Quantitative detection of Deoxynivalenol in food and feed based on nanogold immunochromatographic assay

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Abstract: A deoxynivalenol (DON) monoclonal antibody (DON-Mab) was labeled with gold nanospherical (AuNSs) with a particle size of 45±1.9 nm. The DON Immunochromatographic test card (DON-GICT) for detecting DON in food and feed was developed. The sample does not require special handling. The detection process takes about 20-25 minutes. In conjunction with the nano gold reader, the detection range of the actual sample by DON-GICT is 6.25-6250 μg/kg. DON standards with a final concentration of 500μg/kg were added to 5 grains and 1 feed sample, respectively, and the recovery was 98.3%-127.0%, and the coefficient of variation was 0.9%-4.7%. Twenty-three feed samples and 11 food samples were tested by DON-ELISA and DON-GICT, respectively, and the results were compared. The linear fitting correlation coefficient $R^2 = 0.9845$. It shows that DON-GICT has good accuracy, precision and wide detection range, which can provide efficient and accurate detection method for detecting DON pollution in food and feed.

1. Introduction
Deoxynivalenol (DON), also known as vomiting toxin, is a trichotheccenes, mainly produced by *Fusarium graminearum* and *Fusarium oxysporum* [1]. DON have a triad effect (ie, carcinogenic, teratogenic, mutagenic) [2, 3]. China requires a maximum of 1.0 mg/kg of DON in food and a maximum of 3 mg/kg of DON in feed [4, 5]. Conventional methods for detecting DON by TLC [6, 7], HPLC [8, 9] and ELISA [10] have complicated sample preparation and long detection time, which is not conducive to screening of a large number of samples. Colloidal gold immunochromatography is an important means for rapid screening on the spot because of its rapidity, specificity, easy operation, and no need for any equipment. However most of DON nanogold immunochromatographic test cards in the market are qualitative tests [11, 12]. Therefore, it is very necessary to develop an immunochromatographic test card with rapid quantitative and wide linear range.

2. Experiments
2.1 Main experimental materials and reagents
C$_6$H$_{12}$Na$_3$O$_7$·2H$_2$O, HAuCl$_4$·3H$_2$O, BSA and PEG20000 were purchased from Sigma. DON antigen and DON monoclonal antibodies were prepared in this laboratory. NC membrane (HF135) was purchased from Millipore Corporation. Sample pad, gold marker bonding pad, absorbent paper and bottom plate were purchased from Shanghai Jinbiao Biotechnology Co., Ltd. The DON-ELISA kit was purchased
2.2 Apparatus
UV-Vis spectrophotometer is manufactured by Kim Elmer Perkine Lmer. Multiskan MK3 microplate reader and transmission electron microscope FEI are manufactured by Thermo Company, USA. High-speed refrigerated centrifuge (manufactured by Eppendorf, Germany, model number 5804R). XYZ-3D spray gold filming machine HM3035, micro automatic cutting machine ZQ2000 is produced by Shanghai Jinbiao Biotechnology Co., Ltd. Zeta Plus potentiometer (produced by BROOKHAVEN INSTUMENTS CORP.ATON, USA).

2.3 Procedures

2.3.1 Preparation of gold Nanospherical (AuNSs)
Pour 100 mL of ultrapure water into a clean Erlenmeyer flask equipped with a stir bar and heat to boiling while stirring. Add 3 mL of 1% tetrachloroauric acid. After the solution is boiled again, add 5.4 mL of 1% trisodium citrate. The solution slowly turned into deep burgundy within 1 minute and began to count for 4 minutes after the color stabilized. After the end, the mixture was cooled to room temperature with stirring. The cooled colloidal gold solution was then passed through a 0.22 μm filter and placed in a clean room at room temperature for use.

2.3.2 Preparation of gold-labeled DON antibody (AuNS- DON-Mab)
10 mL of AuNSs was placed in a clean beaker with a magnetic stirrer, 50 μL of 0.2 M K2CO3 was added to adjust the pH of the solution, then 15 μg/ml of DON antibody was slowly added, and the reaction was stirred at room temperature for 30 minutes. After the reaction was completed, 1 ml of 10% BSA was added for blocking for 30 minutes. After the end, all the solutions were placed in a centrifuge at 900 g, centrifuged for 5 minutes, and finally the supernatant was centrifuged at 4500 g for 30 minutes, and the lower layer was diluted with 1 ml of the suspension (5% trehalose, 0.5% BSA, 0.5% glycine, 0.5% Tween 20 dissolved in 10 mM PBS). Place the AuNS-DON-Mab at 2~8 °C for later use.

2.3.3 Preparation of DON Nano gold Quantitative Immunochromatography Test Card (DON-GICT).
DON-BSA (0.5 mg/m) and goat anti-mouse (0.3 mg/ml) were placed at the T and C lines of the NC membrane, respectively. The AuNS-DON-Mab was sprayed onto the pretreated conjugate pad (5% sucrose, 0.5% T-20, 0.04% casein, 0.1% PEG20000 dissolved in 2 mM boric acid buffer) at a spray rate of 2μL/cm. The glass fibers were cut to a size of 300*18 mm, and the glass fibers were soaked for 10 minutes with a sample pad pretreatment solution (0.5% Tetronic 1307, 0.5% BSA dissolved in 100 mM BBS, pH = 7.0 ± 0.1). The above-prepared components were dried in an incubator at 37 °C and a relative humidity of 35% for 12 hours. The conjugate pad, sample pad and NC film were assembled into a large plate in a conventional manner. Cut the large plate into 65mm*3.95mm test strips and load them into a WE-2 plastic card to assemble the test card.

3. Results and discussion

3.1 Characterization of AuNSs and AuNS-DON-Mab
In Figure 1, the maximum absorption peak of AuNSs is 528 nm. The effective particle size of AuNSs measured by a particle size analyzer is 45±1.9 nm. The maximum absorption peak of AuNS-DON-Mab is 534 nm, indicating that the DON antibody has been labeled on AuNS. TEM shows (Figure 3) the structure and morphology of AuNSs. The dispersion is good and the particle size is uniform. The particle size is about 40 nm according to the electron micrograph scale, which is basically consistent with the particle size measurement data. In Figure 2, the zeta potential of AuNSs is -28.19 mV ± 3.92,
which is in a colloidal state capable of stable storage. After the AuNSs were stored at room temperature for 12 months, AuNSs still maintained good colloidal properties, indicating that the AuNSs have good stability.

![Figure 1. UV-visible absorption Spectrum of AuNSs and AuNS-DON-Mab](image1)

![Figure 2. Zeta potential of AuNSs](image2)

![Figure 3. Photographic images and TEM images of AuNSs](image3)

### 3.2 Establishment of DON-GICT standard curve

DON standard (0, 2.5, 10, 50, 100, 250, 500, 1000, 2000 ng/mL) was measured with DON-GICT and the signal value of each standard was read with a nano gold reader. The DON series standards (X-axis) and the measured signal value (Y-axis) were curve-fitted with a nonlinear four-parameter to obtain a curve fitting equation. The results in Figure 4 shows that when DON-GICT measures DON series standards (2.5-250 ng/mL), the nonlinear four-parameter curve fitting correlation coefficient $R^2=0.996$, When the dilution factor is 25, the detection range of the sample is 6.25-6250μg/kg. The minimum detection limit (LOD) was defined as: DON-GICT was used to detect food and feed samples without DON, and each sample was tested 20 times each time to obtain 20 measured signal values. The average value (M) and the standard deviation (SD) were calculated to obtain the signal value of
M+2SD. The M+2SD signal values of grain and feed were substituted into the DON-GICT nonlinear four-parameter fitting equation to obtain their detection limits of 40μg/kg and 53μg/kg, respectively.

![Figure 4. DON-GICT nonlinear four-parameter curve fitting](image)

3.3 DON-GICT precision and accuracy testing

The DON standard at a final concentration of 500μg/kg was separately added to 5 parts of the grain and 1 part of the feed sample, and the original sample and the spiked sample were measured with the same batch of DON-GICT, and each spiked sample was repeatedly tested 10 times. The data (Table 1) shows that the spike recovery is between 98.3% and 127.0% with a coefficient of variation between 0.9% and 4.7%. The test results show that DON-GICT has better accuracy and precision.

| Sample name | Test result (μg/kg) | Add 500μg/kg test result (μg/kg) | Recovery rate | CV (n=10) |
|-------------|---------------------|----------------------------------|---------------|-----------|
| Millet      | 24.79               | 567.29                           | 108.5%        | 4.5%      |
| Sorghum     | 7.40                | 692.58                           | 127.0%        | 4.7%      |
| brown rice  | 17.39               | 565.32                           | 109.6%        | 0.9%      |
| Wheat       | 0                   | 519.98                           | 104.0%        | 2.8%      |
| Corn        | 87.70               | 667.98                           | 116.1%        | 2.6%      |
| Pig feed    | 234.30              | 725.73                           | 98.3%         | 2.2%      |

3.4 Comparison of results between DON-GICT and DON-ELISA for actual samples

Twenty-three feed samples and 11 food samples were selected for simultaneous detection by DON-ELISA and DON-GICT. The DON-ELISA kit sample pretreatment method and detection method were measured according to the self-contained instructions. DON-GICT: Take 5 g of the uniform sample in a 50 ml centrifuge tube, add 25 ml of ultrapure water or distilled water for 5 minutes by vortexing, and centrifuge at 5000 r/min for 5 minutes. After centrifugation, the supernatant was diluted with a sample dilution (0.004% SDS, 0.03% T-20 dissolved in 10 mM PBS) and mixed 5 times. Mix 100 μL of the test solution and add it to the sample well of the test card for 20 minutes. The correlation between the two test results is shown in Figure 5. The results show that the correlation between the two test results is $R^2=0.9845$, indicating that DON-GICT has a good correlation with the commercial DON-ELISA test results.
Figure 5. Comparison of DON-GICT and DON-ELISA results

4. Conclusion
DON-GICT can quantitatively detect DON content in feed and food. The sample does not require special handling. The detection process takes about 20-25 minutes. In conjunction with the nano gold reader, the detection range of the actual sample by DON-GICT is 6.25-6250 μg/kg. DON standards with a final concentration of 500μg/kg were added to 5 grains and 1 feed sample, respectively, and the recovery was 98.3%-127.0%, and the coefficient of variation was 0.9%-4.7%. Comparison of DON-GICT and DON-ELISA results for 23 feeds and 11 food samples. The linear fitting correlation coefficient $R^2=0.9845$. It shows that DON-GICT has good accuracy, precision and wide detection range, which can provide efficient and accurate detection method for detecting DON pollution in food and feed.

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