1 **Detection of kanamycin and gentamicin residues in animal-derived food using**
2 **IgY antibody based ic-ELISA and FPIA**

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Abstract:

Our aim in this study is to show that IgY antibody based immunoassays could be used to detect antibiotic residues in animal-derived food. Briefly, full antigens of gentamicin (Gent) and kanamycin (Kana) were used to immunize the laying chickens to prepare IgY antibodies. Then, these antibodies were evaluated by FPIA and ic-ELISA to detect Gent/Kana in animal-derived samples. The IC_{50} of FPIA and ic-ELISA based anti-Gent IgY were $7.70 \pm 0.6 \mu\text{g/mL}$ and $0.32 \pm 0.06 \mu\text{g/mL}$, respectively. The IC_{50} of FPIA and ic-ELISA based anti-Kana IgY were $7.97 \pm 0.9 \mu\text{g/mL}$ and $0.15 \pm 0.01 \mu\text{g/mL}$. The limits of detection (LOD, IC_{10}) for FPIA based anti-Gent/Kana IgY were 0.17 and 0.007 $\mu\text{g/mL}$, respectively. The LOD for ic-ELISA were both 0.001 $\mu\text{g/mL}$. These results indicated that the ic-ELISA might more suitable for antibiotic residues detection than FPIA.

Keywords: Immunoglobulin Y (IgY); fluorescence polarization immunoassay (FPIA); indirect-competitive ELISA (ic-ELISA); gentamicin; kanamycin

1. Introduction

Chicken IgY antibodies are superior alternatives to mammalian antibodies for several reasons (Schade, Behn, Erhard, Hlinak, & Staak, 2014; Schade, Calzado, Sarmiento, Chacana, Porankiewicz-Asplund, & Terzolo, 2005; Tan, Mohamedali, Kapur, Lukjanenko, & Baker, 2012). The method of isolation of IgY is non-invasive, as it is isolated from eggs rather than blood, which meets the requirements of animal welfare. The yield of IgY is high (1~2.8 g chicken IgY/month) compared to mammalian antibody (0.2 g rabbit IgG/month) (Schade, Calzado, Sarmiento, Chacana, Porankiewicz-Asplund, & Terzolo, 2005). Further, compared to IgG, IgY is more stable at high temperatures, different pH conditions low cross-reaction with other receptors (Shimizu, Nagashima, Sano, Hashimoto, Ozeki, Tsuda, et al., 1992). Moreover, it does not activate the complement system nor does it interact with rheumatoid factor (Schade, Behn, Erhard, Hlinak, & Staak, 2014; Schade, Calzado, Sarmiento, Chacana, Porankiewicz-Asplund, & Terzolo, 2005), thus, the results of IgY based detection of antibiotic residues would be more accurate than that of IgG based detection.

IgY antibody has been widely used in the detection of virus (Parma, Chacana, Lucchesi, Rogé, Velandia, Krüger, et al., 2014), cancer (Grzywa, Łupicka-Słowik, Walczak, Idzi, Bobrek, Boivin, et al., 2014; Xiao, Gao, Taratula, Treado, Urbas, Holbrook, et al., 2009), parasite (Liu, Jing, Cui, Fu, & Wang, 2013; Nie, Wang, Lu, Liu, Li, & Lei, 2014) and chemical contaminants (H. Zhang, Mi, Khan, Sheng, Eremin, Beier, et al., 2015). It has therapeutic application as well. IgY is usually used to treat rotavirus (Vega, Bok, Saif, Fernandez, & Parreño, 2015), allergic asthma (Wei-xu, Qin, Zhu, Yuan-yi, Li-feng, Zhi-yong, et al., 2014), alimentary tract diseases (Rahman, Van Nguyen, Icatlo Jr, Umeda, & Kodama, 2013), etc. Though chicken IgY antibodies have been widely used in vitro immunological detection and diagnosis, very few reports are available on the applications for antibiotic residue detection (He, Hu, Thirumalai, Schade, Du, & Zhang, 2016; He, Wang, & Zhang, 2016; H. Zhang, et al., 2015).

Antibiotic residues in animal-derived food and in the environment represent a major

concern. Prolonged exposure to antibiotics is a health hazard, as it might lead to the development of bacterial resistance against different antibiotics, especially the aminoglycoside antibiotics (Farouk, Azzazy, & Niessen, 2015). Aminoglycosides are the most widely used antibiotics because of their low cost and excellent activity against common bacterial diseases. However, widespread negligence of proper withdrawal time has led to residual aminoglycoside antibiotics in foodstuffs and in the environment. The hazards of antibiotic residue in foodstuffs of animal origin on humans include nephrotoxicity, neurotoxicity and ototoxicity, which have been a serious concern and intensive topic in recent years (Darwish, Eldaly, El-Abbasy, Ikenaka, Nakayama, & Ishizuka, 2013; Martone, Giordano, Dagna, Carulli, Albera, & Rossi, 2014; Musiime, Seale, Moxon, & Lawn, 2015). The European Union (EU) has issued strict maximum residue levels (MRLs) for aminoglycoside antibiotics in various animal-origin foods, such as Kana: 100 µg/kg in pork and 150 µg/kg in milk, Gent: 50 µg/kg in pork and 100 µg/kg in milk. Hence, various detection methods such as liquid chromatography–electrospray-tandem mass spectrometry (Arsand, Jank, Martins, Hoff, Barreto, Pizzolato, et al., 2016), visual detection with gold nanoparticles (Radhakumary & Sreenivasan, 2014), HPLC with Direct UV Detection (Blanchaert, Huang, Wach, Adams, & Schepdael, 2016), Chemiluminescent Enzyme Immunoassay (Peng, ZHANG, Jian, WANG, CHEN, ZHAO, et al., 2016), aptamer-based signal-on bio-assay (J. Chen, Li, Ge, Yang, Zhang, Qu, et al., 2015) and electrochemical immunosensor (Schoukrounbarne, Wagan, & White, 2014; Zhao, Wei, Xu, Li, Wu, Cai, et al., 2011) have been used to ensure the food safety. However, these detection technologies are not applicable in poor economies and developing countries, because these techniques are too expensive and require highly trained human resources.

In the present communication, we have described two immunoassays (fluorescence polarization immunoassay (FPIA) and ELISA) using chicken IgY as capture antibody to detect antibiotics in animal-derived foodstuffs. This study also demonstrates that IgY antibody could be a promising antibody for antibiotic residues detection.

2. Materials and methods

2.1. Materials and solution preparation

Bovine serum albumin (BSA, Vetec, V900933, purity: $\geq 98\%$), Ovalbumin from chicken egg white (OVA, Sigma, A5503, $\geq 98\%$), kanamycin sulfate (Kana, Sigma-Aldrich, E004000), gentamicin sulfate (Gent, Sigma-Aldrich, E003632), 25% glutaraldehyde solution (GDA, Sigma-Aldrich, G6257, Grade II), Freund's complete adjuvant (FCA, Sigma, F5881), Freund's incomplete adjuvant (FIA, Sigma, F5506), Fluorescein isothiocyanate isomer I (FITC, Sigma, F7250), triethylamine (TEA, Sigma-Aldrich, T0886, $\geq 99\%$), tetramethylbenzidine (TMB, Aldrich, 860336, $\geq 99\%$) and Rabbit anti-chicken IgG antibody conjugate to Horseradish peroxidase (Sigma, A9046) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ampicillin sodium (A8180) was purchased from Solarbio (Solarbio, Beijing, China). Anti-Gent chicken single chain variable fragment (scFv) was prepared by our lab (Li, He, Ren, Zhang, Du, & Li, 2016).

Borate buffer (BB, 19.07 g $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ in 1 L distilled water, pH=9, containing 1% sodium azide (w/v) as preservative was used as working buffer. Phosphate-buffered saline (PBS: 0.2 g of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 2.9 g of $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 8.0 g of NaCl in 1 L distilled water, pH 7.4) was stored at 4 °C. PBST (10 mM, pH 7.4, 0.05 % [v/v] Tween 20 in PBS) was stored at 4 °C. Carbonate buffer solution (CBS: 0.75 g Na_2CO_3 , 1.466 g NaHCO_3 in 0.5 L of distilled water, pH = 9.6) was stored at 4 °C. Blocking solution was 5% (m/v) skim milk powder in PBS (PBSM).

2.2. Experimental animals

Four 20-week-old White Leghorn Hens were procured from a local poultry farm. All animal and experimental protocol was reviewed and approved by the Ethics Committee of Northwest A&F University for the use of Laboratory Animals.

2.3. Synthesis of Gent-FITC and Kana-FITC

Gent-FITC and Kana-FITC were synthesized as previous reported (Beloglazova, Shmelin, & Eremin, 2016). Firstly, the 10 μmol Gent/Kana and the equal FITC were dissolved in 1 mL methanol containing 50 μL TEA. Then the mixture was incubated overnight with stirring at 4 °C. The conjugate reaction mixture was identified and purified by using thin-layer chromatography (TLC). The main yellow bands for Gent-FITC at $R_f = 0.1$ were collected and stored at 4 °C.

2.4. Preparation of immunogen and coating antigen

Micromolecules have no immunogenicity due to their low molecular weight. In order to increase the immunogenicity, Gent and Kana was conjugated with BSA or OVA according to the procedure described by Yiqiang Chen (Y. Chen, Wang, Wang, Tang, Zhu, & Xiao, 2008) and Loomans, Elma EMG (Loomans, van Wiltenburg, Koets, & van Amerongen, 2003). In brief, Gent and Kana (each 10 mg) and BSA or OVA (each 10 mg) were dissolved in 1 mL of PBS (pH 8~9). Then 50 μ L of GDA solution was added drop by drop. Then, the reaction mixture was gently stirred for 30 min, sodium borohydride was added to a final concentration of 10 mg/mL, and the solution was incubated for 1 h at 4 °C. Subsequently, the reaction product was dialysed (72 h at 4 °C) in PBS. Finally, the conjugates (Gent-BSA, Gent-OVA, Kana-BSA, and Kana-OVA) were identified by UV spectroscopy and SDS-PAGE.

2.5. Immunization of chicken

Hens were immunized with Gent-BSA or Kana-BSA. The first dose was consisted of 280 μ g of immunogen for injection intramuscularly in four different sites of pectoral muscles as an emulsion of PBS and FCA. Four subsequent booster immunizations were given at two-week intervals with the same dosage of immunogen emulsified in FIA. All eggs were collected from the first immunization and were stored at 4 °C for future use.

2.6. Extraction of IgY Antibody

IgY antibody was extracted from egg yolk by polyethylene glycol (PEG) precipitation (Pauly, Chacana, Calzado, Brembs, & Schade, 2011). Briefly, twice the egg yolk volume of PBS was mixed with the yolk, 3.5% (w/v) PEG 6000 of the total volume was added and vortexed to remove lipids and lipoprotein, followed by 30 min rolling on a rolling mixer. Then, the mixtures were centrifuged at 4 °C for 20 min (10,000 rpm, HC-3018R Anhui USTC Zonkia Scientific instruments, China)). The supernatant was poured through a folded filter and transferred to a new tube and 12 % (w/v) PEG 6000 were added to the tube, vortexed and rolled on a rolling mixer. The mixture was centrifuged at 4 °C for 20 min and the pellet was carefully dissolved in 10 mL PBS. Subsequently, 12 % PEG 6000 was added into the tubes for further precipitated. After final, centrifugation, the pellet was dissolved in 1.2 mL PBS and the mixtures were

dialysed overnight in PBS at 4 °C. The next morning, IgY-extract was taken out from the dialysis capsule by a pipette and transferred to 2 mL tubes and stored at –20 °C for future use. SDS-PAGE was used to evaluate the purity of IgY extract.

2.7. Determination of specific IgY titre by indirect ELISA

In order to monitor IgY antibody titre, an indirect ELISA had been performed as previous described (He, Wang, & Zhang, 2015). Firstly, the concentrations of antibodies and coating antigens were optimized by checkerboard titration. Then, Gent-OVA and Kana-OVA was respectively dissolved in 5 mL CBS (the final concentration is 247.5 ng/mL and 250 ng/mL) and 100 µL per well was added in 96-microplates (NUNC; Roskilde, Denmark) and kept at 4 °C overnight. After washing two times with PBST and one time with PBS, unbound sites were blocked by 110 µL PBSM for 2 h at 37 °C. Plate was again washed as above. After three washing cycles, the rabbit anti-chicken antibody IgG labeled with Horseradish peroxidase was added at the dilution of 1:5000 in PBS and incubated for 1 h at 37 °C. After washing three times with PBST and two times with PBS, the color was developed using TMB for 10 min and then two moles H₂SO₄ was added to the ELISA plate to terminate the reaction. Finally, the absorbance was read at 450 nm in a microplate reader.

2.8. Fluorescence polarization immunoassay (FPIA)

The optimization of the analysis procedure, we first determined the optimal immunoreagents concentrations. The working concentration of tracer was defined as the solution that possesses total final fluorescence intensity 5~10 times higher than the background signal (the blank control contained only 50 mM BB. The working concentration of the antibody was identified from the graphical relation between their dilutions and the degree of fluorescence polarization (FP). The dilution curve was built in semi logarithmic scale, and the optimal antibody dilutions corresponded to 70% of the tracer's binding response to the antibodies.

The competitive FPIA was performed with Gent/Kana standard solutions by using Gent-FITC or Kana-FITC tracers and IgY at the optimized concentrations. Briefly, 50 µL of Gent/Kana standard solutions or samples and 500 µL of working tracer solution were mixed. Then, 500 µL of working antibody solution was added to each tube. After

1-5 min incubation at room temperature, the fluorescence polarization was measured and expressed in “milli-polarization” units (mP). Standard FPIA curves were plotted in a semi logarithmic scale with relative FP values (mP/mP₀) on the y-axis, and the logarithm of the analyte concentration in the x-axis. The sensitivity of FPIA was reflected by half-maximum inhibition concentration (IC₅₀) and the detection ranges were shown between IC₂₀ and IC₈₀. Cross-reactivity (CR) was carried out to evaluate the specificity of antibodies with a group of medicine, including gentamicin, kanamycin and ampicillin (Amp). The equation was $CR\% = IC_{50} (Gent/Kana) / IC_{50} (Analytes) \times 100\%$.

2.9. Indirect competitive ELISA (ic-ELISA)

Methodology of indirect competitive ELISA was similar as described in the previous section except the addition of 50 µL of Gent/Kana standard solutions and 50 µL of diluted IgY (at different dilutions) to each well successively. Gent/Kana standard solution was prepared by diluting with PBS at different concentrations (10000, 1000, 100, 10, 1, 0.1, 0.01, 0.001 and 0 µg/mL). The sigmoid curve that was set as an inhibition curve was fit to a logistic equation in OriginPro8.0 software package with relative OD values (B/B₀) on the y-axis, and the logarithm of the analyte concentration in the x-axis. Sensitivity of immunoassay was evaluated by IC₅₀ value. The evaluation of specificity of antibodies was similar as FPIA.

2.10. Sample preparation for FPIA and ic-ELISA

Milk, chicken and pork samples were purchased from the local market. Samples were prepared as previously reported (S. Zhang, Wang, Irinas, Sergeia, & Shen, 2007). In brief, aliquots of chicken and pork sample (1 g) were homogenized in 1mL PBS, respectively. Then 0, 100 and 200 µL Gent/Kana solutions (1 mg/mL) were added in the homogenates to give tissue levels of 0, 100 and 200 µg/g of Gent/Kana. The spiked chicken and pork samples were extracted with 3 mL of acetonitrile/water (84:16, v/v) for 30 min at room temperature with shaking. The samples were centrifuged at 12000 rpm for 10 min and dried under a steady flow of dry nitrogen gas. The residues were re-dissolved in 1 mL BB buffer and the resulting solutions were used for subsequent analysis. In the case of milk sample, aliquots of 1 mL of

saturated ammonium sulfate were added to aliquots of 500 μ L of milk and centrifuged at 12000 rpm for 10 min. The top fat layer was removed. The rests with 0, 100 and 200 μ g Gent/Kana were mixed respectively by stirring for 10 min and then centrifuged for 6 min at 12000 rpm. The supernatant was taken and subjected to the subsequent analysis. All samples have been test triple times and the relative standard deviation (RSD) was obtained after calculated.

3. Results and discussion

3.1. Preparation of Immunogen and Coating Antigen for Gent/Kana

Haptens are low molecular weight chemicals that can elicit an immune response only when attached to a large carrier such as proteins (e.g., KLH, BSA, OVA). In this study, Gent and Kana were conjugated to BSA and OVA respectively by glutaraldehyde method. UV spectroscopy and SDS-PAGE analyses were carried out to predict the success of coupling (Fig. 1). As is shown in Fig. 1A and 1B, the maximum absorption peaks of OVA and BSA were at 287 and 289 nm; the Gent-OVA and Gent-BSA conjugates had the maximum absorption peaks at 285 and 287 nm, which were different from the maximum absorption peak of Gent at 256 nm. The SDS-PAGE was performed to confirm the conjugation of Gent-OVA and Gent-BSA with the different ratio of mobility indicated that successful conjugation between Gent and carrier protein occurred (Fig. 1C). The results of Kana conjugates were similar to Gent in Fig. 1 and therefore not shown.

Here is Fig.1

3.2. Preparation of anti-Gent/Kana IgY antibody and monitor the titer change

Chicken IgY can be extracted by various methods as previously reported such as the water dilution (WD) method, PEG precipitation method, caprylic acid (CA) extraction, chloroform (CF) extraction, phenol (PHE) extraction, carrageenan (CARRA) extraction (Ren, Yang, Thirumalai, Zhang, & Schade, 2016). Taking both purity and yield into consideration, IgY antibodies were extracted from egg yolk by PEG 6000 precipitation method in present study. Outcome was confirmed by SDS-PAGE, which indicated that IgY had been preliminary purified (Fig. 2A). Specific anti-Gent IgY was determined by indirect ELISA showed that the titer increased after the first

booster immunization and peaked (128000) after the fourth booster immunization (Fig. 2B). The highest titer could maintain about one month and then it started to decrease. Specific anti-Kana IgY was similar to anti-Gent IgY and its highest titer was 256000.

Here is Fig. 2

3.3. Development of FPIA

FPIA is a homogeneous assay technique and it had been widely used in the detection of small molecules (Dinis-Oliveira, 2014). Compared to other immunoassays, it is a rapid, simple, and sensitive method. In this study, the optimization of the analysis procedure had been carried out to reach the highest possible sensitivity to antibodies raised against five anti-aminoglycoside antibiotics. Under the optimal assay conditions, serial competitive inhibition curves were constructed with different tracer ($n=3$, Fig. 3) and the sensitivity of the FPIA was detected and expressed by IC_{50} value. The IC_{50} of anti-Gent IgY with Gent-FITC was $7.70 \pm 0.6 \mu\text{g/mL}$, the IC_{50} of anti-Gent scFv with Gent-FITC was $4.98 \pm 0.7 \mu\text{g/mL}$ and the IC_{50} of anti-Kana IgY with Kana-FITC was $7.97 \pm 0.9 \mu\text{g/mL}$. The limits of detection (LOD, IC_{10}) were 0.17, 0.16 and $0.007 \mu\text{g/mL}$, respectively. The working ranges of quantitative detection (determination) of target analyte (IC_{20} to IC_{80}) were from 0.69 to $86.15 \mu\text{g/mL}$, 0.59 to $57.56 \mu\text{g/mL}$ and 0.09 to $680.51 \mu\text{g/mL}$, respectively. The specificity of anti-Gent IgY (specific identification to Gent) was evaluated by the CR and the results showed that the CR of Gent, Kana and Amp were 100%, less than 1% and less than 1%, respectively. Similarly, the anti-Kana IgY possessed a good specificity (specific identification to Kana).

Here is Fig. 3

3.4. Development of ic-ELISA

ELISA is a heterogeneous assay technique and a most widely used immunoassay (Dinis-Oliveira, 2014). In present study, the ic-ELISA was performed to construct standard curves. As could be seen in the representative inhibition curves (Fig. 4), the IC_{50} values were $0.32 \pm 0.06 \mu\text{g/mL}$ and $0.15 \pm 0.01 \mu\text{g/mL}$ for Gent and Kana respectively. The working ranges of quantitative detection (determination) of target analyte (IC_{20} to IC_{80}) were 0.004 to $27.21 \mu\text{g/mL}$ and 0.003 to $8.07 \mu\text{g/mL}$. The LODs

for both were 0.001 µg/mL. The specificity of IgY was evaluated by the CR and the results were similar with FPIA. The CR results of the anti-Gent scFv have been reported in our previous paper (Li, He, Ren, Zhang, Du, & Li, 2016).

Here is Fig. 4

3.5. Analysis of artificially-spiked animal-derived samples by FPIA and ic-ELISA

In order to evaluate the reliability of these two immune methods, the FPIA and ic-ELISA were used to quantify Gent/Kana in chicken, pork and milk samples. 0, 100 and 200 µg Gent/Kana were added in these samples as described in 2.10. The recoveries and RSD at various spiking levels for the quantification of Gent/Kana in real samples were listed in Table 1 and Table 2. As shown in Table 1, the recoveries of Gent in edible food ranged from 67.44% to 82.29% with RSDs of 0.42–2.77% based FPIA, the recoveries of Gent in edible food ranged from 63.05% to 99.62% with RSDs of 4.63–11.59% based ic-ELISA. The Table 2 showed that the recoveries of Kana in edible food ranged from 55.34 to 94.81% with RSDs of 0.74–3.97% based FPIA. In the ic-ELISA, the recoveries of Kana in edible food ranged from 66.27 to 133.51% with RSDs of 4.08–13.02%.

Here is Table 1

Here is Table 2

There is an urgent need to develop antibiotics detection techniques and product with high sensitivity and accuracy. The immunoassays have been attracted considerable interest due to sensitivity, simplicity, rapidity and so on. However, in small molecule detection, the immunological methods are highly susceptible to many factors, especially the quality of the antibiotics and the antibody used. In this study, FPIA and ic-ELISA have been used to detect Gent/Kana residues in animal-derived food. Their IC₅₀ values in FPIA and ic-ELISA were high, while the LOD of Kana in FPIA was 0.007 µg/mL and the LODs of Gent/Kana in ic-ELISA were 0.001 µg/mL, which meet the detection limit in different major authorities, including EU. While the LODs of Gent in FPIA were 0.17 or 0.16 µg/mL by using specific IgY and scFv, respectively, which is not considered suitable in detection with the respect to the MRL (100 µg/kg in milk) in EU. The ranges of detection (IC₂₀ to IC₈₀) were wider in ic-ELISA (0.004 to 27.21 µg/mL) than that of FPIA (0.69 to 86.15 µg/mL). The recoveries of

Gent/Kana in ic-ELISA were higher than that of FPIA. Taken all together, in our present work, the ic-ELISA might provide higher accuracy in detection as compared to FPIA, despite FPIA offers easy rapid and portable which could be a direction of immunoassay and product.

4. Conclusion

Two immunoassays (FPIA and ic-ELISA) have been developed to evaluate Gent/Kana residue with IgY/scFv. The recovery test from spiked food showed that the recoveries were ranged from 55.34% to 133.51% in FPIA and ic-ELISA. The LODs of FPIA with anti-Gent IgY, anti-Gent scFv or anti-Kana IgY were 0.17, 0.16 and 0.007 $\mu\text{g/mL}$, the LODs of ic-ELISA were both 0.001 $\mu\text{g/mL}$. These analytical results are meeting the mandated MRLs of EU for such antibiotics (except the LODs of FPIA with anti-Gent IgY, anti-Gent scFv) in animal-derived food. These results also indicated that IgY antibody could be used in antibiotic residues detection with different immunoassays in animal-derived food and that chicken IgY antibody would be as an alternative strategic in antibiotic residue detection to ensure food safety.

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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Figure Captions

Fig. 1 Antigen identified by UV and SDS-PAGE. (A) UV scanning spectrum of OVA, Gent and Gent-OVA; (B) UV scanning spectrum of BSA, Gent and Gent-BSA; (C) Appraisal of Gent-OVA and Gent-BSA by SDS-PAGE.

Fig. 2 SDS-PAGE and ic-ELISA analysis of IgY antibody. (A) SDS-PAGE analysis of the purified IgY antibody by PEG 6000; (B) Monitoring of anti-Gent IgY antibody titer.

Fig. 3 Standard curves for Gent/Kana determination by FPIA. x-axis represents the logarithm of the concentration of Gent/Kana; y-axis represents the inhibition rate (mP/mP_0), mP: fluorescence polarisation value at the different concentration competition; mP_0 : fluorescence polarisation value at zero competition.

Fig. 4 Standard curves of ic-ELISA for Gent/Kana. x-axis represents the logarithm of the concentration of Gent/Kana; y-axis represents the inhibition rate (B/B_0), B: OD values of the Gent/Kana at the different concentration; B_0 : OD values of Gent/Kana at zero concentration.

Table 1

Table 1 Results from analysis of Gent in animal derived food by FPIA and ic-ELISA based IgY							
Samples	Spiked (µg/mL)	FPIA			Ic-ELISA		
		Found (µg/mL)	Recovery (%)	RSD (%)	Found (µg/mL)	Recovery (%)	RSD (%)
Milk	0	<LOD			<LOD		
	100	76.49	76.49	0.50	86.29	86.29	9.29
	200	164.58	82.29	1.44	126.10	63.05	6.95
Chicken	0	<LOD			<LOD		
	100	73.61	73.61	1.76	99.62	99.62	4.63
	200	134.88	67.44	2.77	153.08	76.54	5.14
Pork	0	<LOD			<LOD		
	100	81.54	81.54	0.42	89.66	89.66	11.30
	200	162.03	81.02	1.92	156.48	78.24	11.59

Table 2

Table 2 Results from analysis of Kana in animal derived food by FPIA and ic-ELISA based IgY							
Samples	Spiked (µg/mL)	FPIA			Ic-ELISA		
		Found	Recovery	RSD (%)	Found	Recovery	RSD (%)
		(µg/mL)	(%)		(µg/mL)	(%)	
Milk	0	<LOD			<LOD		
	100	65.36	65.36	2.86	83.22	83.22	13.02
	200	140.07	70.03	1.25	132.54	66.27	9.46
Chicken	0	<LOD			<LOD		
	100	71.19	71.19	1.62	71.83	71.83	8.93
	200	189.62	94.81	3.97	166.37	83.19	11.07
Pork	0	<LOD			<LOD		
	100	88.20	88.20	0.74	102.79	102.79	4.08
	200	110.67	55.34	0.97	267.03	133.51	8.17

Figure 1

Figure 2

Figure 3

(A)

(B)

(C)

Figure 4