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CRISPR/Cas12a-based Simplified Lateral Flow Assay for Sensitive Detection of OTA and AFB1

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Abstract

Ochratoxin A (OTA) and Aflatoxin B1 (AFB1) rank among the most widespread mycotoxins in contaminated agricultural products, presenting significant risks to human health via dietary and inhalation exposure. Their harmful impacts encompass hepatotoxicity, nephrotoxicity, neurotoxicity, and immunosuppressive effects. A multiplex CRISPR/Cas12a-assisted lateral flow assay (MC-LFA) was developed for the simultaneous detection of OTA and AFB1, achieving detection limits as low as 50 pg/mL and 250 pg/mL, respectively. The visual detection sensitivity for both mycotoxins was enhanced by 1 to 2 orders of magnitude compared to commercial assays. The experimental procedures have been streamlined by integrating streptavidin lines onto the test strip, eliminating conventional magnetic separation steps. Additionally, the approach eliminates the requirement for crRNA replacement, thereby substantially reducing operational costs. This approach exhibits considerable potential for broad application, given its high sensitivity, specificity, operational simplicity, and cost-effectiveness. This work underscores the significant potential of the MC-LFA strategy for food safety monitoring in resource-limited settings, establishing it as a versatile tool with broad applications in public health, agriculture, and environmental surveillance.

1. Introduction

Ochratoxin A (OTA) and Aflatoxin B1 (AFB1), as fungal secondary metabolites, are among the most widespread mycotoxins found in contaminated agricultural commodities such as wheat, maize, and rice [1, 2]. These toxins can enter the human body through dietary intake and inhalation, posing serious risks to human and animal health [3, 4]. Their adverse effects include hepatotoxicity, nephrotoxicity, neurotoxicity, and immunosuppressive properties [5, 6]. Consequently, numerous organizations have established regulatory thresholds for these contaminants in food products. For instance, the European Commission has set maximum allowable levels of AFB1 at 2 to 12 μg/kg, depending on the specific commodity, and a maximum limit of 5.0 μg/kg for OTA in raw cereals [7, 8]. According to the European Food Safety Authority, the tolerable weekly intake of OTA is set at 120 ng/kg body weight [8]; however, this threshold may be insufficient in light of its genotoxic potential [9]. Thus, developing accurate and efficient detection methods for mycotoxins is crucial to ensure food safety.

Conventional methods for mycotoxin detection include liquid chromatography, enzyme-linked immunosorbent assay (ELISA), and gas chromatography-tandem mass spectrometry [10–12]. These techniques typically require costly and bulky instruments, time-intensive sample preparation, and specialized operators [13]. In contrast, lateral flow assays have emerged as a promising point-of-care testing (POCT) solution for downstream detection, owing to their cost-effectiveness, environmental friendliness, portability, and operational simplicity [14, 15]. For example, Hao et al. designed an innovative lateral flow immunoassay platform utilizing magneto-gold nanohybrids for OTA detection, attaining a highly sensitive detection limit of 0.094 ng/mL [16]. While gold nanoparticles are commonly used as signal labels in commercial mycotoxin test strips, their inherent instability in complex matrices necessitates improved sensitivity [5, 17–19]. Zhong et al. broadened the application of human chorionic

gonadotropin (HCG) test strips by incorporating catalytic hairpin assembly, which enabled the detection of AFB1, OTA, and zearalenone (ZEN) with detection limits of 20 pg/mL, 50 pg/mL, and 20 pg/mL, respectively [20]. These results provide valuable insights for the development of efficient platforms for mycotoxin detection.

Recently, the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins system, originally identified as an adaptive immune mechanism in prokaryotes that defends against foreign nucleic acids [21–23], has not only become a fundamental tool for gene editing but also a promising platform for biosensing. This potential stems from its flexible design, precise target recognition, and the development of biomarker detection methods based on Cas technology [24–26]. Nevertheless, there is a pressing need to improve multiplexing capacity, simplify operational procedures, and enhance overall user-friendliness.

Building upon the aforementioned research background, this study presents a multiplex CRISPR/Cas12a-assisted lateral flow assay (MC-LFA) platform. By leveraging enzyme-assisted signal amplification along with the distinct activation and cleavage specificities of Cas12a, the platform enables simultaneous detection of OTA and AFB1. Multiplex detection can be accomplished simply by replacing the aptamer while retaining the same Cas12a and crRNA sequences, which reduces experimental costs. Additionally, a dedicated streptavidin (SA) line is integrated into the test strip to replace conventional magnetic separation steps, thereby simplifying the experimental workflow. Notably, high sensitivity and accuracy are achieved without additional signal amplification. The test strip operates similarly to an “OR” logic gate, yielding a positive result upon detection of either AFB1 or OTA. Consequently, the MC-LFA strategy exhibits strong potential as an on-site method for the quantitative and sensitive detection of diverse mycotoxins.

2. Experimental

2.1 Materials and Reagents

The LbCas12a were obtained from GenDx Biotech (Suzhou, China). Tris Base, KCl, polyvinyl pyrrolidone (PVP), NaCl, and N, N’-Methylenebisacrylamide were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Polyethylene glycol (PEG) was sourced from Yuanye Biotechnology Co., Ltd. Ammonium persulfate (APS) and Tetramethylethylenediamine (TEMED) were bought from Shanghai Macklin Biochemical Co., Ltd. Gel-Red, 1× phosphate-buffered saline (PBS), and tween 20 were acquired from Beyotime Biotechnology. Streptavidin (SA), bovine serum albumin (BSA), and Triton X-100 were purchased from Sigma-Aldrich. Ochratoxin (OTA) and aflatoxin B1 (AFB1) were bought from Pribolab (Qingdao, China). Aflatoxin B2 (AFB2), deoxynivalenol (DON), and fumonisin B1 (FB1) were acquired from Meryer (Shanghai, China). Zeralenone (ZEN) was made by Nature Standard (Shanghai, China). Citrillin (CIT) was provided by Anpel (Shanghai, China), while sterigmatocystin (STC) was procured from ACMEC (Shanghai, China). Carboxyl-modified CdTe/CdS QDs were procured from Jiayuan Quantum Dots Co., Ltd (Wuhan, China). All oligonucleotide sequences, 6× loading buffer, Anti-FAM, Anti-Dig, and Anti-

rabbit-antibody were provided by Sangon (Shanghai, China). Anti-TAMRA was acquired from Huading Biology (Nanjing, China). The colloidal gold test strip was provided by Finde (Shenzhen, China). Nitrocellulose membrane (Sartorius CN 140) was purchased from Jieyi Biotechnology Co. Ltd. (Shanghai, China). Sample pad, polyvinyl chloride backing card, absorption pad, programmable strip cutter, and XYZ platform dispenser were supplied by Goldbio Co., Ltd. (Shanghai, China).

2.2 Probe preparation

The aptamer sequences of OTA and AFB1 are those that have been reported in the literature [27, 28]. The double-loop stem structures were designed, and the thermal kinetic parameters were simulated using the Oligo Analyzer Software. Probe 1 was rapidly chilled on ice after being heated at 95 ℃ for 5 min to maintain its single-stranded conformation. Probe 2 was heated to 95 ℃ for 5 min and then gradually cooled to ambient temperature to preserve its hairpin structure. Subsequently, Probe 1 and Probe 2 were combined in a concentration ratio of 3:1 to form Switch, ensuring a low background ratio at 37 ℃ for a duration of 30 min. Equivalents of HBR1 and HBR2 were annealed at a final concentration of 4 μM in hybridization buffer (20 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM KCl, 100 mM NaCl, pH 7.3) by heating to 95 ℃ for 5 min and gradual cooling to ambient temperature to obtain the half-blocking reporter (HBR). The oligonucleotide sequences employed in this investigation are provided in **Table S1** and **Fig. S1**.

2.3 Electrophoresis Characterization

DNA products were analyzed by 12% native polyacrylamide gel electrophoresis (PAGE). The gel was prepared by mixing 10 mL of 30% acrylamide/bisacrylamide solution (29:1, w/w), 8.25 mL of distilled water, 6.25 mL of 5× TBE buffer, 250 μL of 10% ammonium persulfate (APS) solution, and 25 μL of N,N,N’,N’-tetramethylethylenediamine (TEMED). Prior to loading into the DYY-6D electrophoresis apparatus (Beijing Liuyi Instrument Co., Ltd., China), 5 μL of each DNA sample was mixed with 1 μL of 6× loading buffer. The resulting mixtures were loaded onto the pre-cast gel and subjected to electrophoresis at 180 V for an initial 6 min stabilization period, followed by separation at 135 V for 30 min to achieve optimal resolution of nucleic acid species. Upon electrophoresis, the native PAGE gels were stained with GelRed, and the gel images were captured with a Tanon imaging system (Tanon, China).

2.4 Preparation of Anti-FAM-QDs Conjugates

For Anti-FAM-QDs preparation, carboxyamine condensation was performed using a modified coupling method, as previously reported [29, 30]. Initially, 8 μL of the quantum dot stock solution was pipetted into 1 mL of ultrapure water contained in a glass vial. Subsequently, 4 μL of EDC (1 mg/mL), 20 μL of PEG (5 wt%), 20 μL of HEPES (pH 7.3, 1 mol/L), and 60 μL of Anti-FAM (0.55 mg/mL) were sequentially introduced into the mixture. The resulting solution was then incubated on a rotator at ambient temperature in the dark for 3 h. The Anti-FAM-QDs were purified via centrifugation at 3000 rpm for 2 min. To prevent aggregation, Triton X-100 (2.5%) was subsequently added with a volume of 80 μL.

2.5 Construction of Test Strips

A test strip consists of a sample pad, a nitrocellulose membrane, an absorbent pad, and a polyvinyl chloride backing. Initially, the sample pad was immersed in a pretreatment buffer solution (0.01% BSA, 0.05% PVP,1× PBS, 0.1% Triton X-100) for 30 min, then dried at 37 ℃ for 12 h. Next, the sample pad, nitrocellulose membrane, and absorbent pad were sequentially assembled on a PVC backing card with 2 mm overlaps to construct the test strip. Then, the SA (1 mg/mL), Anti-Dig (0.5 mg/mL), Anti-TAMRA (0.05 mg/mL), and Anti-rabbit-antibody (1 mg/mL) were dispensed onto the nitrocellulose membrane at 1 μL/cm using an XYZ platform. Ultimately, the strips were accurately cut to 6 cm × 2 mm with a programmable strip cutter and stored at ambient temperature in light-protected bags.

2.6 Detection Procedure of MC-LFA

Using OTA as an example, 10 μL Switch releases all of Probe 2 after reacting with 10 μL of OTA at a variable concentration for 45 min at 37°C. Subsequently, the 3 μL of the above mixed solution, 2 μL 10× HOLMES buffer, 0.3 μL Cas12a, 0.3 μL crRNA, 8 μL HBR, and 6.4 μL ddH_2O were mixed. In tube A, the solution with a total volume of 20 μL was reacted for 15 min at 37°C. AFB1 reacts in tube B under the same conditions as tube A. Following incubation, the contents of Tubes A and B were pooled, and then 54 μL of ddH_2O and 6 μL of Anti-FAM-QDs were added to yield a total volume of 100 μL. The lateral flow test strip was then carefully immersed in the mixture, and fluorescence signals were recorded after a 5 min interval.

2.7 “OR” Logic Gate

After putting 10 μL of Switch for OTA and 10 μL of Switch for AFB1 in one tube, adding the target (10 μL, 2 μg/mL), and incubating for 45 min at 37°C, Probe 2 was fully released. Then, 3 μL of the aforementioned mixed solution was taken, and 2 μL of 10× HOLMES buffer, 0.3 μL of Cas12a, 0.3 μL of crRNA, 8 μL of HBR, and 6.4 μL of ddH_2O were added. The 20 μL of reagent solution was placed in one tube and reacted at 37 ℃ for 15 min. After that, 74 μL of ddH_2O and 6 μL of Anti-FAM-QDs were added to bring the total amount to 100 μL. Finally, the test strip was dipped into the liquid, and fluorescence results were recorded after 5 min.

2.8 Real Sample Analysis

Maize and wheat flour were obtained from a local supermarket. Sample preparation followed established protocols with slight modifications [31, 32]. Briefly, 200 mg of each flour was dissolved in 2 mL of a methanol/water mixture (6/4, v/v), and thoroughly homogenized by vortexing at ambient temperature for 30 min. After centrifugation at 10,000 rpm for 12 min, the supernatant was diluted 10-fold with 1× PBS and spiked with varying concentrations of target analytes. Ultimately, target analyte recovery was assessed using MC-LFA under optimized conditions.

3. Results and Discussion

3.1 The Detection Principle of MC-LFA

The detection principle of MC-LFA is illustrated in Fig. 1A, with the oligonucleotide sequences used in this study provided in **Table S1**. Following sample preprocessing, the interaction between the target and Probe 1 on the Switch involves various intermolecular forces such as van der Waals forces, electrostatic attractions, hydrogen bonding, and π–π stacking of aromatic rings [27, 33–35]. This interaction leads to the release of Probe 2, whose red sequence then binds complementarily to crRNA, thereby activating the trans-cleavage activity of Cas12a. The structure and thermodynamic parameters of Probe 2 are depicted in **Fig. S1**. To enable compatibility with lateral flow assays, a half-blocking reporter (HBR) for Cas12a was designed by site-specifically conjugating digoxin (Dig)/TAMRA, biotin, and FAM groups to the terminal ends of its oligonucleotide strands. Upon binding of the released Probe 2 to crRNA, Cas12a cleaves the single-stranded DNA (ssDNA) portion of the HBR, generating double-stranded Dig/FAM and TAMRA/FAM products. These products exhibit high affinity for Anti-FAM-QDs. The cleaved dsDNA substrates labeled with Dig/FAM are captured at T1 via specific recognition by anti-digoxin antibodies, while those labeled with TAMRA/FAM are immobilized at T2 through binding to anti-TAMRA antibodies. Consequently, OTA and AFB1 were individually detected using Tube A and Tube B, employing the corresponding HBR probes Dig/FAM-Biotin and TAMRA/FAM-Biotin, respectively.

As shown in Fig. 1B, four test lines were designed to detect MC-LFA output signals, with streptavidin, anti-digoxin, anti-TAMRA, and anti-rabbit antibodies immobilized on the SA, T1, T2, and C lines, respectively. Noteworthy, intact HBR and single-stranded sequences were successfully captured at the SA line via biotin-streptavidin interaction, serving as an alternative to conventional magnetic separation methods. The digoxin- and FAM-labeled cleaved dsDNA products are immobilized at the T1 line through specific binding to anti-digoxin antibodies, enabling the detection of OTA. Similarly, cleaved dsDNA substrates labeled with TAMRA/FAM are captured at the T2 line by specific binding to anti-TAMRA antibodies for AFB1 detection. The control (C) line contains immobilized anti-rabbit antibodies that specifically bind to anti-FAM quantum dots (QDs), thereby verifying the validity of the lateral flow assay. Fluorescence signals from the target analytes can be visualized under a handheld UV lamp (365 nm) using a smartphone camera, and quantitative analysis is performed with ImageJ software by calculating the fluorescence intensity ratio between the T-line and C-line, expressed as FIT/FIC. In this study, a duplex Switch consisting of two distinct DNA molecules, designated as "Probe 1" and "Probe 2", was employed. Probe 1 incorporates both aptamer and non-aptamer sequences. The non-aptamer portion and a segment of the aptamer are complementary to a region of Probe 2, facilitating the formation of a stable duplex structure between the two probes. The formation of this stable duplex is engineered to effectively block the red sequence in probe 2 from binding to the crRNA, thus inhibiting the activation of Cas12a's trans-cleavage activity. Upon target binding, Probe 1 selectively forms an aptamer–target complex, which enhances the accessibility of the red sequence linked to the crRNA. Consequently, the trans-cleavage activity of Cas12a is activated. Under non-target conditions, the non-aptamer region and a segment of the aptamer domain in Probe 1 sterically hinder the Cas12a-crRNA complex from binding to the complementary sequence in Probe 2. This inhibition is alleviated upon aptamer–target complex formation. A key advantage of this switching mechanism lies in the functional independence of the

aptamer from the activator, enabling theoretical adaptation to any target through modification of the aptamer sequence alone.

3.2 Feasibility of the Method

The preparation and characterization of Anti-FAM-QDs are illustrated in **Fig. S2**. The UV-vis absorption profiles of the aptamer and its conjugates are provided in **Fig. S3**. As indicated in Fig. 2A, Cas12a displayed no catalytic activity toward the cleavage of ssDNA, HBR (lanes 2 and 4), or ssRNA (**Fig. S4**) in the absence of a target sequence. PAGE analysis in Fig. 2B reveals that the presence of Probe 2 or S (red sequence) induced degradation of the ssDNA substrates (lanes 3 and 5). To further evaluate the system's response to the target, a fluorescence-based cleavage assay was performed. As anticipated, target activation resulted in a marked increase in fluorescence intensity (Fig. 2C). Since Cas12a specifically cleaves ssDNA rather than dsDNA [28], a HBR was incorporated into the MC-LFA design (Fig. 2D). Consistent with this specificity, the PAGE results in Fig. 2D confirm that the double-stranded region remained intact, with cleavage confined exclusively to the single-stranded portion. Furthermore, the feasibility of the HBR was verified by fluorescence measurements, in which one strand was labeled with HEX and the other was dual-labeled with FAM and BHQ1. In the absence of target activation, no fluorescence signals were observed at 520 nm (FAM) or 556 nm (HEX). Following Cas12a-mediated cleavage, however, a significant increase was detected only in the FAM signal (Fig. 2E), confirming that cleavage was restricted to the single-stranded region labeled with FAM. The decrease in the HEX signal observed in Fig. 2F likely results from enhanced quenching efficiency of BHQ1 after FAM cleavage. This preferential cleavage of the HBR by Cas12a highlights its suitability for integration with downstream lateral flow strip detection systems. To facilitate multiplexed visual detection, lateral flow strips were designed with four separate test lines for use in the MC-LFA system. Intact HBR and ssDNA substrates were captured at the SA line, while cleavage of the dsDNA substrate produced signals at the T1 and T2 lines. The performance of the MC-LFA system was assessed across a range of antibody concentrations, with optimization of the T1 and T2 concentration ratios presented in **Fig. S5**.

3.3 Analytical Performance of MC-LFA

To achieve optimal analytical performance of the proposed mycotoxin detection system, a comprehensive evaluation of key experimental parameters was performed (**Fig. S6-S7**). Using the optimized parameters obtained above, the MC-LFA method was subsequently applied to detect OTA and AFB1. As shown in Fig. 3A and 3B, a strong linear correlation was observed between the fluorescence intensity ratio and the corresponding target concentrations. The linear detection ranges for OTA and AFB1 were determined to be 0.05μg/mL and 0.5 ng/mL to 5 μg/mL, respectively, with visual detection limits of 50 pg/mL for OTA and 250 pg/mL for AFB1. Compared with previous studies (**Table S2**), the MC-LFA approach exhibits enhanced performance, characterized by a wider dynamic range and higher sensitivity. The study revealed a logarithmic relationship between analyte concentration and signal intensity. The binary Cas12a complex was demonstrated to recognize single-stranded DNA independently of a protospacer adjacent motif (PAM), leading to robust and non-specific cleavage of

ssDNA. Cas12a, an endonuclease comprising roughly 1,200-1,500 amino acids [28], exhibits trans-cleavage activity upon binding to its activator molecule. Stoichiometric titration experiments determined the catalytic efficiency of this non-specific ssDNA cleavage to be $5.1 \times 10^8\ s^{-1}M^{-1}$. This logarithmic correlation can thus be attributed to Cas12a's specific target recognition coupled with its highly efficient non-specific cleavage activity.

The detection performance of the sensors was further assessed using a commercial colloidal gold immunoassay. The MC-LFA presents a marked improvement in visual sensitivity, achieving a two-order-of-magnitude enhancement for OTA (0.05 ng/mL compared to 5 ng/mL) (Fig. 3C) and a one-order-of-magnitude increase for AFB1 (0.25 ng/mL versus 5 ng/mL) (Fig. 3D). Considering that a spiked concentration of 0.1 ng/mL corresponds to a sample concentration of 1.1 ng/g,20 the estimated visual detection limits for OTA and AFB1 are approximately 0.55 ng/g and 2.75 ng/g, respectively. Notably, the MC-LFA complies with the strictest regulatory standards set by the European Commission, which specify maximum limits of 5.0 μg/kg for OTA and 12 μg/kg for AFB1[20].

3.4 Double-Blind Experiment

Qualitative analysis is frequently employed for on-site detection owing to its efficiency and rapidity, especially when binary outcomes such as "positive" or "negative" are required. In this work, the accuracy of the MC-LFA detection system was assessed using a double-blind experimental design. One investigator prepared 36 samples in total, comprising 18 negative controls and 18 positive samples spiked with AFB1 (2 μg/mL) and OTA (2 μg/mL), respectively. A second investigator, blinded to sample identities, independently performed the detection using the MC-LFA platform. As depicted in Fig. 3E, the OTA test strip correctly identified all 18 positive samples and 17 of the 18 negative samples, yielding a sensitivity of 100%, a specificity of 94.44%, and an overall accuracy of 97.22%. For AFB1, the assay accurately classified 17 positive and 17 negative samples, resulting in both sensitivity and specificity of 94.44% and an overall accuracy of 94.44% (Fig. 3F). These findings demonstrate that the proposed method provides highly reliable on-site qualitative detection, with particularly strong performance in identifying positive cases. Subsequently, the test strips were applied to artificially simulated negative and positive samples to evaluate potential inter-batch variability (**Fig. S8-S9** and **Table S3-S4**).

3.5 "OR" Logic Gate Detection

As illustrated in Fig. 4A, by co-localizing the detection probes for OTA and AFB1 in a single reaction tube, Probe 2 is generated upon recognition of either OTA or AFB1. This activates Cas12a, leading to cleavage of the HBR reporter (Dig/FAM-Biotin). The detection of either analyte yields a positive signal, thereby realizing an "OR" logic gate analogous to Boolean algebra. Consequently, only one test line is required on the lateral flow strip (Fig. 4B). In this design, OTA and AFB1 act as input signals. The output is defined as 1 when one or both inputs are present, and 0 otherwise. This "OR" logic-based approach has been successfully applied to detect OTA and AFB1 contamination in spiked maize and wheat samples, highlighting its potential as an efficient strategy for food safety assessment. Furthermore, the strip's practicality and accuracy were evaluated by spiking commercial maize and wheat flour samples with

varying concentrations of the target analytes, alongside naturally mold-contaminated food samples obtained by exposing maize and wheat flour to a high-risk contamination environment at ambient temperature for several days (**Table S5** and **Fig. S10**).

4. Conclusions

In summary, the CRISPR/Cas12a-assisted lateral flow assay has been established as a highly versatile platform for multiplex detection of OTA and AFB1. This strategy requires only aptamer modification, while Cas12a and crRNA remain unaltered, significantly reducing experimental costs. Relative to commercially available colloidal gold test strips, the developed method demonstrates a 10- to 100-fold enhancement in detection sensitivity. Integration of an “OR” logic gate into the test strip design effectively broadens the spectrum of detectable targets. Notably, the signal-on mode is achieved directly, eliminating the need for complex steps such as magnetic bead separation. This work highlights the substantial potential of the MC-LFA strategy for food safety monitoring in resource-limited settings, positioning it as a promising tool across diverse analytical fields, including public health, agriculture, and environmental surveillance.

Declarations

CRediT authorship contribution statement

This manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contribution

W.J. and Y.Z. wrote the original draft, performed visualization, validation. J.K. and Z.Z. conducted formal analysis and data curation. K.Q. provided resources and methodology support. Q.X. performed writing - review & editing, project administration, and supervision. Q.W. provided resources and methodology support. Y. Q. and L.W. conducted writing - review & editing, validation, supervision, and conceptualization. H.J. conducted funding acquisition, supervision, conceptualization, and project administration. All authors reviewed the manuscript.

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Data Availability

All data supporting the findings of this study are available within the paper and its Supplementary Information.

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Figures

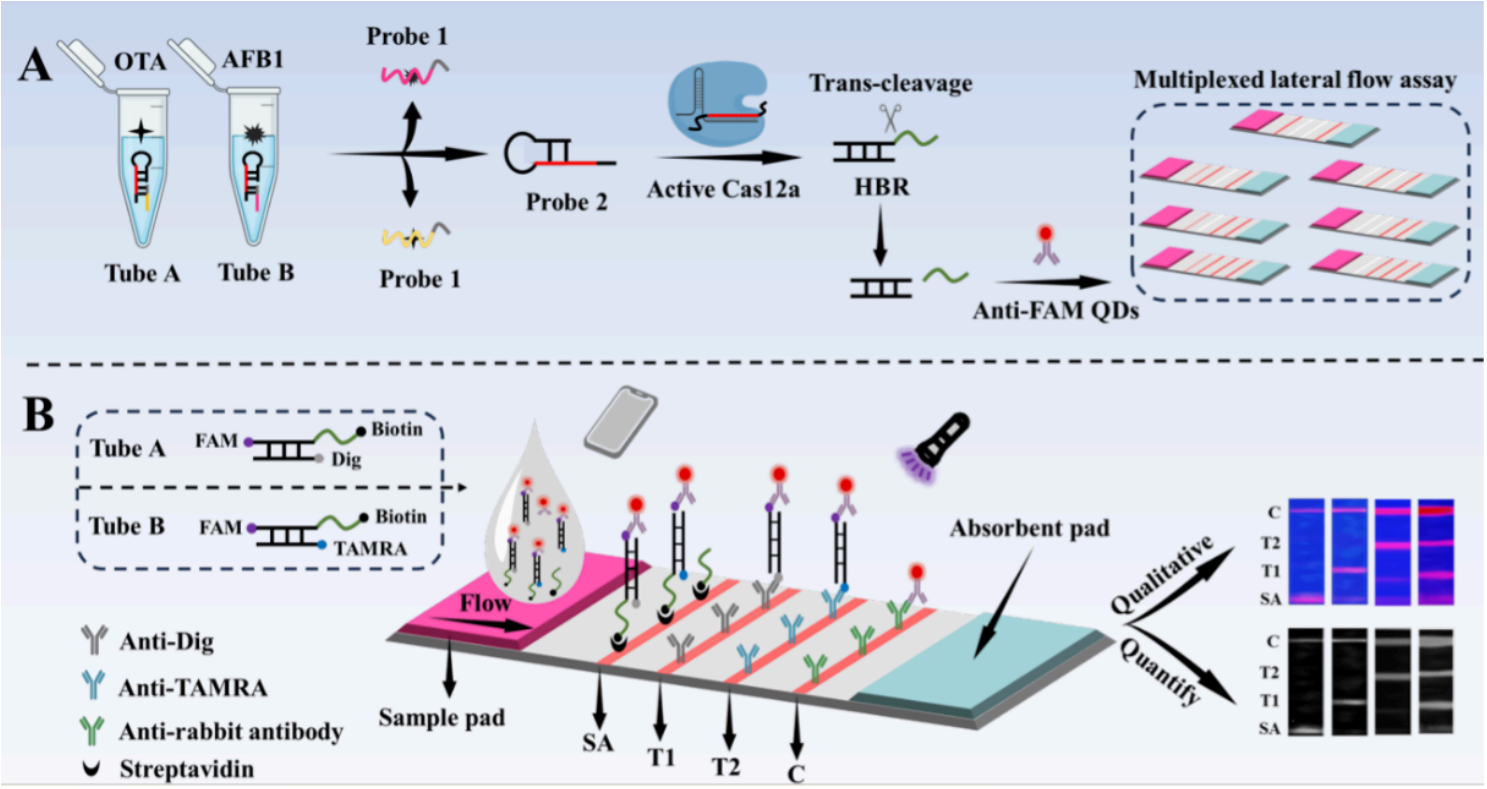


Figure 1

(A) The principle of MC-LFA. (B) The design and downstream detection of MC-LFA.

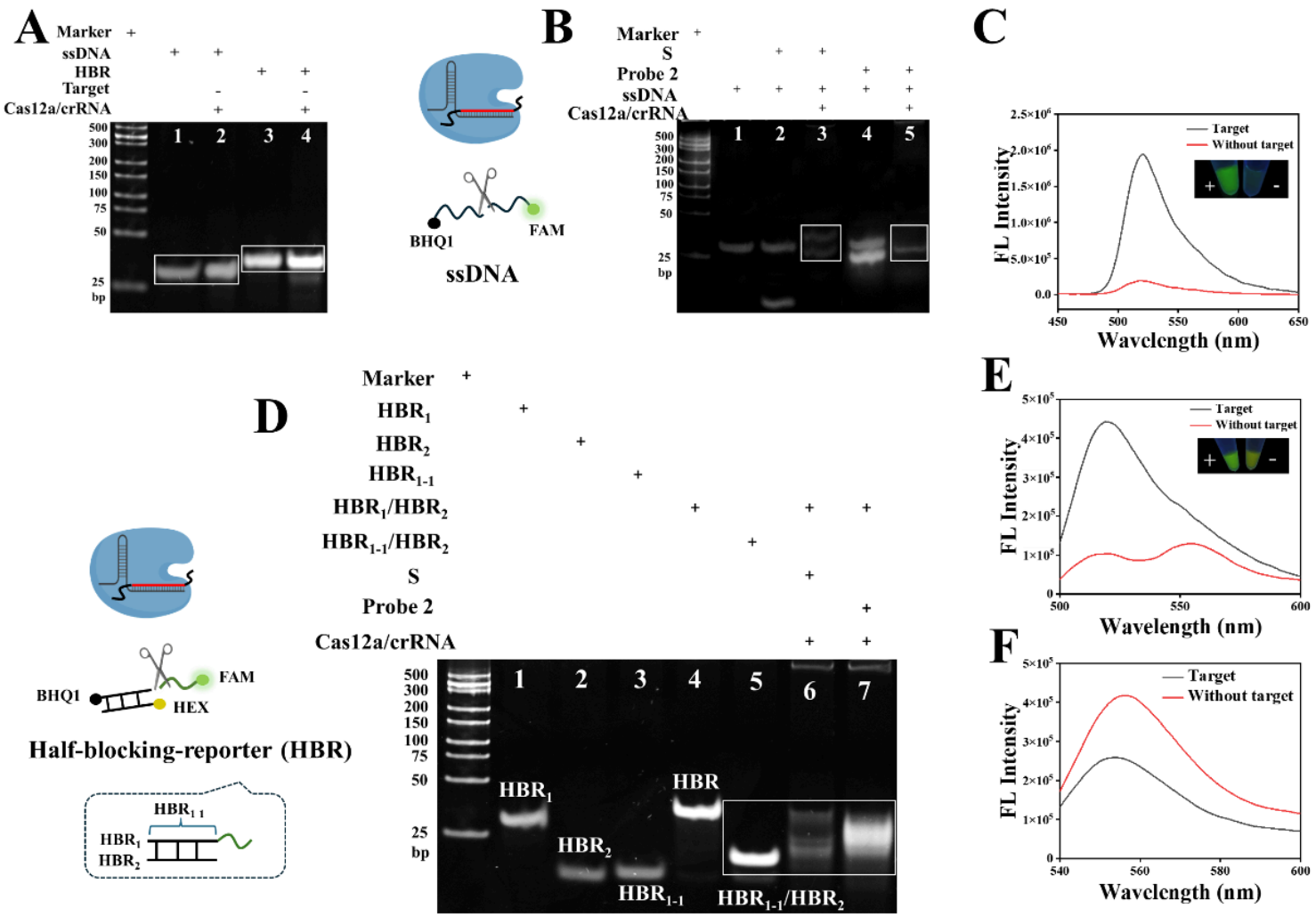


Figure 2

Verification of the CRISPR-Cas12a system. (A) Illustration of the Cas12a-mediated ssDNA and HBR cleavage without a target (probe 1 of OTA hybridizes with probe 2 + Cas12a/crRNA + reporter). Native PAGE analysis (B) and fluorescence intensity (C) were performed to evaluate the *trans*-cleavage activity of Cas12a towards ssDNA substrates upon target activation. Native PAGE analysis (D) and fluorescence intensity (E is of FAM, F is of HEX) were performed to evaluate the *trans*-cleavage activity of Cas12a towards HBR substrates upon target activation.

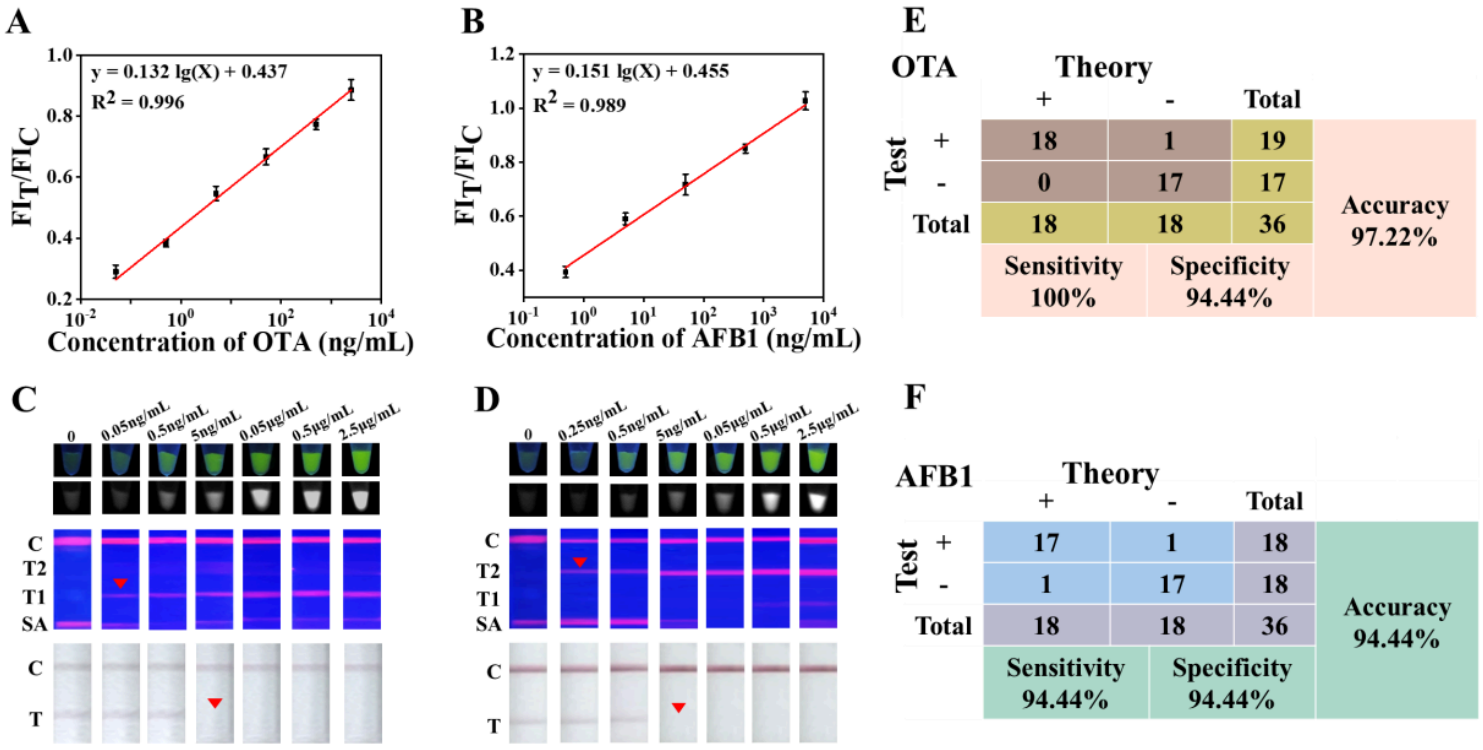
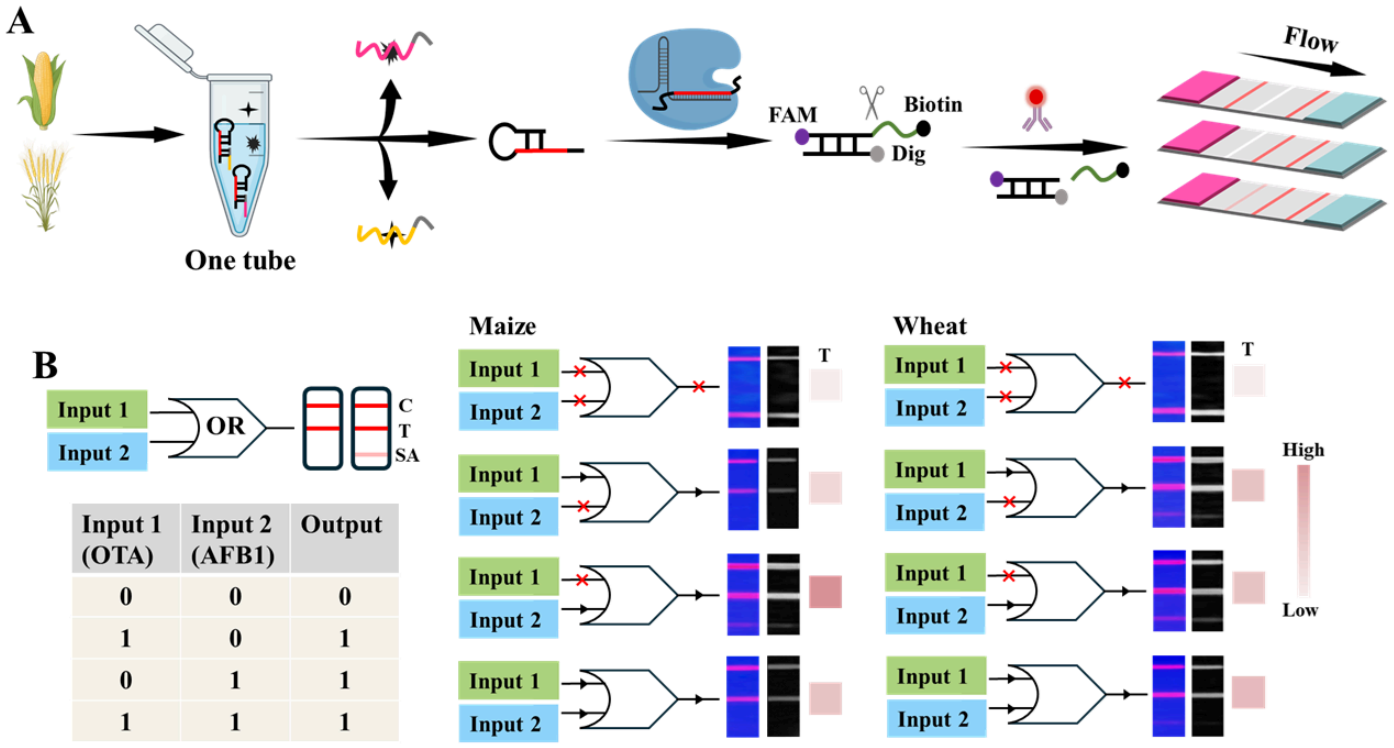


Figure 3

Linear correlation of OTA (A) and AFB1 (B), respectively. Photographs of a series of tubes, MC-LFA, and commercial colloidal gold test strips in response to different concentrations of OTA (C) and AFB1 (D). Double-blind experiment of MC-LFA (E, F). The error bars were derived from three parallel measurements.



Input 1 (OTA)	Input 2 (AFB1)	Output
0	0	0
1	0	1
0	1	1
1	1	1

Figure 4

(A) The principle of MC-LFA in one tube. (B) Two-input “OR” logic gate strategy in spiked maize and wheat.

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