A novel colloidal gold labeled antigen for the detection of Deoxynivalenol using an immunochromatographic assay method

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Abstract: In this paper, an immunochromatographic assay card was developed for the detection of DON in feed and cereals using a novel colloidal gold labeling method. For the colloidal gold immunochromatographic rapid detection (GICD) card, a monoclonal antibody DON-mAb and a goat anti-chicken IgY were drawn on NC membrane as the test line (T line) and the control line (C line) respectively. A gold labeled DON-CBSA conjugate and a gold labeled chicken IgY were sprayed onto the conjugate pad. The GICD card has cut-off levels of 50ng/mL for DON, which is invulnerable to matrix interference, and applicable to a wide range of samples. The GICD detecting results of feed and grain samples were compared with the results of ELISA testing, which showed good consistency.

1. Introduction
Deoxynivalenol (DON), also known as vomitoxin (vomitoxin), is a fungus compounds. DON is highly toxic to humans and animals, which can cause vomiting, diarrhea, skin irritation, antifeedant, neurological disorders, abortion, and stillbirth and so on [1 ~ 3]. DON have three effects (ie, carcinogenic, teratogenic, mutagenic) [4, 5], in the International Cancer Research Institute published evaluation report, DON was classified as a class III carcinogen. China rules that DON in cereals and feeds should be less than 1.0 mg / kg [6, 7]. So the detection and monitoring of DON is very necessary.

Colloidal gold immunochromatography has the advantages of fastness, specificity, simple operation, no need of any equipment and so on. It has become the important ways for rapid screening at the scene. Most of the current DON test cards are based on colloidal gold labeled monoclonal antibody technology developed, the sensitivity of the DON standard is generally around 100ng/ml [8, 9]. According to the literature found that most of the DON test card is only applicable to the matrix composition comparison Simple samples, usually cereal grain samples [10, 11]. We have studied the DON Immunochromatographic assay card based on colloidal gold labeled antigen technology (GICD card). After preliminary experimental study, the minimum detection limit of the test card to the DON standard is 50ng/ml or less. The GICD card anti-matrix interference ability is strong, it is suitable for the detection of DON in samples such as cereals and feedstuffs, more capable to meet the needs of the market.
2. Experiments

2.1. Materials and chemicals
C₆H₅Na₃O₇·2H₂O, HAuCl₄·3H₂O and Bovine serum albumin (BSA) were purchased from Sigma Aldrich; Chicken IgY and Goat anti-Chicken IgY were obtained from Luoyang Bai aotong Experimental Materials Center (Luoyang China). DON-CBSA and monoclonal antibodies against DON (Anti-DON MAb) were prepared in our laboratory. All other chemicals and reagents are of analytical grade or better and are purchased from Xilong Scientific Co., Ltd. The Nitrocellulose membrane HF13502S25 was purchased from Millipore Company;. DON ELISA kits were provided by Qingdao Pribolab Bioengineering Co., Ltd.

2.2. Apparatus
XYZ-3D Crossed and Spraying Machine HM3035 and Microcomputer Automatic Cutting Machine ZQ2000 were purchased from Shanghai Kinbio Tech Co., Ltd. (Shanghai, China). The centrifuge 5804R purchased from eppendof Co., Ltd. The ultraviolet-visible spectrophotometer was purchased from Perkinelmer.

2.3. Procedures
2.3.1. Preparation of 40 nm nano-gold particles, colloidal gold labeled DON antigen and colloidal gold labeled chicken IgY polyclonal antibody.
2.3.2. Prepare colloidal gold immunochromatographic rapid detection cards. The GICD card includes the following parts, the PVC plate, Absorbent Pad, Nitrocellulose membranes (NC), Colloidal gold bond pad and sample pad et al. The above components were assembled into GICD cards in accordance with Figure 1 way.

![GICD cards assembly decomposition diagram](image)

Figure 1. GICD cards assembly decomposition diagram.

2.3.3. Detection of GICD cards sensitivity. DON standards were diluted with ultrapure water into a series of concentrations (0, 10, 20, 40, 50, 100ng/ml); GICD cards were used to measure the concentrations of the all standards when testing sensitivity.
2.3.4. Extraction of cereals and feed samples. For GICD cards, weigh the sample with 5.0 g homogenized (over 20 mesh sieve), add 25 ml of ultra-pure water to shake for 5 minutes, then centrifuge at 5000r/min for 5 minutes; Diluted 3 times with sample diluent (0.03% T-20, 0.004% SDS in 10mM PBS) for GICD detection; Diluted 5 times with sample diluent (0.03% T-20, 0.004% SDS in 10mM PBS) for ELISA detection.
2.3.5. Detection of natural samples. 13 cereals samples and 21 feed samples were tested by GICD cards. The results were compared and validated by ELISA kit. The ELISA kits were operated according to the instructions.

3. Results and discussion

3.1. Identification of colloidal gold, colloidal gold labeled DON antigen and labeled chicken IgY antibody
The gold nanoparticles were identified by UV-Vis spectrophotometer. It can be seen that the gold particles have Maximum absorption peak at about 525nm in figure 2. Between 500nm and 600nm, the peak type is smooth and the peak width is small, indicating that the diameter of the gold nanoparticles...
is about 40nm, and the dispersibility of the gold particles is better. The right side of the electron microscope scan of colloidal gold particles confirmed the UV extrapolation.

As shown in Figure 2, the highest absorption peak of the two markers has a redshift phenomenon, indicating that the diameter of colloidal gold particles increases. It was confirmed that DON-Ag and IgY antibodies had been labeled on colloidal gold particles, respectively.

3.2. Optimization of GICD Parameters

For the GICD cards, the pH of the reaction system, the type of buffer solution, the ionic strength of the buffer solution will have an effect on the reaction of antigen and antibody. Use the ImageJ software to measure the gray value of the T-line of the test card. The ratio of the gray value of the positive T line and the gray value of the negative T line indicates the binding rate of the antigen and antibody in this positive condition.

As shown in Figure 3, T represents the negative T line gray value, Tp represents the positive T line gray value, and Tp/T represents the binding rate. The lower the binding rate, the better the blocking effect, indicating that the higher the sensitivity. The best condition was 30 mM PBS with a pH of 6.5 ± 0.1.

3.3. The GICD cards sensitivity

Detection of DON standard gradient solution, the results in Figure 4 show that the GICD card to detect 40ng/ml standard solution, T line has a very weak color, detection of 50ng/ml standard solution, T line does not show color, so the card detection DON standard solution sensitivity is 50ng/ml.
3.4. The actual samples were tested and compared with the ELISA results
As shown in Table 1: (+) Indicates positive, (-) indicates negative, and (+/-) indicates weak positive. The 21 feed samples were obtained from Qingdao Pribolab Bioengineering Co., Ltd.; these samples have been confirmed with Elisa kit, samples 4, 11, 16, their DON content from 1080 to 4112.5ug / kg. GICD cards test results for only a red quality control line, indicating that the results were positive. 12 and 14 were detected by GICD cards, and the results were weak positive. ELISA results showed that the contents of 12 and 14 were 600ng / ml and 625ng / ml, which were located in the gray area (600-800ng/ml) of GICD card. 13 samples of cereal samples were purchased in supermarkets or collected in Beijing and Shandong provinces. Samples 21, 31 and 34 had DON levels of 730 ng / ml, 2472.5ng/ml and 2477.5ng/ml, respectively. The remaining sample had DON content below 700ng/ml. These samples were examined by GICD cards. Only samples of samples 21, 31 and 34 showed only C lines, indicating that the results were positive; the other samples were colored C and T lines, indicating that the results were negative. Sample No. 21 is in the gray area of GICD card.

GICD cards test results and ELISA test results are basically consistent.

Table 1: Comparison of ELSIA test results with GICD cards test results.

| Sample serial number | sample name          | ELISA test results (ng/g) | GICD cards test results (+/-) |
|----------------------|----------------------|---------------------------|------------------------------|
| 1                    | Peanut meal          | 95                        | (-)                          |
| 2                    | Citric acid residue  | 135                       | (-)                          |
| 3                    | Starch slag          | 185                       | (-)                          |
| 4                    | Sugar residue        | 4112.5                    | (+)                          |
| 5                    | Cottonseed meal      | 112.5                     | (-)                          |
| 6                    | Corn gluten meal     | 102.5                     | (-)                          |
| 7                    | Bran                 | 132.5                     | (-)                          |
| 8                    | Sorghum              | 145                       | (-)                          |
| 9                    | White DDGS           | 75                        | (-)                          |
| 10                   | DDGS 2254            | 515                       | (-)                          |
| 11                   | DDGS 705             | 1535                      | (+)                          |
| 12                   | Wheat germ           | 600                       | (+/-)                        |
| 13                   | Wheat flour          | 0                         | (-)                          |
| 14                   | Feed No. 1           | 625                       | (+)                          |
| 15                   | Laying hens feed     | 112.5                     | (-)                          |
| 16                   | Feed 510             | 1080                      | (+)                          |
| 17                   | Soybean meal         | 215                       | (-)                          |
| 18                   | Test 19              | 55                        | (-)                          |
| 19                   | Corn flour 2511      | 105                       | (-)                          |
| 20                   | Corn                 | 305                       | (-)                          |
|   | Description                         | Price   | Unit |
|---|-------------------------------------|---------|------|
| 21 | Corn powder                         | 320     |      |
| 22 | Sorghum (good supermarket)          | 0       |      |
| 23 | Corn flour                          | 22.5    |      |
| 24 | Sorghum (good supermarket)          | 57.5    |      |
| 25 | Soybeans (good supermarkets)        | 262.5   |      |
| 26 | Corn flour (good supermarket)       | 730     |      |
| 27 | Corn flour (Lok Jiajia Supermarket) | 95      |      |
| 28 | Soybeans (Lok Jiajia Supermarket)   | 315     |      |
| 29 | Rice (Lok Jiajia Supermarket)        | 102.5   |      |
| 30 | Brown rice (Lok Jiajia Supermarket) | 82.5    |      |
| 31 | Corn (Beijing)                      | 2472.5  |      |
| 32 | Wheat flour                         | 252.5   |      |
| 33 | Wheat (Shandong Yimeng)             | 130     |      |
| 34 | Wheat flour                         | 2477.5  |      |

4. Conclusion
GICD card is a quick diagnostic product, the entire detection process for about 10 minutes, which can be used for a large number of samples on-site screenings of qualitative testing experiments. The GICD card's visual inspection line for DON was 50ng/ml. The sensitivity to the sample was 750ng/ml.

Coupled with a simple and fast sample preparation, the described assay format can be used as a simple, rapid, cost-effective and robust on-site screening tool for DON contamination in feed and cereals samples.

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