Food Sci. Biotechnol. 22(2): 585-591 (2013)
DOI 10.1007/s10068-013-0118-5

RESEARCH ARTICLE

Rapid Detection of *Campylobacter jejuni* Using Fluorescent Microspheres as Label for Immunochromatographic Strip Test

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Received: 20 July 2012 / Revised: 16 October 2012 / Accepted: 26 October 2012 / Published Online: 30 April 2013

Abstract *Campylobacter jejuni* is a worldwide foodborne pathogen recognized as a leading cause of human gastrointestinal enteritis. A rapid, sensitive, and specific method is required to monitor food and water in cases of contamination by this pathogen. This report presents a novel immunochromatographic test (ICT) using fluorescent microspheres labeled with polyclonal antibodies of *C. jejuni* as the capture reagent dispensed onto the conjugate pad. Polyclonal antibodies against the outer membrane protein PEB1 of *C. jejuni* were used as the detective reagent at the test line, whereas the goat anti-rabbit IgG was used on the control line. PEB1 was obtained by gene cloning and expression to prepare its antibody. In this study, a simple and rapid ICT is reported for detecting *C. jejuni* for the first time with a detection limit of 10^6 CFU/mL.

Keywords: *Campylobacter jejuni*, protein PEB1, fluorescent microsphere, polyclonal antibody, immunochromatographic strip test

Introduction

Campylobacter jejuni, which colonizes the intestinal tract of both wild and domestic animals, is recognized worldwide as a leading cause of bacterial gastrointestinal enteritis (1,2). It is widely present in poultry meat and slaughterhouses (3), especially raw and undercooked chicken (4). Thus establishment of a simple and quick detection method for *C. jejuni* is a valid aim for preventing and controlling this foodborne disease.

Usually the detection methods for *C. jejuni* include traditional biochemical tests, molecular biology methods and enzyme-linked immunosorbent assay (ELISA). Typical detection methods frequently require the contaminated food sample or feces to be incubated in enriched media for 6-18 h. A portion of the broth is then plated on agar media and then analyzed by biochemical tests and serological reactions which are time-consuming and laborious (5). Other methods such as PCR-based methods (6-8), require trained personnel, and expensive equipment for gene amplification. Although enzyme immunoassay had been used widely in many laboratories (9,10), this method also requires specialist instruments and trained personnel. So this simple and quick technology for the detection of *C. jejuni* which even can be achieved by poor condition laboratory.

In this study, a new immunochromatographic test (ICT) has been established using anti-PEB1 polyclonal antibodies as the detective reagent at the test line. Reports showed that the gene encoding the outer membrane protein PEB1 was relatively conserved and the protein is expressed in different serotypes of *C. jejuni* (11). PEB1 (28 kDa) is a common antigen and a major cell adherence molecule of *C. jejuni*. It is involved both in binding to intestinal cells and in amino acid transport (12). Significantly, the immunogenicity and immunoprotective nature of recombinant PEB1 had been identified (12,13).

Kawatsu *et al.* (14) has developed a colloidal gold immunochromtographic test using monoclonal antibody (MAb) for detection of *C. jejuni*. But it is the first time to develop an ICT using fluorescent microspheres labeled with polyclonal antibodies instead of MAb since not many

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laboratories have the conditions to prepare MAb. The specificity and sensitivity of the immunochromatographic strip has been estimated by pure cultured bacteria and cultured bacteria mixed with other bacteria in chicken carcass and fish shellfish chylous.

Materials and Methods

Bacterial strains, plasmids, growth conditions, chemicals, and reagents All strains were stored in 20% glycerol at −80°C in our laboratory. *Campylobacter jejuni* strain NC002163 was the source of genomic DNA, provided by National Institutes for Food and Drug Control, China. *Escherichia coli* BL21 (DE3) was the host of pET-28a-*peblA* plasmid and used for production of PEB1 protein. *E. coli* DH5α was used as intermediate host strain for the vectors. *E. coli* strains were grown at 37°C in Luria-Bertani (LB) broth or on solid LB agar. The *C. jejuni* strains were grown at 37°C on Columbia agar under microaerobic conditions. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), bovine serum albumin (BSA), isopropyl-β-D-thiogalactopyranoside (IPTG), and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). The sample pad, conjugate release pad, nitrocellulose membrane, and absorbent pad were obtained from Millipore (Bedford, MA, USA). All solvents and other chemicals were analytical reagent grade.

Construction of the expression vector for PEB1 The *peblA* gene from *C jejuni* NC002163 was amplified by PCR using primers *peblA*-UP (5'-GCGGATCCGCAGAA GGTAAACTTGAGTCTAT-3') and *peblA*-DOWN (5'-CC GCTCGAGTTATAAACCCCATTTTTTCGCT-3'). The PCR product was cleaved with restriction enzymes *BamH*I and *Xho*I, and ligated into the pET-28a expression vector treated with the same 2 enzymes. The constructed expression vector was transformed into *E. coli* BL21 (DE3). Positive clones were selected according to the standard molecular method. The vector with correct sequence was used for the expression of PEB1. Expression of PEB1 was induced by the addition of 1 mM IPTG and the cells were grown for another 4 h. An aliquot (0.5 mL) of cells was harvested by centrifugation and resuspended in 100 μL of SDS-PAGE loading buffer: 50 mM Tris-HCl, pH 6.8, 10%(v/v) glycerol, 2%(w/v) SDS, 100 mM dithiothreitol, 0.1%(w/v) bromophenol blue. Samples were boiled for 5 min and cellular debris was removed by centrifuging at 15,000×*g* at 4°C for 10 min. The supernatant (whole cell extract) containing the PEB1 fusion protein was analyzed by SDS-PAGE and used for further purification.

Purification of the PEB1 protein The purification of the expressed protein was carried out by affinity chromatography. A 0.5 mL aliquot of the whole cell extract was loaded onto a nickel ions affinity column (GE Healthcare Biosciences, Pittsburgh, PA, USA) and then eluted with 1 mL of 250 mM iminazole. The eluted PEB1 was concentrated by ultrafiltration (MWCO 10,000; Millipore, Billerica, MA, USA) and further purified with molecular sieve Superdex 75 (GE Healthcare Biosciences). The concentrated solution was loaded onto the column in 0.5-mL volume each time. The column was eluted with phosphate-buffered saline (PBS) at a flow rate of 0.5 mL/min. Elution fractions were collected and lyophilized. SDS-PAGE assay was performed to monitor the presence and purity of the target protein.

PEB1 protein coupled with magnetic beads The PEB1 protein was conjugated to magnetic beads by active ester method. Twenty mg of magnetic beads powder was washed 3 times with NaH_2PO_4-HCl buffer (0.2 mM, pH 5.0). Magnetic beads solution was suspended in 0.5 mL NaH_2PO_4-HCl buffer (0.2 mM, pH 5.0). Then 1.52 mg EDC and 0.86 mg sulfo-*N*-hydroxysuccinimide (NHS) were added to it and reacted with shaking at 25°C for 40 min. The pH of the solution was adjusted to 7.8 and 500 μg PEB1 protein was added and reacted at 25°C for 3 h and eventually kept at 4°C overnight. The PEB1 protein conjugated magnetic beads were washed 3 times with PBS to remove the reaction solution and un-coupled protein, and kept at 4°C.

Preparation of antisera Four male New Zealand white rabbits (2 months old, purchased from Nanchang University School of Medicine) were intraperitoneally injected with 200 μg of heat killed *C. jejuni* mixed with complete Freund's adjuvant (Sigma-Aldrich) and subsequently intraperitoneally boosted 3 more times with the same antigens mixed with incomplete Freund's adjuvant. Sera were collected 1 week after the last injection. Anti- PEB1 polyclonal antibodies were prepared in the same way.

Purification of anti-*C. jejuni* polyclonal antibody The antisera against the whole proteins of *C. jejuni* obtained from the immunized rabbits cross reacted with the species *Pseudomonas aeruginosa, Staphylococcus aureus, Lactobacillus bulgaricus, Shigella flexneri,* and others (totally 23 strains tested). Bacteria that cross reacted with the original serum were cultivated to obtain pellets. After 3 washes with PBS, the pellets were suspended in 5 mL of PBS in a 10-mL tube; the obtained pellets (1 g) and prepared protein PEB1 coupled to magnetic beads were mixed together with 10 μL of the serum for 1 h at room temperature to remove the non-*C. jejuni* specific immunoglobulins. The supernatant was collected by centrifugation (6,000×*g*, for 10 min at 4°C) and purified using octylic

acid-ammonium sulfate precipitation. In brief, the supernatant was diluted 4-fold with acetate buffer (60 mM, pH 4.0) and adjusted to a pH 4.5 with 1 N HCl. Octylic acid (25 μL/mL of serum diluted solution) was added under constant stirring. After 30 min, the precipitate was removed by centrifugation (13,000×*g*, for 30 min at 4°C). The supernatant was harvested, and the pH was adjusted to 7.4 with 1 N NaOH. Ammonium sulfate was added to the solution (0.277 g/mL) and stirred for 30 min. Finally, the precipitate containing immunoglobulin was collected by centrifugation (13,000×*g*, for 30 min at 4°C), then dissolved in a minimum volume of PBS (0.01 M, pH 7.4), and dialyzed for 2 days at 4°C. After dialysis, the specific antiserum was adjusted to 1 mg/mL, divided to 1 mL aliquots, and stored at 70°C as described above. Anti-*C. jejuni* polyclonal antibodies were further purified by Protein A montage spin columns (according to the manual of Millipore).

Purification of anti-PEB1 polyclonal antibody Ten mg prepared magnetic beads were added to 500 μL 1% BSA to block for 1 h at 37°C and washed 3 times with PBS with Tween 20 (PBST). Then 10 μL antiserum against PEB1 was added to conjugate with magnetic beads for 1 h at 37°C. The non-conjugated antibodies were removed by washing 3 times with PBST. A 50 μL glycine-HCl solution (500 mM NaCl, 0.5% Tween 20, pH 2.3) was mixed with the beads for 1 min. Immediately afterwards 2.6 μL Na_3PO_4 (0.1 M) was added to adjust to neutral pH to avoid the irreversible denaturation of antibodies. The supernatant containing the antibodies was collected and lyophilized before use.

Conjugation of anti-*C. jejuni* polyclonal antibodies with fluorescent microspheres (FM) A solution containing 2.5 mg of fluorescent microspheres (400 nmol) was initially sonicated for 5 min, and then added to PBS. Thirty μg anti-*C. jejuni* polyclonal antibodies (/mg FM) and 0.1917 mg EDC, whose molar ratio to FMs was 1:1, were slowly added to the mixture to promote the coupling. The final volume of solution (5 mL) was incubated for 2 h at room temperature (RT) with light stirring to allow antibodies to adhere to the microsphere surface. The anti-*C. jejuni* polyclonal antibody-FMs were blocked in 1% BSA for 30 min, collected by centrifuge (9,000×*g*, 10 min), and washed once with PBS. Finally, FMs-labeled anti-*C. jejuni* polyclonal antibodies were stored in 1/10 volume suspension at 4°C.

Preparation of immunochromatographic strips Seven-hundred μL of 1.5 mg/mL anti-PEB1 polyclonal antibodies were dispensed onto the lower part of a nitrocellulose membrane strip with a Bio-strip Dispenser HGS102, as the test line (T), while 500 μL of 1 mg/mL goat anti-rabbit IgG was dispensed 5 mm above the test line, as the control line (C). The FMs labeled anti-PEB1 polyclonal antibodies were dispensed onto the conjugate pad at the speed of 8 μL/cm. After drying for 3 h at 37°C, the nitrocellulose membrane was blocked in 0.5% ovalbumin for 10 min, and then continued drying. Sample pad made of glass fibers was treated with PBS (pH 7.4) containing 1% BSA, 1% trehalose, 0.02% sodium azide, and 0.1% Tween 20, and dried at 37°C. A filter paper absorption pad was applied immediately without pretreatment. After drying for 1 h at 37°C, the nitrocellulose membrane, conjugate pad, sample pad, and absorbent pad were assembled, laminated and pasted to a plastic backing plate. The assembled membrane was cut into strips of 4 mm width and 60 mm length with a programmable strip cutter HGS201, and the strips were stored in drying cylinder.

ICT procedures *C. jejuni* suspensions in PBS (pH 7.4) were adjusted to concentrations of 10^4-10^8 CFU/mL. Eighty μL of each pure *C. jejuni* suspensions were pipetted into the well on the sample pad of a test strip. Samples of chicken carcass and fish shellfish chylous were prepared by mixing 2 g of chicken carcass and fish shellfish chylous with 20 mL of PBS spiked with *C. jejuni* at concentrations ranging from 10^4-10^8CFU/mL mixed with 10^6 CFU/mL *E. coli O157:H7* PELI1264, *E. sakazakii* YC633B, and *Salmonella* ATCC13311. Then the samples were homogenized by stomaching for 1 min so that the heavier particulates could settle before pipetting 80 μL of the clear supernatant into a vial for analysis. Chicken carcass and fish shellfish chylous extract in PBS without pathogen were used as negative control. After that, the labeled anti-*C. jejuni* polyclonal antibody-FMs on the conjugate pad were dissolved and owed with the solution toward the membrane on which capture reagents were immobilized. Additionally, stools were examined by ICT strip (ICTS). The stool sample collected from healthy *Balb/c* mice without pretreatment was served as control. In the treatment groups, mice were administered 10^{10} CFU/mL *C. jejuni* NC002163 by gavage. Then the stools were collected the next day. The stools were diluted to 10%(w/v) in PBS solution for ICTS. The intensity of uorescence on the test line could be observed by portable card reader device. The overall assay time for this ICT was within 10 min.

Results and Discussion

Fusion expression and purification of PEB1 protein To obtain pure PEB1 for immunization, the gene encoding PEB1 was cloned into pET-28a-*peblA* (Fig. 1). The *peblA* fragments was obtained by digesting with *BamH*I and *Xho*I. Recombinant HIS-tagged fusion protein was expressed

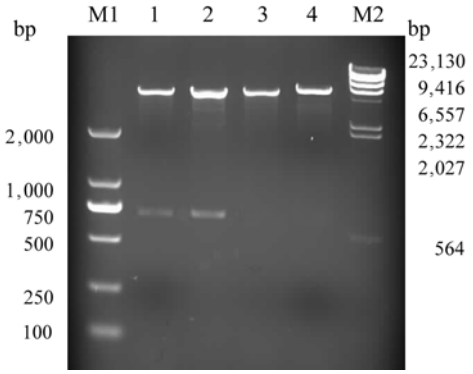

Fig. 1. Gel electrophoresis of extracted recombination plasmid and its dual enzyme digested products. Lane: 1-2, recombination plasmids (dual enzyme digestion); 3, plasmid pET28a (dual enzyme digestion); 4, recombination plasmid (single enzyme digestion); M1, DL2000 DNA marker; M2, 1 kb ladder DNA marker. Sizes of pET28a and the target gene were about 6 kb and 700 bp, respectively. After digestion by *BamH*I and *Xho*I, the plasmid appeared as 2 bands of the expected sizes.

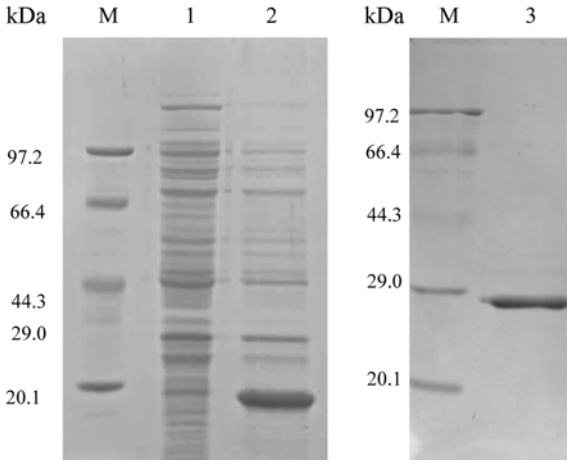

Fig. 2. SDS-PAGE analysis of the expressed and purified PEB1. Lane: 1, expression product of *E.coli* containing pET28a as a control; 2, expression product of *E.coli* containing pET-28a-*peblA* which had a clear target protein band at about 28 kDa; 3, fusion protein of PEB1 purified by affinity chromatography; M, protein molecular weight markers. Samples were analyzed on 12% polyacrylamide gels.

in *E. coli* BL21 (DE3) and purified by nickel ions affinity column (Fig. 2). The molecular mass of the recombinant PEB1 was determined as about 28 kDa by gel filtration and agreed with the expected size. As shown in Fig. 2, the purity of the fusion PEB1 was sufficient without other bands.

Polyclonal antibody production and purification Using the protein to immunize rabbits, after 4 injections the antiserum was collected. Then the antiserum was purified by octylic acid-ammonium sulfate precipitation. The purified antiserum was used for immunblotting to test its cross

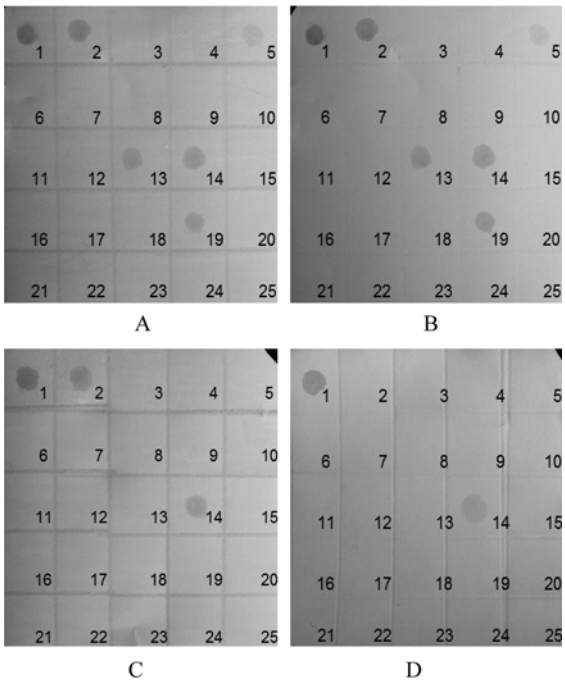

Fig. 3. Cross reactions of antisera and purified antibodies with common foodborne pathogens and probotics by colony immunoblotting. (A), Purified anti-PEB1 polyclonal antibodies by octylic acid-ammonium sulfate precipitation; (B), purified anti-*C. jejuni* polyclonal antibodies by octylic acid-ammonium sulfate precipitation; (C), further purified anti-PEB1 polyclonal antibodies by reverse absorption; (D), further purified anti- *C. jejuni* polyclonal antibodies by reverse abosorption

reaction (Fig. 3A, 3B). Both the purified anti-*C. jejuni* antibodies and anti-PEB1 antibodies reacted with PEB1, and cross reacted with the species *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Lactobacillus bulgaricus*, and *Shigella flexneri* (Table 1, 2). After reverse absorption to remove the non-*C. jejuni* immunoglobulins, the purified antiserum was used to examine whether its cross-reactions had been cleared up by immunblotting (Fig. 3C, 3D). The cross reactions with *P. aeruginosa*, *L. bulgaricus*, and *S. flexneri* had been removed except *St. aureus* because Staphylococal protein A has the ability to capture most antibody. And the anti-*C. jejuni* polyclonal antibodies had been removed the reactions with PEB1 by reverse absorption in the similar way as strains. In this way, the antibody pairs were obtained for ICTS. Purified anti-PEB1 polyclonal antibodies and anti-*C. jejuni* polyclonal antibodies were analyzed by SDS-PAGE (Fig. 4). So polyclonal antibodies labeled with the fluorescent microspheres was used to prepare the immunochromatographic strip test. It saves time and manpower in fusing cells and screening the antibody using hybridoma methodology (15,16). In contrast, production of polyclonal antibody involves a quicker process and less cost. Moreover, the polyclonal antibody could recognize more antigen epitopes (17), thus increasing

Table 1. Cross reactions of antiserum against outer membrane protein PEB1 and its purified antibodies

No.	Strain	Original antiserum[1)]	Purified antibody
1	*C. jejuni* NC002163	+	+
2	Protein PEB1	+	+
3	*Vibrio parahaemolyticus* 1616	-	-
4	*Salmonella* ATCC13311	-	-
5	*P. aeruginosa* CMCC10104	+	-
6	*Cronobacter sakazakii* ATCC29544	-	-
7	*C. sakazakii* YC633B	-	-
8	*Listeria monocytogenes* CMCC54001	-	-
9	*L. monocytogenes* PLMO1344	-	-
10	*E. coli* O157:H7 PELI1264	-	-
11	*E. coli* O157:H7 PELI0480	-	-
12	*Micrococcus luteus*	-	-
13	*S. exneri* ATCC29903	+	-
14	*S. aureus* CMCC26003	+	-
15	*Candida albicans* Z1	-	-
16	*Bifidobacterium longum* WBLO01	-	-
17	*B. lactis* WLABO9	-	-
18	*B. animalis* WBBR05	-	-
19	*L. bulgaricus* F1	+	-
20	*B. bifidum* WBBI01	-	-
21	*B. adolescentis* WBAD08	-	-
22	*L. plantarum* ATCC 8014	-	-
23	*L. acidophilus* ATCC 4356	-	-
24	*L. rhamnosus* GG ATCC 7469	-	-
25	PBS	-	-

[1)]+, positive signal; -, negative signal

Table 2. Cross reactions of antiserum against *C. jejuni* and its purified antibody

No.	Strain	Original antiserum[1)]	Purified antibody
1	*C. jejuni* NC002163	+	+
2	Protein PEB1	+	-
3	*Vibrio parahaemolyticus* 1616	-	-
4	*Salmonella* ATCC13311	-	-
5	*P. aeruginosa* CMCC10104	+	-
6	*C. sakazakii* ATCC29544	-	-
7	*C. sakazakii* YC633B	-	-
8	*L. monocytogenes* CMCC54001	-	-
9	*L. monocytogenes* PLMO1344	-	-
10	*E. coli* O157:H7 PELI1264	-	-
11	*E. coli* O157:H7 PELI0480	-	-
12	*Micrococcus luteus*	-	-
13	*S. exneri* ATCC29903	+	-
14	*S. aureus* CMCC26003	+	-
15	*Candida albicans* Z1	-	-
16	*Bifidobacterium longum* WBLO01	-	-
17	*B. lactis* WLABO9	-	-
18	*B. animalis* WBBR05	-	-
19	*L. bulgaricus* F1	+	-
20	*B. bifidum* WBBI01	-	-
21	*B. adolescentis* WBAD08	-	-
22	*L. plantarum* ATCC 8014	-	-
23	*L. acidophilus* ATCC 4356	-	-
24	*L. rhamnosus* GG ATCC 7469	-	-
25	PBS	-	-

[1)]+, positive signal; -, negative signal

the chance of better capture the antigen than monoclonal antibody. Since the polyclonal antibody was eliminated the cross-reactions in our study, its specificity was not necessarily worse than monoclonal antibody. By specific purification of polyclonal antibodies, the effective antibody pairs were obtained as the capture antibody and test antibody.

Sensitivity and specificity of the test strip To facilitate the detection of *C. jejuni* in food samples, we developed an ICT method using fluorescent microspheres for the first time. Under optimal assay conditions, different concentrations of *C. jejuni* suspensions were detected by ICT in triplicate (Fig. 5A). ICT is based on the double antibodies sandwich theory. When sample solution was added to the sample pad, it bound to the fluorescent microspheres-labeled anti-*C. jejuni* polyclonal antibodies coated on the conjugate pad. When the antigen-antibody binding solution passed the test line on which anti-PEB1 polyclonal antibodies was immobilized, the analyte would bind with this polyclonal

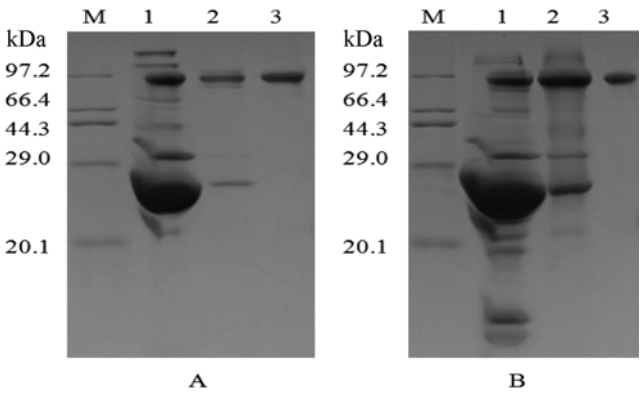

Fig. 4. SDS-PAGE electrophoresis pattern of the purified antibodies. Lanes in (A): M, protein molecular weight markers; 1, anti-PEB1 polyclonal antibodies; 2, purified anti-PEB1 polyclonal antibodies by octylic acid-ammonium sulfate precipitation; 3, further purified anti-PEB1 polyclonal antibodies by Protein A montage spin columns. Lanes in (B): M, protein molecular weight markers; 1, anti-*C. jejuni* polyclonal antibodies; 2, purified anti-*C. jejuni* polyclonal antibodies by octylic acid-ammonium sulfate precipitation; 3, further purified anti-*C. jejuni* polyclonal antibodies by magnetic beads

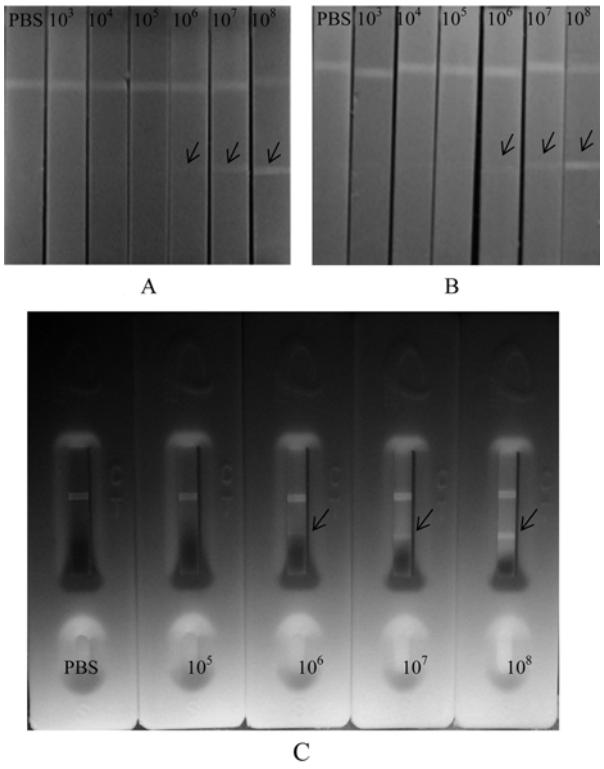

Fig. 5. ICT with different concentrations of *C. jejuni* suspensions in PBS (A), mixed with 10^6 CFU/mL of other bacterium in fish shellfish chylous (B), and mixed with 10^6 CFU/mL of other bacterium in chicken carcass (C) (ranging from 10^3 to 10^8 CFU/mL). PBS solution, control; chicken carcass and fish shellfish chylous extract in PBS without pathogen, negative control. Arrows point to the test line.

antibody in different antigenic determinants. When both the test line and the control line appeared fluorescent, the result was considered to be positive. If the concentration of *C. jejuni* was below the detection limit or absent in the sample, only the control line appeared, indicating a negative result. If no control line emerges, the test was invalid. The color intensities could be easily seen by portable card reader device 5 min later at concentrations ranging from 10^6 to 10^8 CFU/mL. As a result, the detection limit is considered to be 10^6 CFU/mL. The specificity of the strip test was determined by testing *C. jejuni* suspensions mixed with 10^6 CFU/mL of other bacteria in chicken carcass and fish shellfish chylous (Fig. 5B). The results indicated the detection limit of *C. jejuni* is the same without interference by other bacteria.

Stool assay Stools were collected from mice for the detection of *C. jejuni* by ICTS in triplicate (Fig. 6). The results showed that the *C. jejuni* could be detected in treated mice fecal. The breakthrough of our protocol is the use of fluorescent microspheres as the label. Fluorescent microspheres as a special class of functional microspheres,

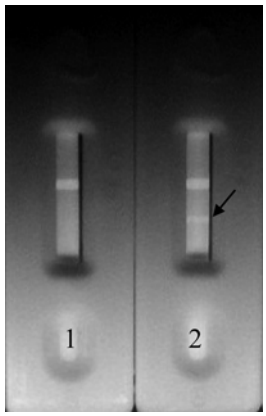

Fig. 6. Stool samples examined by ICTS. 1, ICTS with stool sample from healthy mice; 2, ICTS with stool sample from mice administered *C. jejuni* by gavage. Arrow points to the test line.

have stable morphology, narrow particle size, good dispersion, and high luminous efficiency (18). Although fluorescent microspheres have a very wide range of uses, they are rarely employed for the bacteria detection in foods. It is firstly appled into the detection of *C. jejuni* for ICT, and shows a 10-fold increase in sensitivity compared with using colloidal gold (data not shown). However, the detection limit of our ICTS did not show a 10-fold increase in sensitivity compared with colloidal-gold ICT by Kawatsu *et al*. (14) because the titer of prepared polyclonal antibodies were not high enough especially after purified by reverse absorption.

To date, there are some commercially method for detection of *C. jejuni*. There exists the TaqMan *C. jejuni* detection kit which uses real-time PCR technology. This method is very fast and excellent in specificity and sensitivity, but it requires expensive instruments and equipment. Endtz *et al.* (19) evaluated a new commercial enzyme immunoassay (ProspecT Campylobacter Microplate Assay; Alexon-Trend, Minnieapolis, MN, USA) for the detection of *C. jejuni* and *C. coli* in stool samples. The detection limit was approximately 3×10^6 CFU/mL in faecal suspensions. But the total performance time of the test was approximately 2 h. Compared to our ICTS, it takes longer time and needs specific intrusments and trained person. Kawatsu *et al.* (14) has labeled the monoclonal antibody against 15 kDa cell surface protein of *C. jejuni* with colloidal gold (14). In our study, we used polyclonal antibody instead of monoclonal antibody. The MAb were effective in the capture of the specific antigen, but large amounts of laboratory do not have the conditions to prepare MAb.

However, only 1 standard strain of *C. jejuni* was detected by ICTS as the strain resources that we owe is too limited. The food samples that we obtained is too rare to be isolated with *C. jejuni*, so we added *C. jejuni* to chicken carcass and fish shellfish chylous samples for detection.

Therefore, our ICTS could provide food industry and clinical medicine an alternative tool for a rapid, simple, and convenient detection of *C. jejuni*.

Acknowledgments This project was sponsored by 'Twelfth Five-Year Plan' for National Science and Technology Support Program (2011BAK10B06, 2011BAK10B01, 2011BAK10B02), National Natural Science Foundation (31000048, 31260363), Science and Technology Cooperation Project of Jiangxi province, China (2010EHA02200), and the Research Program of the State Key Laboratory of Food Science and Technology of Nanchang University (SKLF-TS-200916, SKLF-MB-201002).

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