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Postprint: Development of a Rapid Quantitative Colloidal Gold Detection Kit for Aflatoxin B1

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Abstract

This study aimed to develop a rapid quantitative colloidal gold detection kit for aflatoxin B1 in grains and feed by employing colloidal gold immunochromatographic technology combined with a colloidal gold quantitative reader. The kit utilized colloidal gold-labeled highly specific monoclonal antibodies and conducted orthogonal experiments with conjugated antigens to determine optimal conditions. Quantitative detection of aflatoxin B1 content in samples was achieved through color comparison between the control and test lines using a colloidal gold quantitative reader. The results demonstrated that the aflatoxin B1 colloidal gold rapid quantitative detection kit offered a simple, rapid, and stable detection method that yielded quantitative data, thereby eliminating variations associated with human visual observation. The kit exhibited no cross-reactivity with common mycotoxins, and the relative error between sample detection results and those obtained by high-performance liquid chromatography remained within 20%. Consequently, the aflatoxin B1 colloidal gold rapid quantitative detection kit developed in this study is suitable for rapid quantitative detection of aflatoxin B1 content in grains and feed.

Full Text

Development of a Colloidal Gold Rapid Quantitative Test Kit for Aflatoxin B1

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Abstract: This study aimed to develop a rapid quantitative colloidal gold test kit for detecting aflatoxin B1 content in grains and feed by applying colloidal gold immunochromatography technology combined with a colloidal gold quantitative reader. The kit utilized colloidal gold-labeled highly specific monoclonal antibodies and performed orthogonal experiments with conjugated antigens to determine optimal conditions. Simultaneously, by comparing the color intensity between the control and test lines and employing a colloidal gold quantitative reader, the aflatoxin B1 content in samples could be quantitatively determined. Results demonstrated that the aflatoxin B1 colloidal gold rapid quantitative test kit offered a simple, rapid, and stable detection method that yielded specific numerical data, avoiding variations inherent in visual observation. The kit showed no cross-reactivity with common mycotoxins, and the relative error between sample detection results and high-performance liquid chromatography (HPLC) results was within 20%. These findings indicate that the aflatoxin B1 colloidal gold rapid quantitative test kit developed in this study can be used for rapid quantitative detection of aflatoxin B1 in grains and feed.

Keywords: aflatoxin B1; colloidal gold; test strip
Classification Code: S816.17

Introduction

Aflatoxin B1 is the most prevalent mycotoxin in agricultural products and exhibits strong toxicity, causing reduced feed intake, decreased production performance, and compromised immunity in livestock, with severe cases resulting in animal death and substantial losses to the animal husbandry industry. Furthermore, when dairy cows ingest feed containing aflatoxin B1, aflatoxin M1 can be detected in milk through metabolic processes, affecting milk quality. Consequently, countries worldwide have established strict maximum residue limits for aflatoxin B1, and it remains a mandatory testing item in feed raw materials and finished products.

Currently, commonly used methods for detecting aflatoxin B1 include high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and colloidal gold immunochromatography. In recent years, various immunological detection methods for aflatoxins have been developed. Among these, colloidal gold immunochromatography has been widely applied for detecting aflatoxin B1 in grains, though it only enables qualitative analysis. This study aimed to develop a rapid quantitative detection kit for aflatoxin B1 in grains and feed based on colloidal gold immunochromatography combined with a quantitative reader, providing an accurate, rapid, and simple method for detecting aflatoxin B1 content in feed.

Materials and Methods

1.1 Test Materials and Equipment

Test materials included: aflatoxin B1, zearalenone, deoxynivalenol, fumonisin, ochratoxin, and T-2 toxin standards (Sigma-Aldrich, USA); chloroauric acid ($HAuCl_4 \cdot 4H_2O$, Sinopharm Chemical Reagent Co., Ltd.); aflatoxin B1 conjugated ovalbumin (AFB1-OVA, Beijing Longkefangzhou Bio-Engineering Technology Co., Ltd.); aflatoxin B1 monoclonal antibody (AFB1-Ab, Beijing Longkefangzhou Bio-Engineering Technology Co., Ltd.); goat anti-mouse immunoglobulin G (IgG, Shanghai Gold Standard Biotechnology Co., Ltd.); nitrocellulose membrane (Millipore, USA); colloidal gold conjugation pads, sample pads, and absorbent paper (Shanghai Gold Standard Biotechnology Co., Ltd.); and aflatoxin B1 immunoaffinity chromatography columns (Beijing Longkefangzhou Bio-Engineering Technology Co., Ltd.).

Equipment included: three-dimensional membrane spraying instrument HM3030 (Shanghai Gold Standard Biotechnology Co., Ltd.); automatic microcomputer cutting machine ZQ2000 (Shanghai Gold Standard Biotechnology Co., Ltd.); high-speed refrigerated centrifuge H-2050R (Changsha Xiangyi Centrifuge Instrument Co., Ltd.); UV continuous scanning spectrophotometer (Thermo Fisher Scientific, USA); digital magnetic stirring heating mantle (Tianjin Labtech Instruments Co., Ltd.); electric thermostatic blast drying oven (Shanghai Yiheng Scientific Instruments Co., Ltd.); colloidal gold quantitative reader (Shanghai Jiehao Scientific Instruments Co., Ltd.); and constant temperature incubator (Hangzhou Aosheng Technology Co., Ltd.).

1.2.1 Preparation of Colloidal Gold Solution

In a round-bottom flask, 1 L of ultrapure water was heated to boiling, followed by addition of 10 mL of 1% chloroauric acid solution. After 2 minutes, 12 mL of 1% trisodium citrate solution was added, causing the solution color to gradually change from black to wine red. The solution was boiled for an additional 15 minutes, then cooled to room temperature and stored at 4 °C. The quality of the colloidal gold solution was verified by UV scanning.

1.2.2 Determination of Optimal pH for Antibody Labeling

One milliliter of colloidal gold solution was aliquoted, and 1, 3, 5, 8, 10, 15, or 20 µL of potassium carbonate (K_2CO_3) solution was added to adjust pH. Aflatoxin B1 monoclonal antibody was labeled at 5 µg/mL, blocked with a final concentration of 1% BSA, centrifuged at 4,000 r/min for 5 minutes, washed once with phosphate-buffered saline (PBS), centrifuged again at 4,000 r/min for 5 minutes, and resuspended in 300 µL of suspension buffer. The labeled antibody was sprayed onto colloidal gold conjugation pads at 10 µL/cm and dried at 37 °C for 2 hours.

Aflatoxin B1 conjugated antigen and goat anti-mouse IgG were each diluted to

0.5 mg/mL and coated onto nitrocellulose membrane at 1 µL/cm, then dried at 37 °C for 1 hour. After assembling the master card and cutting into strips, PBS solution was used for testing. The test line (T line) color development was observed, and the buffer volume yielding the strongest color without gold aggregation was selected as the optimal labeling pH.

1.2.3 Determination of Optimal Antibody Labeling Concentration and Antigen Coating Concentration

Using the determined optimal pH, orthogonal experiments were conducted with antibody labeling concentrations of 2, 3, 4, or 5 µg/mL and conjugated antigen coating concentrations of 0.1, 0.2, 0.3, or 0.4 mg/mL at the T line position. Aflatoxin B1 standards at concentrations of 0, 2, 5, 10, 20, and 50 µg/kg were tested. The combination showing clear color development at 0 µg/kg and highest sensitivity was selected as optimal.

Under these conditions, goat anti-mouse IgG was coated at the control line (C line) position at concentrations of 0.05, 0.10, 0.15, or 0.20 mg/mL. Aflatoxin B1 standard at 10 µg/kg was tested, and the coating concentration producing C line intensity comparable to the T line was selected as optimal.

1.2.4 Assembly of Aflatoxin B1 Colloidal Gold Rapid Quantitative Test Kit Cards

Nitrocellulose membranes coated with aflatoxin B1 conjugated antigen and goat anti-mouse IgG, polyester pads sprayed with aflatoxin B1 monoclonal antibody-conjugated colloidal gold, glass fiber filter paper, and absorbent paper were assembled onto polyvinyl chloride (PVC) backing plates. The assembled cards were cut into 3.88 mm-wide test strips using a cutting machine and loaded into test card shells to produce the final detection cards.

1.2.5 Determination of Sample Pretreatment Method

Aflatoxin B1-free corn samples were ground and divided into four 1 g portions. Aflatoxin B1 standard solution was added to each portion to achieve a final concentration of 10 µg/kg. Each sample was extracted with 5, 6, 7, or 8 mL of sample extraction solution, then diluted six-fold with dilution buffer. The prepared aflatoxin B1 colloidal gold rapid quantitative test kit cards were used to determine the optimal extraction solution and dilution buffer volumes.

1.2.6 Establishment of Standard Curve

A series of aflatoxin B1 standards at different concentrations (0, 10, 20, 30, 40, 50, and 60 µg/kg) were prepared. After extraction and dilution according to the determined ratio, 100 µL of each standard was added to the sample well of detection cards and incubated at 37 °C for 10 minutes. The cards were then placed in the colloidal gold quantitative reader to obtain the peak area ratio

of T line to C line (T/C value). Each concentration was tested five times, and the average value was calculated. A standard curve was constructed with the average T/C value as the x-axis and aflatoxin B1 standard concentration as the y-axis.

1.2.7 Spiked Recovery Test

Corn and corn dried distillers grains with solubles (DDGS) samples were tested for aflatoxin B1 content, then spiked with different concentrations (0, 10, 20, and 50 µg/kg) of aflatoxin B1 standard and retested to calculate the recovery rates.

1.2.8 Kit Specificity Testing

Zearalenone, deoxynivalenol, fumonisin, ochratoxin, and T-2 toxin standards were prepared at concentrations of 100, 500, 1,000, and 2,000 µg/kg and tested with the aflatoxin B1 colloidal gold rapid quantitative test kit to evaluate specificity.

1.2.9 Intra- and Inter-batch Reproducibility Testing

The same batch and three different batches of aflatoxin B1 colloidal gold rapid quantitative test kits were used to detect aflatoxin B1 standards at concentrations of 5, 10, 20, and 40 µg/kg. Intra-batch testing was performed with five replicates per concentration, while inter-batch testing was performed with two replicates per concentration per batch to evaluate reproducibility.

1.2.10 Kit Stability Testing

Aflatoxin B1 colloidal gold rapid quantitative test kits were stored in a 45 °C incubator and tested at weeks 0, 2, 4, 6, and 8.

1.2.11 Sample Testing

Ten randomly selected commercial grain and feed samples with aflatoxin B1 content ranging from 2–50 µg/kg were tested using both HPLC and the developed aflatoxin B1 colloidal gold rapid quantitative test kit for comparison.

1.3 Statistical Analysis

Experimental data were statistically analyzed and used for standard curve preparation using Excel 2007, with results expressed as mean values.

Results

2.1 Colloidal Gold Solution Scanning Results

Using double-distilled water as a reference, UV spectrophotometry scanning from 400–700 nm was performed to determine the absorption curve and peak. The maximum absorption peak (λmax) was 527 nm with a maximum absorbance value (ODmax) of 0.109. The prepared colloidal gold solution contained particles approximately 40 nm in size, exhibited a transparent wine-red color, and showed no oily floating matter on the surface, confirming its suitability for test strip preparation.

2.2 Optimal Antibody Labeling pH

When 5 µL of K_2CO_3 solution was added to the colloidal gold to adjust pH, no gold aggregation occurred during antibody labeling, and color development was strongest. The pH of the adjusted colloidal gold solution was approximately 8.0.

2.3 Optimal Antibody Labeling Concentration and Antigen Coating Concentration

Through orthogonal experiments, the optimal conditions were determined as: antibody labeling at 3 µg/mL, T line conjugated antigen coating at 0.2 mg/mL, and C line goat anti-mouse IgG coating at 0.1 mg/mL. Under these conditions, the detection limit of the test strip for aflatoxin B1 standards was 2 µg/kg.

2.4 Sample Pretreatment Method

The final detection conditions were established as: 1 g of ground sample extracted with 8 mL of sample extraction solution, diluted six-fold with dilution buffer, with 100 µL applied to the card, and incubated at 37 °C for 10 minutes.

2.5 Standard Curve

Testing of a series of aflatoxin B1 standards at various concentrations determined the linear range and corresponding T/C values as shown in Table 1 . The standard curve was established with T/C value as the x-axis and standard concentration as the y-axis, as illustrated in Figure 1 [Figure 1: see original paper].

2.6 Spiked Recovery Test Results

Corn and corn DDGS samples were tested for aflatoxin B1 content, then spiked with 0, 10, 20, and 50 µg/kg aflatoxin B1 standard for detection. Recovery rates were calculated and are presented in Table 2 . The spiked recovery rates for corn samples ranged from 98% to 125%, while those for corn DDGS samples ranged from 96% to 136%.

2.7 Kit Specificity Results

As shown in Table 3 , when various mycotoxin standards were tested using the aflatoxin B1 colloidal gold rapid quantitative test kit, all results were below 2 μg/kg, indicating no cross-reactivity with zearalenone, deoxynivalenol, fumonisin, ochratoxin, or T-2 toxin.

2.8 Intra- and Inter-batch Reproducibility Results

Intra-batch reproducibility was evaluated by testing aflatoxin B1 standards using the same batch of kits with five replicates per concentration, as shown in Table 4 . Results demonstrated that intra-batch coefficient of variation was less than 10% for all concentrations.

Inter-batch reproducibility was assessed using three different batches of kits, with two replicates per concentration per batch, as presented in Table 5 . The inter-batch coefficient of variation was also less than 10% for all concentrations.

2.9 Kit Stability Results

Aflatoxin B1 colloidal gold rapid quantitative test kits stored at 45 °C were tested at weeks 0, 2, 4, 6, and 8 for aflatoxin B1 standard concentration. Stability results are shown in Table 6 . The kit maintained acceptable performance after 8 weeks of storage at 45 °C, with coefficient of variation less than 15%.

2.10 Sample Detection Results

Ten samples were tested using the aflatoxin B1 colloidal gold rapid quantitative test kit, with results presented in Table 7 . The kit results correlated well with HPLC detection, with relative error within 20%.

Discussion

Aflatoxin B1 is the most common mycotoxin in agricultural products, exhibiting strong toxicity that causes reduced feed intake, decreased production performance, and compromised immunity in livestock, with severe cases leading to animal death and substantial economic losses. Additionally, when dairy cows consume feed contaminated with aflatoxin B1, aflatoxin M1 can be detected in milk through metabolic processes, compromising milk quality. Consequently, stringent maximum residue limits for aflatoxin B1 have been established worldwide, and it remains a mandatory testing parameter in feed raw materials and finished products.

Currently, HPLC, ELISA, and colloidal gold immunochromatography are commonly used methods for aflatoxin B1 detection. The aflatoxin B1 colloidal gold rapid quantitative test kit developed in this study offers several advantages over existing products. First, the operation is simple and rapid. HPLC requires sample extraction followed by purification through immunoaffinity columns, with

each sample requiring approximately 25 minutes for instrumental analysis. Commercial ELISA kits used for aflatoxin B1 detection in grain and oil products require refrigerated storage, temperature equilibration before use, and a 45-minute reaction at 25 °C after sample extraction. Both methods demand skilled technical personnel. In contrast, the developed kit can be stored at room temperature; stability tests demonstrated that the coefficient of variation remained below 15% after 8 weeks at 45 °C, equivalent to 6 months at ambient temperature. During testing, samples require only simple extraction and direct application after dilution, with a 10-minute reaction in a 37 °C incubator that is independent of ambient temperature conditions, enabling even shorter average processing times for batch analyses.

Second, the results are accurate with good reproducibility. Existing colloidal gold test strips determine results through visual comparison of T line and C line color intensity, where T line color equal to or darker than C line indicates a negative result, and T line lighter than or absent of color indicates a positive result. This approach only permits qualitative detection, judging whether mycotoxin content exceeds the cutoff limit based on positive/negative outcomes. Visual interpretation can introduce errors when T line and C line colors are similar. The developed kit employs a quantitative reader to provide specific mycotoxin concentrations, eliminating subjective visual assessment and associated human error. Spiked recovery rates for corn samples ranged from 98% to 125%, and for corn DDGS samples from 96% to 136%. Both intra- and inter-batch coefficients of variation were less than 10%.

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