monocytogenes* ATCC 19111, 3 *L. monocytogenes* ATCC 19115, 4 *L. monocytogenes* ATCC 19118, 5 *L. monocytogenes* CMCC 54003

protein [22, 36, 41] have been prepared. A sandwich ELISA against *L. monocytogenes* based on P60 mAb and polyclonal Ab (pAb) was developed by Yu; however, it's based on pAb and sensitivity was not reported. Recently, a phage display antibody prepared by Tu [10] recognized the popular serotypes 1/2a, 1/2b, and 4b of *L. monocytogenes*, and the detection limit of the sandwich ELISA was 10^4 CFU·mL^{-1}. However, the ICG strip test reported by Shim [25] cross reacted with *Listeria* spp. (10^7 CFU·mL^{-1}) and was not strictly specific to *L. monocytogenes*. The ICG strip assay developed by Kim [26] used IMS for pre-concentration. The sensitivity decreased to 6.6×10^3 CFU·mL^{-1} following IMS. However, the cross-reactivity against different serotypes of *L. monocytogenes* was unknown. As reviewed in Table 2, the sensitivity of our method was a little lower but still comparable with the classical lateral flow assays. However, the existing immunoassays are all insufficient to detect the low contaminant level (1–9 CFU·mL^{-1}) in the food sample and enrichment was inevitable. The advantage of our method was the specific and homogenous identification of different serotypes of *L. monocytogenes*, which provided a resolution to the current cross-reactivity issue with other existing ICG strip assay.

The Pep D pAb prepared by Bubert [36] recognized *L. monocytogenes*, and the P60 protein mAb prepared by Yu [22] recognized *L. monocytogenes* and *Listeria* spp. These results were confirmed in our study. It is noteworthy that the two types of mAbs can be paired in the sandwich ELISA and be effective in the lateral flow assay, probably because the epitopes of the two types of mAbs are different and both are part of the P60 protein. Our results overcome the main limitations of the current ICG strip immunoassay including the lack of mAb specific to *L. monocytogenes* and the lack of a mAb pair.

Conclusion

We prepared mAb 1C1 that recognized specific peptide Pep D of *L. monocytogenes* and mAb 10E7 that recognized P60 protein of *Listeria* spp. The mAb pair was used in the development of an AuNP-based ICG strip assay. Based on the results, the ICG strip assay detected the eight tested *L. monocytogenes* strains of different popular serotypes (1/2a, 1/2b, 4b, 4e), without any cross-reactivity with other *Listeria* spp. (*L. ivanovii*, *L. innocua*, *L. welshimeri*, and *L. grayi*) or common Gram-positive and Gram-negative bacteria. Milk samples spiked with *L. monocytogenes* were detected by the ICG strip assay in 13 h. Therefore, the ICG strip represents a portable and effective tool for the identification of *L. monocytogenes* in foods.

Table 2 An overview on recently reported immunoassay for determination of *L. monocytogenes*

Method	Detection limit	Extra step	Accuracy	Cross-reactivity	Reference
Lateral flow assay	2.5 ng (p60) 3.7×10^6 CFU·mL^{-1}	None	8/8	None	This work
Competitive fluorescence immunoassay	100 ng (P60) Unknown	None	6/6	None	[41]
Nucleic acid lateral flow immunoassay	0.05 ng (DNA) 10^5 CFU·mL^{-1}	PCR	9/9	None	[42]
Lateral flow immunoassay	10^5 CFU·mL^{-1}	None	8/8	React with listera spp.	[25]
Lateral-flow enzyme immunoconcentration	9.5×10^1 CFU·mL^{-1}	None	1/1	None	[29]
superparamagnetic Lateral flow immunoassay	10^4 CFU·mL^{-1}	None	1/1	None	[32]
Immuno-magnetic based lateral flow immunoassay	6.6×10^3 CFU·mL^{-1}	None	1/1	Unknown	[26]

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Compliance with ethical standards The author(s) declare that they have no competing interests.

References

1. Auvolat A, Besse NG (2016) The challenge of enumerating listeria monocytogenes in food. Food Microbiol 53:135
2. Lee SH, Ahn JY, Lee KA, Um HJ, Sekhon SS, Park TS, Min J, Kim YH (2015) Analytical bioconjugates, aptamers, enable specific quantitative detection of listeria monocytogenes. Biosens Bioelectron 68:272
3. Liao YH, Zhou XM, Xing D (2014) Quantum dots and Graphene oxide fluorescent switch based multivariate testing strategy for reliable detection of listeria monocytogenes. ACS Appl Mater Interfaces 6:9988
4. Yeni F, Yavas S, Alpas H, Soyer Y (2016) Most common foodborne pathogens and Mycotoxins on fresh produce: a review of recent outbreaks. Crit Rev Food Sci Nutr 56:1532
5. Gasanov U, Hughes D, Hansbro PM (2005) Methods for the isolation and identification of listeria spp. and listeria monocytogenes: a review. FEMS Microbiol Rev 29:851
6. Weller D, Andrus A, Wiedmann M, 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
7. Day JB, Basavanna U (2015) Magnetic bead based immunodetection of listeria monocytogenes and listeria ivanovii from infant formula and leafy green vegetables using the bio-Plex suspension array system. Food Microbiol 46:564
8. Jamali H, Paydar M, Ismail S, Looi CY, Wong WF, Radmehr B, Abedini A (2015) Prevalence, antimicrobial susceptibility and virulotyping of listeria species and listeria monocytogenes isolated from open-air fish markets. BMC Microbiol 15:144
9. Zhou MY, Jiang MJ, Ren CY, Liu SJ, Pu QK, Goldfine H, Shen H, Wang C (2016) Listeria ivanovii infection in mice: restricted to the liver and lung with limited replication in the spleen. Front Microbiol 7:790
10. Tu Z, Chen Q, Li YP, Xiong YH, Xu Y, Hu N, Tao Y (2016) Identification and characterization of species-specific nanobodies for the detection of listeria monocytogenes in milk. Anal Biochem 493:1
11. Tait E, Perry JD, Stanforth SP, Dean JR (2014) Bacteria detection based on the evolution of enzyme-generated volatile organic compounds: determination of listeria monocytogenes in milk samples. Anal Chim Acta 848:80
12. Kong DZ, Liu LQ, Xing CR, Kuang H, Xu CL (2015) Sensitive and highly specific detection of Cronobacter sakazakii based on monoclonal sandwich ELISA. Food Agric Immunol 26:566
13. Li P, Zhang Y, Lei H, Wang H, Xu Z, Shen Y, Sun Y, Pang J, Yang J (2015) Development of chemiluminescent enzyme immunoassay for the determination of aflatoxin M1 in milk products. Food Agric Immunol 26:157
14. Marusov G, Sweatt A, Pietrosimone K, Benson D, Geary SJ, Silbart LK, Challa S, Lagoy J, Lawrence DA, Lynes MA (2012) A microarray biosensor for multiplexed detection of microbes using grating-coupled surface Plasmon resonance imaging. Environ Sci Technol 46:348
15. Wang W, Wang W, Liu L, Xu L, Kuang H, Zhu J, Xu C (2016) Nanoshell-enhanced Raman spectroscopy on microplate for staphylococcal enterotoxin B sensing. ACS Appl Mater Interfaces 8: 15591
16. Wu X, Wang W, Liu L, Kuang H, Xu C (2015) Monoclonal antibody-based cross-reactive sandwich ELISA for the detection of salmonella spp. in milk samples. Anal Methods 7:9047
17. Law JWF, Ab Mutalib NS, Chan KG, Lee LH (2015) An insight into the isolation, enumeration, and molecular detection of listeria monocytogenes in food. Front Microbiol 6:1227
18. Wang HY, Zhang CS, Xing D (2011) Simultaneous detection of salmonella enterica, Escherichia Coli O157:H7, and listeria monocytogenes using oscillatory-flow multiplex PCR. Microchim Acta 173:503
19. Wang W, Liu L, Song S, Xu L, Kuang H, Zhu J, Xu C (2016) Gold nanoparticle-based strip sensor for multiple detection of twelve salmonella strains with a genus-specific lipopolysaccharide antibody. Science China Materials 59:665
20. Wang WB, Liu LQ, Song SS, Tang LJ, Kuang H, Xu CL (2015) A highly sensitive ELISA and Immunochromatographic strip for the detection of salmonella typhimurium in milk samples. Sensors 15: 5281
21. Hearty S, Leonard P, Quinn J, O'Kennedy R (2006) Production, characterisation and potential application of a novel monoclonal antibody for rapid identification of virulent listeria monocytogenes. J Microbiol Methods 66:294
22. Yu K-Y, Noh Y, Chung M, Park H-J, Lee N, Youn M, Jung BY, Youn B-S (2004) Use of monoclonal antibodies that recognize p60 for identification of listeria monocytogenes. Clin Diagn Lab Immunol 11:446
23. Wang W, Liu L, Xu L, Kuang H, Zhu J, Xu C (2016) Gold-nanoparticle-based multiplexed immunochromatographic strip for simultaneous detection of staphylococcal enterotoxin a, B, C, D, and E. Part Part Syst Charact 33:388
24. Wang WB, Feng M, Kong DZ, Liu LQ, Song SS, Xu CL (2015) Development of an immunochromatographic strip for the rapid detection of pseudomonas syringae pv. Maculicola in broccoli and radish seeds. Food Agric Immunol 26:738
25. Shim WB, Choi JG, Kim JY, Yang ZY, Lee KH, Kim MG, Ha SD, Kim KS, Kim KY, Kim CH, Ha KS, Eremin SA, Chung DH (2007) Production of monoclonal antibody against listeria monocytogenes and its application to immunochromatography strip test. J Microbiol Biotechnol 17:1152
26. Kim HS, Cho IH, Seo SM, Jeon JW, Paek SH (2012) In situ immuno-magnetic concentration-based biosensor systems for the rapid detection of listeria monocytogenes. Mater Sci Eng, C 32:160
27. Chen Q, Lin JH, Gan CQ, Wang YH, Wang D, Xiong YH, Lai WH, Li YT, Wang MH (2015) A sensitive impedance biosensor based on immunomagnetic separation and urease catalysis for rapid detection of listeria monocytogenes using an immobilization-free interdigitated array microelectrode. Biosens Bioelectron 74:504
28. Chen R, Huang X, Xu H, Xiong Y, Li Y (2015) Plasmonic enzyme-linked immunosorbent assay using Nanospherical brushes as a catalase container for colorimetric detection of ultralow concentrations of listeria monocytogenes. ACS Appl Mater Interfaces 7:28632
29. Cho IH, Irudayaraj J (2013) Lateral-flow enzyme immunoconcentration for rapid detection of listeria monocytogenes. Anal Bioanal Chem 405:3313
30. Huang XL, Xu ZD, Mao Y, Ji YW, Xu HY, Xiong YH, Li YB (2015) Gold nanoparticle-based dynamic light scattering immunoassay for ultrasensitive detection of listeria monocytogenes in lettuces. Biosens Bioelectron 66:184
31. Oaew S, Charlermroj R, Pattarakankul T, Karoonuthaisiri N (2012) Gold nanoparticles/horseradish peroxidase encapsulated polyelectrolyte nanocapsule for signal amplification in listeria monocytogenes detection. Biosens Bioelectron 34:238

32. Shi L, Wu F, Wen YM, Zhao F, Xiang JJ, Ma L (2015) A novel method to detect listeria monocytogenes via superparamagnetic lateral flow immunoassay. Anal Bioanal Chem 407:529
33. Karoonuthaisiri N, Charlermroj R, Teerapornpuntakit J, Kumpoosiri M, Himananto O, Grant IR, Gajanandana O, Elliott CT (2015) Bead array for listeria monocytogenes detection using specific monoclonal antibodies. Food Control 47:462
34. Mendonca M, Conrad NL, Conceicao FR, Moreira AN, da Silva WP, Aleixo JAG, Bhunia AK (2012) Highly specific fiber optic immunosensor coupled with immunomagnetic separation for detection of low levels of listeria monocytogenes and L. Ivanovii. BMC Microbiol 12:275
35. Ronholm J, van Faassen H, MacKenzie R, Zhang ZY, Cao XD, Lin M (2013) Monoclonal antibodies recognizing the surface autolysin IspC of listeria monocytogenes serotype 4b: epitope localization, kinetic characterization, and cross-reaction studies. PLoS One 8: e55098
36. Bubert A, Schubert P, Köhler S, Frank R, Goebel W (1994) Synthetic peptides derived from the listeria monocytogenes p60 protein as antigens for the generation of polyclonal antibodies specific for secreted cell-free L. Monocytogenes p60 proteins. Appl Environ Microbiol 60:3120
37. Guan D, Guo L, Liu L, Kong N, Kuang H, Xu C (2015) Development of an ELISA for nitrazepam based on a monoclonal antibody. Food Agric Immunol 26:1
38. Kuang H, Wang WB, Xu LG, Ma W, Liu LQ, Wang LB, Xu CL (2013) Monoclonal antibody-based sandwich ELISA for the detection of staphylococcal enterotoxin a. Int J Environ Res Public Health 10:1598
39. Frens G (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. Nat Phys Sci 241:20
40. Wu S, Wu QP, Zhang JM, Chen MT, Guo WP (2016) Analysis of Multilocus sequence typing and virulence characterization of listeria monocytogenes isolates from Chinese retail ready-to-eat food. Front Microbiol 7:168
41. Beauchamp S, D'Auria S, Pennacchio A, Lacroix M (2012) A new competitive fluorescence immunoassay for detection of listeria monocytogenes. Anal Methods 4:4187
42. Blazkova M, Koets M, Rauch P, van Amerongen A (2009) Development of a nucleic acid lateral flow immunoassay for simultaneous detection of listeria spp. and listeria monocytogenes in food. Eur Food Res Technol 229:867