Development of ic-ELISA and lateral-flow immunochromatographic assay strip for the detection of vancomycin in raw milk and animal feed

Dezhao Kong, Zhengjun Xie, Liqiang Liu, Shanshan Song, Hua Kuang and Chuanlai Xu

State Key Lab of Food Science and Technology, Jiangnan University, Wuxi, People’s Republic of China

ABSTRACT
A highly sensitive monoclonal antibody (mAb) 3H4 against vancomycin (VAN) was prepared. Indirect competitive enzyme-linked immunosorbent assay (ic-ELISA) and lateral-flow immunochromatographic assay (ICA) were developed based on the mAb. The 50% inhibition concentration (IC50) value and limit of detection (LOD) value of ic-ELISA method for vancomycin were 0.59 and 0.06 ng/mL, and for norvancomycin were 1.51 and 0.13 ng/mL under optimized conditions as pH 7.4, 0.4% (m/v) NaCl, and 5% (v/v) acetonitrile. In lateral-flow ICA, the visual limit of detection (vLOD) value and cut-off values for vancomycin were 1 and 2.5 ng/mL, and for norvancomycin were 5 and 10 ng/mL under optimized conditions as pH 8.6 with 1 mg/mL coating antigen and 1 µg/mL gold nanoparticle-labeled mAb. In raw milk and animal feed samples, recovery rates from ic-ELISA ranged from 89.2% to 121.6%. The vLOD and cut-off value were 5–10 ng/g and 100–200 µg/kg, respectively. Therefore, both methods were sensitive, rapid, and effective for the on-site detection and rapid mass screening of samples.

ARTICLE HISTORY
Received 25 November 2016
Accepted 19 December 2016

KEYWORDS
Vancomycin; ic-ELISA; lateral-flow immunochromatographic strip; raw milk; animal feed

Introduction
Vancomycin, a glycopeptide antibiotic obtained from microorganisms, has strong bactericidal activity against gram-positive bacteria but not against anaerobic or gram-negative bacteria (Arnold, Ghogawala, & Tamerler, 2016). Specifically, vancomycin inhibits bacterial cell wall synthesis (Malabarba & Ciabatti, 2001), RNA synthesis and affects bacterial cell membrane permeability. In humans, vancomycin has toxicity as ototoxic and nephrotoxic effects (Finch & Eliopoulos, 2005; Katikaneni, Lwin, Villanueva, & Yoo, 2016). However, the excessive use or misuse of antibiotics has contributed to the emergence of antibiotic-resistant bacterial strains (McAdam et al., 2012; Rho et al., 2012). Additionally, the presence of antibiotic residues in animal-derived foods poses a threat to human health. Therefore, a sensitive and rapid analytical method for detecting vancomycin in foods and animal feed is required to ensure public health.
The current vancomycin detection methods include liquid chromatography, for example, high performance liquid chromatography (HPLC) (Wicha & Kloft, 2016) and liquid chromatography–tandem mass spectrometry (MS) (Oyaert et al., 2015), spectrophotometry, chemiluminescence (Khatatae, Lotfi, & Hasanzadeh, 2015), capillary electrophoresis (Dominguez-Vega, Perez-Fernandez, Crego, Garcia, & Marina, 2014), and microchip micellar electrokinetic chromatography (Wu et al., 2015). However, these detection methods require expensive instruments, trained personnel, and complex preparation steps. Additionally, these methods involve complex antibiotic extraction procedures.

Immunoassays are effective for the detection of antibiotics (Beloglazova & Eremin, 2015; Peng et al., 2016). The enzyme-linked immunosorbent assay (ELISA) is sensitive and applicable for high-throughput sample quantitative screening (Gao et al., 2015; Odekerken et al., 2015). Compared with ELISA, the lateral-flow immunochromatographic assay (ICA) strip is more rapid and simple. As an instrument-free method, the ICA strip could be used for on-site qualitative and semi-quantitative detection (Guo et al., 2015; Zhu et al., 2014). In this study, the mAb was obtained after mouse immunization and cell fusion. Then we developed an indirect competitive ELISA (ic-ELISA) and lateral-flow ICA. Both methods were used to analyze raw milk and animal feed samples.

Materials and methods

Chemicals

Vancomycin hydrochloride, ovalbumin (OVA), bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and carbodiimide (CDI) were acquired from Sigma (St. Louis, MO, USA). Norvancomycin was obtained from Jiangsu Yutong Biotech Co. (Yancheng, Jiangsu, China). Bacitracin, polymyxin B, and polymyxin E were purchased from J&K Scientific (Shanghai, China). Goat anti-mouse immunoglobulin (IgG) antibody was supplied by Jackson ImmunoResearch Laboratories. Other reagents and chemicals were acquired from the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China).

Nitrocellulose (NC) high-flow-plus membrane (Pura-bind RP) was obtained from Whatman-Xinhua Filter Paper Co. (Hangzhou, China). Sample pad (CB-SB08), polyvinylchloride (PVC) backing card, and absorption pad (SX18) were supplied by Goldbio Tech Co. (Shanghai, China).

All buffer solutions were prepared with ultrapure water (Milli-Q purification system, Millipore Co., Bedford, MA, USA).

Antigen preparation

Vancomycin hydrochloride contains different functional groups: hydroxyl group, carboxyl group, and amino group. Therefore, different antigens were prepared as immunogens and coating antigens.

Antigen 1 (VAN-A1) was conjugated to carrier protein (BSA/OVA) by the N-hydroxysuccinimide ester method (Odekerken et al., 2015). Briefly, vancomycin was dissolved in 0.01 M phosphate buffer solution (PBS) at 20 mg/mL, and carrier protein (BSA/
OVA) was dissolved in 0.1 M sodium carbonate-bicarbonate buffer (CB, pH 9.6) at 10 mg/mL. Vancomycin (10 mg), NHS (30 mg), and EDC (45 mg) were allowed to react at room temperature for 2 h under constant stirring. Subsequently, the solution was mixed with the appropriate carrier protein solution and allowed to react at room temperature for 2 h under constant stirring. The conjugates were dialyzed against 0.01 M PBS for 3 d in the dark. Then the antigens VAN-BSA-A1 and VAN-OVA-A1 were obtained.

Antigen 2 (VAN-A2) was conjugated to a carrier protein (BSA/OVA) by the carbonyldimidazole method (Liu, Zhou, & Qinghua, 2007). Briefly, vancomycin was dissolved in dry dimethylsulfoxide at 10 mg/mL. Vancomycin (10 mg) and CDI (120 mg) were allowed to react at 37°C for 4 h. The appropriate carrier protein was added and allowed to react at room temperature for 2 h under constant stirring. The conjugates were dialyzed against 0.01 M PBS for 3 d in the dark. Then the antigens VAN-BSA-A2 and VAN-OVA-A2 were obtained.

The different antigens were analyzed by UV–Vis spectroscopy and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

**Preparation of mAb against vancomycin**

Female BALB/c mice (8–10 weeks of age) were injected with different antigens (VAN-BSA-A1 and VAN-BSA-A2), and their serum were analyzed by ic-ELISA. Mice with the highest serum antibody titer and lowest IC₅₀ were selected for cell fusion and hybridoma cell screening (Wang et al., 2009). Hybridoma cells were screened and cloned three times. The selected cell line (1 × 10⁷) was injected into the peritoneal cavity of mice for ascite production. mAbs were purified from ascites by the caprylic acid-ammonium sulfate precipitation method (Kuang et al., 2013).

Antibody subtype was identified using a subtype kit. The affinity constant (Ka) of mAb was determined by the simple antibody dilution analysis method (Lew, 1984).

**Development and optimization of ic-ELISA**

Ic-ELISA was developed as previously reported (Guan et al., 2015; Kong et al., 2015). Coating antigen was diluted by coating buffer (0.05 M, CB, pH 9.6), added into each well of 96-well plates (100 µL) and incubated at 37°C for 2 h. After three washes, each well was blocked with 200 µL blocking buffer (2% gelatin in 0.05 M CB, pH 9.6) and incubated at 37°C for 2 h. Following three washes, 50 µL anti-vancomycin mAb and 50 µL vancomycin standard solution were added into each well and incubated at 37°C for 30 min. After three washes, 100 µL HRP-labeled goat anti-mouse IgG was added and incubated at 37°C for 30 min. Following four washes, 100 µL 3,3′,5,5′-tetramethylbenzidine substrate was added and incubated at 37°C for 15 min in the dark. The reaction was terminated with the addition of 50 µL sulfuric acid (2 M) per well. The results were measured at 450 nm in a microplate reader.

In consideration of the detection sensitivity and sample extraction, the developed ic-ELISA was optimized as previously reported (Yan, Liu, Xu, Kuang, & Xu, 2015). Different factors were evaluated including pH (5.0, 6.0, 7.4, 8.6, and 9.6), ionic strength (NaCl concentration: 0.4%, 0.8%, 1.6%, and 3.2%, m/v), and acetonitrile concentration (0%, 5%, 10%, 20%, and 40%) of the standard dilution buffer.
**Cross-reactivity**

The specificity of mAb was evaluated by measuring cross-reactivity (CR). Norvancomycin, bacitracin, polymyxin B, and polymyxin E were analyzed by ic-ELISA. CR was calculated by the following equation (Liu, Yan, Zhang, Kuang, & Xu, 2015):

\[
CR(\%) = \frac{IC_{50} \text{ of vancomycin}}{IC_{50} \text{ of competitor}} \times 100\%.
\]

**Preparation of the lateral-flow ICA strip**

The ICA strip consists of four sections: the PVC backing card, the NC membrane, the sample pad, and the absorption pad. The NC membrane was attached to the middle of the PVC backing card. The sample pad was first immersed in 0.01 M PBS (containing 1% BSA and 0.2% Tween-20) to reduce matrix interference (Liu, Xing, Yan, Kuang, & Xu, 2014) and dried at 37°C. The sample pad and absorption pad were attached on each end of the PVC backing card with a 2-mm overlap on the NC membrane (Figure 1(A)). Goat anti-mouse IgG (0.5 mg/mL) was sprayed onto the NC membrane toward the absorption pad at 1 µL/cm using a membrane dispenser (Xinqidian Gene-Technology Co. Ltd., Beijing, China), resulting in the formation of the control line (C line). The coating antigen was sprayed onto the NC membrane toward the sample pad at 1 µL/cm, resulting in the formation of the test line (T line). The assembled strip card was dried at 37°C for 30 min and cut into individual test strips (Kong, Liu, Song, Kuang, & Xu, 2016).

Gold nanoparticles (GNPs) were prepared as previously reported (Feng et al., 2015). The pH value of a GNP solution (1 nM, 10 mL) was adjusted with 0.1 M K$_2$CO$_3$ and conjugated to anti-vancomycin mAb. The conjugates were allowed to react at room temperature for 50 min and mixed with 0.5% BSA (w/v). The BSA solution was used for blocking and stabilizing GNP-labeled mAbs. Following a 2-h incubation at room temperature, the

![Figure 1](image_url). Principle of the lateral-flow ICA strip detection. (A) Composition of the lateral-flow ICA strip and (B) strip detection with negative sample and positive sample.
solution was centrifuged at 7000 g for 30 min. The precipitate was washed three times with 0.02 M PBS (containing 5% sucrose, 1% BSA, and 0.5% PEG 6000), dissolved in 1 mL of 0.02 M PBS (containing 0.02% NaN₃), and stored at 4°C.

The lateral-flow ICA strip is based on an antibody–antigen reaction. GNP-labeled mAb solution (50 µL) is mixed with 150 µL of sample, allowed to react at room temperature for 5 min. By this time, the GNP-labeled mAb would have conjugated with free vancomycin contained in sample. Then the mixture added to the sample pad and migrated toward the absorbent pad. The GNP-labeled mAbs could be captured by the coating antigen and goat anti-mouse IgG antibody, and vancomycin-GNP-labeled mAb could be captured by the goat anti-mouse IgG antibody. Finally, the results can be observed with the naked eyes after 5 min (Xu et al., 2015).

The visual limit of detection (vLOD) was defined as the lowest concentration of vancomycin that results in a weaker T line color intensity compared to that of negative samples. The cut-off value was defined as the threshold concentration of vancomycin that results in the disappearance of the T line (Song et al., 2014).

Sample analysis

Raw milk and animal feed samples were used in this study. Raw milk was first centrifuged at 5000 g for 15 min at 4°C. The lipid layer was removed, and the skim milk sample was used for analysis. Animal feed (2 g) was ground, mixed vigorously with 20 mL acetonitrile-PBS (10:90, v/v) for 15 min, and subjected to ultrasonic extraction for 10 min. The resulting extract was centrifuged at 8000 g for 10 min. The supernatant was diluted with an equal volume of ultrapure water.

Results and discussion

Antigen characterization

Different antigens were characterized by UV–Vis spectroscopy and SDS-PAGE (Figures 2 and 3). The characteristic peaks of the carrier proteins (BSA and OVA) and vancomycin

![Figure 2](image)

Figure 2. The UV–Vis spectroscopy of different antigens. (A) UV–Vis spectroscopy of VAN-BSA immunogens and (B) UV–Vis spectroscopy of VAN-OVA coating antigens.
were obtained at 278 and 280 nm, respectively. At similar concentrations, the antigens had a higher and wider peak than the carrier proteins. Meanwhile, a new characteristic peak of antigens were obtained at 310 nm, which confirmed that the antigens were successfully conjugated to the carrier proteins. The SDS-PAGE images were taken by the gel image analysis system from Furi Science & Technology Co., Ltd. (Shanghai, China). And the results revealed a significant band shift between antigens and carrier proteins, thereby confirming that the antigens were successfully conjugated to the carrier proteins. The molar ratios of antigens and carrier protein were also analyzed by this system as 6.4:1 to VAN-BSA-A1, 8.5:1 to VAN-BSA-A2, 3.2:1 to VAN-OVA-A1, and 6.3:1 to VAN-OVA-A2. VAN-BSA was used as immunogen, and VAN-OVA was used as coating antigen.

**Synthesis of mAb against vancomycin**

Different types and combinations of immunogens and coating antigens were evaluated (Table 1). VAN-BSA-A1 (immunogen) and VAN-OVA-A1 (coating antigen) were selected as the optimum combination. The selected hybridoma cell line was 3H4, and

**Table 1.** The evaluation of different immunogens and coating antigens.

| Antigens     | VAN-OVA-A1 Titer | IC₅₀ (ng/mL) | VAN-OVA-A2 Titer | IC₅₀ (ng/mL) |
|--------------|------------------|--------------|------------------|--------------|
| VAN-BSA-A1   | 27,000           | 10           | 9000             | –            |
| VAN-BSA-A2   | 3000             | –            | 9000             | –            |

**Figure 3.** The SDS-PAGE image of different antigens. (A) The SDS-PAGE image of VAN-BSA immunogens, (1) BSA, (2) VAN-BSA-A1, (3) VAN-BSA-A2 and (B) The SDS-PAGE image of VAN-OVA coating antigens, (4) OVA, (5) VAN-OVA-A1, (6) VAN-OVA-A2.
the corresponding mAbs were purified. The corresponding antibody subtype of mAb 3H4 was IgG1. Based on the simple antibody dilution analysis method by ELISA, the $K_a$ value of mAb 3H4 was $3.65 \times 10^9$ L/mol.

**Development and optimization of ic-ELISA**

To optimize ic-ELISA, we used a coating antigen concentration of 0.1 µg/mL and an antibody concentration of 0.05 µg/mL. The $IC_{50}$ value and antibody titer were chosen as evaluation standard. Different pH and NaCl concentration can affect the sensitivity of ic-ELISA method (Kong, Liu, Song, Kuang, & Xu, 2017). Specifically, pH affects the number of sites available for the antigen–antibody reaction. Extreme pH even could induce changing of antibody molecule and destroy the antigen–antibody reaction. Ionic strength affects the charge of epitope and paratope groups, thereby limiting the antigen–antibody reaction and affecting antibody titer and IC$_{50}$. Figure 4(A,B) shows that the lowest IC$_{50}$ value and optimum antibody titer were obtained at pH 7.4 and 0.4% (m/v) NaCl.

Acetonitrile-PBS was used for the extraction of vancomycin from animal feed even though vancomycin is water soluble. With increasing acetonitrile, antibody titer and

![Figure 4](image.png)

Figure 4. The optimization of ic-ELISA method. (A) The optimization of ic-ELISA method with different pH values; (B) the optimization of ic-ELISA method with different ionic strength (NaCl concentration); (C) the optimization of ic-ELISA method with different acetonitrile concentration; and (D) the standard curve established under the optimum conditions.
IC$_{50}$ were found to increased, thereby reducing the detection sensitivity of ic-ELISA (Figure 4(C)). Based on the optimization experiments, 5% acetonitrile (v/v) was selected. The standard curve for ic-ELISA was established under the optimum conditions, that is, pH 7.4, 0.4% (m/v) NaCl, and 5% (v/v) acetonitrile (Figure 4(D)). The equation of the standard curve was $y = 0.0115 + 1.81/[1 + (x/0.594)^{0.876}]$, the linear regression correlation coefficient ($R^2$) was 0.995, the IC$_{50}$ value was 0.59 ng/mL, and the limit of detection (LOD, IC$_{10}$) was 0.06 ng/mL.

**mAb specificity**

Norvancomycin, bacitracin, polymyxin B, and polymyxin E were analyzed by ic-ELISA (Table 2). MAb 3H4 recognized norvancomycin with a CR value of 40% (Table 2). The IC$_{50}$ value and LOD of norvancomycin was 1.51 and 0.13 ng/mL, respectively.

As norvancomycin was a kind of antibiotics which is similar to vancomycin on the structure, application, and side-effect, a simultaneous detection of these two antibiotics would simplify detected procedure and improved the food safety detection. However, even though these two antibiotics were seldom used together, the cross-reaction would also effected the quantitative determination of vancomycin and norvancomycin, respectively. New specific mAb could overcome this drawback by the new immunogen with special modification. And the new specific mAbs for these two antibiotics had been under preparation in our lab.

**Optimization and characterization of the lateral-flow ICA strip**

The developed lateral-flow ICA strip was optimized by testing different coating antigen concentrations (0.5 and 1 mg/mL), pH value of GNP-labeled mAb (7.4, 8.6, and 9.6), and concentrations of GNP-labeled mAb (0.8 and 1 µg/mL). The results shown in Figure 5 revealed that at 1 mg/mL coating antigen and pH 8.6, deep color intensities were obtained on the C and T lines. The optimized lateral-flow ICA strip was tested with both negative (0 ng/mL) and positive samples (5 ng/mL) using different concentrations of GNP-labeled mAb (Figure 5(D)). In the negative sample, 1 µg/mL GNP-labeled mAb contributed to a deep color intensity on the T line. In the positive sample, a similar detection sensitivity was obtained with 0.8 µg/mL GNP-labeled mAb. Therefore, the lateral-flow ICA strip was developed at pH 8.6 with 1 mg/mL coating antigen and 1 µg/mL GNP-labeled mAb.

The sensitivity of the lateral-flow ICA strip was confirmed with a series of vancomycin standards (0, 0.25, 0.5, 1, 2.5, and 5 ng/mL) and norvancomycin standards (0, 0.5, 1, 2.5, 5, and 10 ng/mL). The vLOD value of VAN and norvancomycin were 1 and 5 ng/mL,

| Table 2. The CR value of mAb against vancomycin by the ic-ELISA method. |
|----------------|----------------|------|
| Analytes      | IC$_{50}$ (ng/mL) | CR (%) |
| Vancomycin    | 0.59            | 100   |
| Norvancomycin | 1.51            | 40    |
| Bacitracin    | >1000           | <0.1  |
| Polymyxin B   | >1000           | <0.1  |
| Polymyxin E   | >1000           | <0.1  |
respectively. The cut-off values were 2.5 ng/mL for vancomycin and 10 ng/mL for norvancomycin (Figure 6).

Sample analysis

For ic-ELISA and lateral-flow ICA strip, raw milk and animal feed samples were spiked with different concentrations of vancomycin standards. Both ic-ELISA and lateral-flow ICA were performed six times.

Figure 5. The optimization of the lateral-flow ICA strip. (A) GNP-labeled mAb with pH of 7.4: (1) coating antigen at 1 mg/mL; (2) coating antigen at 0.5 mg/mL; (B) GNP-labeled mAb with pH of 8.6: (1) coating antigen at 1 mg/mL; (2) coating antigen at 0.5 mg/mL; (C) GNP-labeled mAb with pH of 9.6: (1) coating antigen at 1 mg/mL; (2) coating antigen at 0.5 mg/mL; (D) (1) GNP-labeled mAb concentration of 0.8 µg/mL; (2) GNP-labeled mAb concentration of 1 µg/mL; N, Vancomycin-negative sample (0 ng/mL); P, Vancomycin-positive sample (5 ng/mL).

Figure 6. The sensitive analysis of lateral-flow ICA strip. (A) The sensitive analysis with vancomycin: (1) 0 ng/mL; (2) 0.25 ng/mL; (3) 0.5 ng/mL; (4) 1 ng/mL; (5) 2.5 ng/mL; and (6) 5 ng/mL; (B) The sensitive analysis with norvancomycin: (1) 0 ng/mL; (2) 0.5 ng/mL; (3) 1 ng/mL; (4) 2.5 ng/mL; (5) 5 ng/mL; and (6) 10 ng/mL.
The ic-ELISA results are presented in Table 3. For intra-assay reproducibility, recoveries were from 89.5% to 121.6% with milk sample and 89.2% to 112.3% with animal feed sample. For inter-assay reproducibility, recoveries were from 94.0% to 99.0% with milk sample and 97.4% to 99.7% with animal feed sample. These results revealed that the developed ic-ELISA was sensitive for vancomycin detection in real samples. In the lateral-flow ICA strip, the vLOD value and cut-off value for raw milk were 5 and 10 ng/g, and for animal feed samples were 100 and 200 μg/kg (Figure 7). Therefore, the lateral-flow ICA strip was accurate and suitable for the analysis of real samples.

A comparison was taken between the ic-ELISA, lateral-flow ICA strip, and HPLC-MS/MS with three level of spiked animal feed samples. The result was shown in Table 4. The lateral-flow ICA strip could only obtained semi-quantitative analysis of samples without exacted result. For ic-ELISA and HPLC-MS/MS method, the results did not show a significant difference between them, which indicated a good correspondence between these two methods.

Although immunoassays were general and simply methods for detection of various compounds, the more sensitive determination method for different targets were also important. The last ic-ELISA method for vancomycin had been developed with a detection range of 20–5000 ng/mL in 2015 (Odekerken et al., 2015). Our developed method showed

Table 3. The spiked sample analysis with ic-ELISA method (n = 6).

| Spiked (μg/kg) | Intra-assay | Inter-assay |
|---------------|-------------|-------------|
|               | Recovery (%) | CV (%)      | Recovery (%) | CV (%)  |
| Raw milk sample | 0.5 | 121.60 ± 12.0 | 9.91 | 94.20 ± 8.0 | 8.49 |
|               | 1  | 119.00 ± 8.0  | 6.72 | 99.00 ± 10.0 | 10.10 |
|               | 2  | 89.50 ± 6.0   | 6.70 | 94.00 ± 5.0  | 5.32 |
| Animal feed sample | 10 | 89.20 ± 5.4  | 6.05 | 98.80 ± 6.2  | 6.28 |
|               | 20 | 112.30 ± 5.3  | 4.68 | 99.70 ± 5.1  | 5.12 |
|               | 40 | 105.40 ± 3.2  | 3.04 | 97.40 ± 6.3  | 6.49 |

Figure 7. The sample analysis by lateral-flow ICA strip (n = 6). (A) The raw milk sample: (1) 0 ng/mL; (2) 1 ng/mL; (3) 2.5 ng/mL; (4) 5 ng/mL; (5) 10 ng/mL; and (6) 25 ng/mL; (B) The animal feed sample: (1) 0 μg/kg; (2) 20 μg/kg; (3) 50 μg/kg; (4) 100 μg/kg; (5) 200 μg/kg; and (6) 500 μg/kg.
a great improvement with the detection sensitivity. The obtained sensitive mAb could also be used for the development of other immunoassay as using capillary electrophoresis with laser-induced fluorescence with a minimum detectable concentration of 0.98 ng/mL (Lam & Le, 2002). However, for on-site detection and large sample screening, the common ic-ELISA and ICA strips were more suitable with the close detection sensitivity.

Conclusion

A sensitive anti-vancomycin mAb 3H4 was obtained following mouse immunization and cell fusion. Ic-ELISA and lateral-flow ICA were developed for vancomycin detection in raw milk and animal feed samples. The recovery rates of ic-ELISA ranged between 89.2% and 121.6%. In the lateral-flow ICA strip, the vLOD value and cut-off value for raw milk were 5 and 10 ng/g, and for animal feed samples were 100 and 200 μg/kg. Both detection methods were suitable for vancomycin detection in different samples. Ic-ELISA was sensitive and adequate for high-throughput screening. The lateral-flow ICA strip represents a more rapid and simple diagnostic tool for on-site detection. The developed analytical methods would be applicable for vancomycin monitoring.

Funding

This work is financially supported by the National Natural Science Foundation of China (21522102, 21301073s), National Key R&D Program (2016YFD0401101), and grants from Natural Science Foundation of Jiangsu Province, MOF and MOE (BE2016307, BK20150145, BX20151038, BK20140003, BE2014672, BE2013613, BE2013611).

Notes on contributors

Dezhao Kong got his bachelor’s from Nanjing University of Finance & Economics, Nanjing, China in 2012 and then he began to study in Jiangnan University (Wuxi, China) for a Ph.D. student in food science. His research interests are immunoassay applications in food.

Zhengjun Xie is a full professor of Food science and technology of Jiangnan University. He got his Ph.D. in food science in 2009. His research interests are fast detection technology and food safety evaluation.

Liqiang Liu got his Ph.D. in Food science in 2014 from Jiangnan University, Wuxi, China and then became a faculty in college of Food Science and Technology of Jiangnan University. His research interests are immunochromatographic strip design and application.

Table 4. The comparison between ic-ELISA, lateral-flow ICA strip and HPLC-MS/MS (n = 6).

| Spiked (μg/kg) | ic-ELISA (μg/kg) | Lateral-flow ICA | HPLC-MS/MS (μg/kg) |
|---------------|------------------|------------------|-------------------|
| 50            | 47.76 ± 5.24     | _b               | 49.48 ± 5.62      |
| 100           | 93.33 ± 9.51     | _ ≤c              | 96.63 ± 13.49     |
| 200           | 190.71 ± 11.67   | _ ≥d              | 197.30 ± 16.36    |

^aMean value±standard deviation (n = 6).
^bNegative result. The test line is obviously observed.
^cWeakly positive result. Light test line is observed.
^dPositive result. No test line is observed.
**Shanshan Song** got her master’s degree in Food science in 2012 from Jiangnan University, Wuxi, China and then became a research assistant in college of Food science and technology of Jiangnan University. Her research interests are monoclonal antibody development.

**Hua Kuang** got her Ph.D. from China Agricultural University in 2009 and then began to work as a faculty in college of Food Science and Technology of Jiangnan University. She is currently a full professor in food safety. Her research interests are biosensor development.

**Chuanlai Xu** is a full professor of Food science and technology of Jiangnan University. He got his Ph.D. in food science in 2002. His research interests are fast detection technology and food safety evaluation.

**References**

Arnold, P. M., Ghogawala, Z., & Tamerler, C. (2016). Vancomycin, bone growth, and wound healing. *Journal of Neurosurgery: Spine*, 25, 145–146. doi:10.3171/2015.12.spine151351

Beloglazova, N. V., & Eremin, S. A. (2015). Design of a sensitive fluorescent polarization immunoassay for rapid screening of milk for cephalaxin. *Analytical and Bioanalytical Chemistry*, 407, 8525–8532. doi:10.1007/s00216-015-9006-6

Dominguez-Vega, E., Perez-Fernandez, V., Grego, A. L., Garcia, M. A., & Marina, M. L. (2014). Recent advances in CE analysis of antibiotics and its use as chiral selectors. *Electrophoresis*, 35, 28–49. doi:10.1002/elps.201300347

Feng, M., Kong, D. Z., Wang, W. B., Liu, L. Q., Song, S. S., & Xu, C. L. (2015). Development of an immunochromatographic strip for rapid detection of *Pantoea stewartii* subsp. *Stewartii*. *Sensors*, 15, 4291–4301. doi:10.3390/s150204291

Finch, R. G., & Eliopoulos, G. M. (2005). Safety and efficacy of glycopeptide antibiotics. *Journal of Antimicrobial Chemotherapy*, 55, ii5–ii13. doi:10.1093/jac/dki004

Gao, W., Jiang, L., Ge, L., Chen, M., Geng, C., Yang, G., … Liu, X. (2015). Sterigmatocystin-induced oxidative DNA damage in human liver-derived cell line through lysosomal damage. *Toxicology in Vitro*, 29, 1–7. doi:10.1016/j.tiv.2014.08.007

Guan, D. D., Guo, L. L., Liu, L. Q., Kong, N., Kuang, H., & Xu, C. L. (2015). Development of an ELISA for nitrazepam based on a monoclonal antibody. *Food and Agricultural Immunology*, 26, 611–621. doi:10.1080/09540105.2014.998637

Guo, J. N., Liu, L. Q., Xue, F., Xing, C. R., Song, S. S., Kuang, H., & Xu, C. L. (2015). Development of a monoclonal antibody-based immunochromatographic strip for cephalaxin. *Food and Agricultural Immunology*, 26, 282–292. doi:10.1080/09540105.2014.907242

Katikaneni, M., Lwin, L., Villanueva, H., & Yoo, J. (2016). Acute kidney injury associated with vancomycin when laxity leads to injury and findings on kidney biopsy. *American Journal of Therapeutics*, 23, E1064–E1067.

Khatteea, A., Lotfi, R., & Hasanzadeh, A. (2015). A novel permanganate-morin-CdS quantum dots flow injection chemiluminescence system for sensitive determination of vancomycin. *RSC Advances*, 5, 82645–82653. doi:10.1039/c5ra14708e

Kong, N., Guo, L. L., Guan, D. D., Liu, L. Q., Kuang, H., & Xu, C. L. (2015). An ultrasensitive ELISA for medroxyprogesterone residues in fish tissues based on a structure-specific hapten. *Food Analytical Methods*, 8, 1382–1389. doi:10.1007/s12161-014-0023-4

Kong, D., Liu, L., Song, S., Kuang, H., & Xu, C. (2016). Development of an immunochromatographic strip for the semi-quantitative and quantitative detection of biotin in milk and milk products. *Analytical Methods*, 8, 1595–1601. doi:10.1039/c5ay02659h

Kong, D., Liu, L., Song, S., Kuang, H., & Xu, C. (2017). Development of sensitive, rapid, and effective immunoassays for the detection of Vitamin B12 in fortified food and nutritional supplements. *Food Analytical Methods*, 10(1), 10–18.

Kuang, H., Xing, C. R., Hao, C. L., Liu, L. Q., Wang, L. B., & Xu, C. L. (2013). Rapid and highly sensitive detection of lead ions in drinking water based on a strip immunosensor. *Sensors*, 13, 4214–4224. doi:10.3390/s130404214
Lam, M. T., & Le, X. C. (2002). Competitive immunoassay for vancomycin using capillary electrophoresis with laser-induced fluorescence detection. *The Analyst, 127*, 1633–1637. doi:10.1039/b206531b

Liew, A. M. (1984). The effect of epitope density and antibody affinity on the ELISA as analysed by monoclonal antibodies. *Journal of Immunological Methods, 72*, 171–176. doi:10.1016/0022-1759(84)90445-9

Liu, L. Q., Xing, C. R., Yan, H. J., Kuang, H., & Xu, C. L. (2014). Development of an ELISA and immunochromatographic strip for highly sensitive detection of microcystin-LR. *Sensors, 14*, 14672–14685. doi:10.3390/s140814672

Liu, L. Q., Yan, H. J., Zhang, X., Kuang, H., & Xu, C. L. (2015). Development of an anti-chlorothalonil monoclonal antibody based on a novel designed hapten. *Food and Agricultural Immunology, 26*, 410–419. doi:10.1080/09540105.2014.938319

Liu, R., Zhou, Y. U., & Qinghua, H. E. (2007). Preparation and identification of a monoclonal antibody against citrinin. *Wei sheng yan jiu [Journal of Hygiene Research], 36*, 190–193.

Malabarba, A., & Ciabatti, R. (2001). Glycopeptide derivatives. *Current Medicinal Chemistry, 8*, 1759–1773.

McAdam, A. J., Hooper, D. C., Demaria, A., Limbago, B. M., O’Brien, T. F., & Mccaughey, B. (2012). Antibiotic resistance: How serious is the problem, and what can be done? *Clinical Chemistry, 58*, 1182–1186.

Odekerken, J. C. E., Logister, D. M. W., Assabre, L., Arts, J. J. C., Walenkamp, G., & Welting, T. J. M. (2015). ELISA-based detection of gentamicin and vancomycin in protein-containing samples. *SpringerPlus, 4*, 8. doi:10.1186/s40064-015-1411-y

Oyaert, M., Peersman, N., Kieffer, D., Deiteren, K., Smits, A., Allegaert, K., … Pauwels, S. (2015). Novel LC-MS/MS method for plasma vancomycin: Comparison with immunoassays and clinical impact. *Clinica Chimica Acta, 441*, 63–70. doi:10.1016/j.cca.2014.12.012

Peng, J., Wang, Y. W., Liu, L. Q., Kuang, H., Li, A. K., & Xu, C. L. (2016). Multiplex lateral flow immunoassay for five antibiotics detection based on gold nanoparticle aggregations. *RSC Advances, 6*, 7798–7805. doi:10.1039/c5ra22583c

Rho, H., Shin, B., Lee, O., Choi, Y.-H., Rho, J., & Lee, J. (2012). Antibiotic resistance profile of bacterial isolates from animal farming aquatic environments and meats in a peri-urban community in South Korea. *Journal of Environmental Monitoring, 14*, 1616–1621.

Song, S. Q., Liu, N., Zhao, Z., Njumbe Ediage, E., Wu, S., Sun, C., … Wu A. (2014). Multiplex lateral flow immunoassay for mycotoxin determination. *Analytical Chemistry, 86*, 4995–5001. doi:10.1021/ac500540z

Wang, C. M., Liu, Y. H., Guo, Y. R., Liang, C. Z., Li, X. B., & Zhu, G. N. (2009). Development of a McAb-based immunoassay for parathion and influence of the competitor structure. *Food Chemistry, 115*, 365–370. doi:10.1016/j.foodchem.2008.11.091

Wicha, S. G., & Kloft, C. (2016). Simultaneous determination and stability studies of linezolid, meropenem and vancomycin in bacterial growth medium by high-performance liquid chromatography. *Journal of Chromatography B, 1028*, 242–248. doi:10.1016/j.jchromb.2016.06.033

Wu, M. L., Gao, F., Zhang, Y., Wang, G., Wang, Q. J., & Li, H. (2015). Sensitive analysis of antibiotics via hyphenation of field-amplified sample stacking with reversed-field stacking in microchip micellar electrokinetic chromatography. *Journal of Pharmaceutical and Biomedical Analysis, 103*, 91–98. doi:10.1016/j.jpba.2014.11.004

Xu, N., Xu, L., Ma, W., Liu, L., Kuang, H., & Xu, C. (2015). An ultrasensitive immunochromatographic assay for non-pretreatment monitoring of chloramphenicol in raw milk. *Food and Agricultural Immunology, 26*, 635–644. doi:10.1080/09540105.2014.998640

Yan, H. J., Liu, L. Q., Xu, N. F., Kuang, H., & Xu, C. L. (2015). Development of an immunoassay for carbendazim based on a class-selective monoclonal antibody. *Food and Agricultural Immunology, 26*, 659–670. doi:10.1080/09540105.2015.1007446

Zhu, A. R., Huo, R. L., Zhou, W., Zhang, L., Gao, F., & Zhou, G. H. (2014). Establishment of colloidal gold immunochromatography strip for detection of florfenicol residues. *Current Pharmaceutical Analysis, 10*, 263–270.