

For research article

Response to Reviewer 3 Comments

1. Summary

We highly appreciate the editor's and reviewers' kind consideration of the scientific content of our work. We have studied comments carefully and have made corrections which we hope to meet with approval. Revised portion are marked in red in the manuscript. Detailed reply to the comments and suggestions has been made as follows.

2. Questions for General Evaluation	Reviewer's Evaluation	Response and Revisions
Does the introduction provide sufficient background and include all relevant references?	Yes	Thanks for the review.
Is the research design appropriate?	Yes	Thanks for the review.
Are the methods adequately described?	Can be improved	A response has been made in the point-by-point reply letter.
Are the results clearly presented?	Can be improved	A response has been made in the point-by-point reply letter.
Are the conclusions supported by the results?	Yes	Thanks for the review.

3. Point-by-point response to Comments and Suggestions for Authors

Comments 1: In "Keywords" replace the abbreviation "PCM" with the word "Procymidone".

Response 1: Thanks for checking carefully and asking questions, we have made changes to the abbreviations in the keywords

Keywords: immunochromatographic (IC) test; time-resolved fluorescence; AuNPs; **Procymidone**; vegetables.

Comments 2: Are there maximum permissible concentrations of Procymidone in vegetables? How do the LOD and LR found in this study compare with maximum permissible concentrations?

Response 2: Thanks for your valuable advice. Different countries have different detection amounts for different kinds of vegetables, we added a general range in the paper, and the detection limit of our method is much lower than the allowable detection amount, so the method can meet the detection requirements in application.

By testing various vegetable samples, the characteristics and advantages of colloidal gold and fluorescence immunochromatographic test strips are compared, demonstrating their

applicability in detecting pesticide residues. According to the International Codex Alimentarius, the amount allowed to be detected in vegetables is generally 0.2-5 mg/kg. The combination of both test strips in food safety monitoring prioritizes colloidal gold test strips for rapid screening, followed by fluorescence test strips for precise measurement of contaminant levels, forming a multimodal detection method that leverages the advantages of both technologies.

Comments 3: It is advisable to decipher the abbreviations in the "Method" column of Table 1.

Response 3: Thanks for your reminder, we will explain the method mentioned in Table 1 in the introduction section, and ensure that the abbreviation is marked when it is first mentioned in the paper.

Compared with other published detection methods, this mode can not only realize the field detection of the target, the LOD and detection width are enough to meet the minimum requirements of the detection limit of the target, but the cost per sample is reduced by 50%-80% compared with other methods ,for example Thermal Desorption-comprehensive Gas Chromatography-Mass Spectrometry (TD-cGC-MS), Gas Chromatography-Mass Spectrometry (GC-MS), Matrix Solid-Phase Dispersion-Gas Chromatography-Electron Capture Detection (MSPD-GC-ECD), Enzyme-Linked Immunosorbent Assay (ELISA), and High Performance Liquid Chromatography-Diode Array Detection (HPLC-DAD),while reducing detection time and costs (Table 1).

Comments 4: The text uses the abbreviation of the word "Procymidone" as both PCM and FRM (lines 39, 204, 261, 292-294). Make the abbreviation uniform.

Response 4: Thank you very much for your careful evaluation. We carefully check the abbreviations of the full text to ensure the consistency of abbreviations and uniform use of PCM.

Comments 5: In section 2.2.2, indicate how long the gold sol retains its properties unchanged.

Response 5: Thank you for your valuable comments. What we understand is the storage time of the colloidal gold solution, and since we label the antibody directly after the experimental preparation, no detailed study of the shelf life has been done. Generally, stable colloidal gold solution can be stored for 3-6 months. In the latest experiment, we also used that batch of colloidal gold solution that has been prepared for 6 months, and the experimental effect was good, so we added relevant instructions in the manuscript.

Comments 6: "Negative samples of leeks and peppers were ground and mixed with anhydrous methanol" (line 205). Does methanol affect the T-line color intensity and sensitivity of Procymidone determination using the FLMICS and GLMICS.

Response 6: Thank you for your constructive comments. We carefully considered this issue and conducted comparative experiments on the adjustment of methanol dosage, and found that the experimental results did not change significantly. After reviewing the literature, it can be attributed to the following reasons: Under the experimental conditions, methanol, as an extraction solvent, has undergone subsequent dilution, pH adjustment and volatilization, and its residual amount has no significant influence on the color intensity and sensitivity of T-lines of FLMICS and GLMICS. The strip's material design (e.g., PVA stabilizer, blocking protein) and optimized operation process effectively offset the potential interference of methanol and ensure the reliability of the test results

Comments 7: In section 3.1, describe not only the color of the T-line, but also the C-line with

and without PCM on FLMICS and GLMICS.

Response 7: Thank you for your valuable comments! We have added a detailed description of line C (control line) in Section 3.1 to clarify the color rendering of T and C lines under different sample conditions (with or without PCM). Specific modifications are as follows:

In the part of detection principle of FLMICS and GLMICS, it is explained that both T line and C line show color in negative samples, while T line signal weakens while C line keeps color in positive samples.

The function of C line as a quality control line is emphasized to ensure the effectiveness of the test strip.

These additions enhance the completeness of the description of the method and help the reader to understand more clearly how the strips work and the result interpretation criteria.

In the immunochromatographic test, both T line and C line were chromogenic under the negative sample: T line was chromogenic due to antigen-antibody binding, and C line was chromogenic through the combination of unbound labeled antibody and fixed antibody, verifying the normal function of the test strip. In positive samples, the target competes to suppress T-line color rendering, while C-line color is always displayed to ensure detection validity. C line as the core quality control standard, if there is no color, the result is invalid.

Comments 8: Why is the highest fluorescence signal obtained at pH 8 (lines 250-251)? Please, explain.

Response 8: Thanks for your helpful comments, the experimental results showed that the fluorescence intensity was significantly higher at pH 8 than at other pH values (Figure 5A), indicating that the antigen binding capacity of the coupled probe was strongest at this condition. After reviewing the literature, we found that its theoretical mechanism is:

Activation of carboxyl group (-COOH) on the surface of fluorescent microspheres with EDC and NHS of antibody amino group (-NH₂). In the weakly alkaline environment of pH 8, carboxyl group is fully deprotonated and is more easily activated by EDC/NHS to generate active ester intermediates, which promotes stable covalent binding with antibody and maximizes antibody coupling efficiency.

At the same time, PBS at pH 8 provides moderate ionic strength, inhibits the aggregation of fluorescent microspheres and maintains their monodispersion, thereby increasing the effective coupling surface area. This pH is close to the isoelectric point of the antibody (pI ≈ 6-8), reducing the conformation distortion caused by charge rejection and preserving the antigen binding activity of the antibody

Comments 9: The description of Fig. 5B and 5C is mixed up in the text. Replace Fig. 5B with 5C (lines 254-255) and also replace Fig. 5C with 5B (line 258).

Response 9: Thank you for your careful comment, our BC picture in Figure 5 is in the wrong position. We have corrected the order, both in the text and in the note.

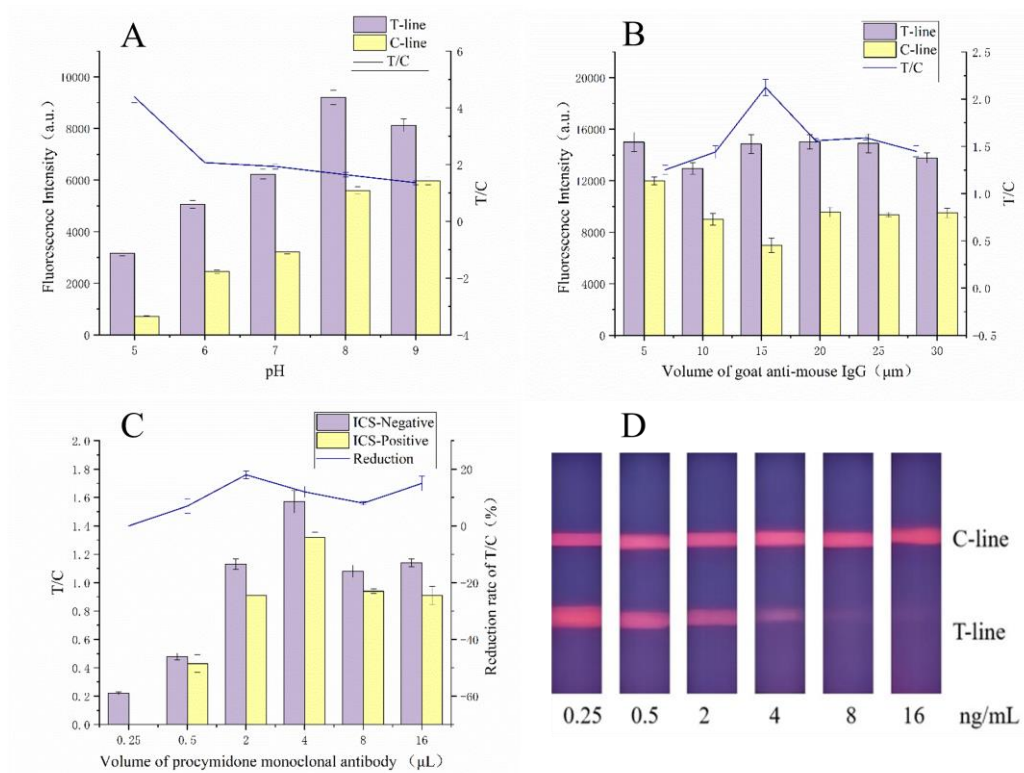


Figure 5. Parameter optimization of FLMICS: (A) pH is 8, the fluorescence signal is the strongest and T/C is stable, which is the best pH value; (B) the volume of GaMIgG is 15μL, T/C reaches its maximum; (C) volume of PCM mAb is 2μL, the fluorescence intensity was excellent and T/C was stable; (D) Performance of test strips.

Comments 10: Decipher the designations N and P on the strips in the caption to Fig. 6.

Response 10: Thank you very much for your careful review of the article, we really did not do enough to explain N and P.

N (negative control) : In the samples without the addition of humus, the color intensity of the T-line reflects the coupling efficiency and the background signal.

P (positive control) : For samples containing saprophyrin, the degree of T-line signal inhibition reflects the detection sensitivity.

We have added an explanation to the diagram.

Figure 6. Parameter optimization of GLMICS, **N (Negative Control)** and **P (Positive Control)** . (A) The colorimetric effect is significant when the added volume is 8μL. (B) The concentration of the antigen is selected 0.3mg/mL.

Comments 11: Has the selectivity of the developed Paper-Based Sensors been tested against structural analogues of Procymidone, such as Vinclozolin, Chlozolate, Iprodione, Imidacloprid?

Response 11: Thank you for your careful review of this article and your valuable comments. As for the selectivity of sensors for PCM structural analogues mentioned by you, **in this paper**, we mainly focus on the development and optimization of FLMICS and GLMICS dual-mode detection methods, and focus on verifying their sensitivity, accuracy, applicability to actual samples and ease of operation. However, it is true that the selectivity of structural analogues of saprophyticus has not been systematically tested. We fully agree with you on the

importance of selective validation, and we will explicitly mention this limitation in the revised draft, and highlight the importance of selective validation and future research directions in the discussion section. Your questions provide us with an important direction to further improve the research, we are deeply grateful!

Comments 12: The fluorescence intensity of the T-line and C-line is a dynamic process that changes over time. How was the moment of fluorescence intensity measurement determined?

Response 12: Thank you very much for your constructive evaluation. There is indeed room for improvement in the calculation of fluorescence intensity. In this study, we observed no significant attenuation of fluorescence signal within 10 minutes after the chromatographic stop through pre-experiment, which was determined based on a standardized 10-minute detection process. The fluorescence intensity measurement time is our next focus. At present, we use a real-time fluorescence imaging system to capture dynamic signals, record the signal change process, and optimize the optimal reading time. However, relevant algorithms need to be further improved.

Comments 13: What is the lifetime of the developed Paper-Based Sensors?

Response 13: Thank you for your valuable evaluation. Our paper-based sensor can be used normally when it is stored in a dry and light-free environment at 4°C for 6 months, but the specific life needs to be further verified by long-term stability experiments. We will supplement relevant data in the future to support reliability evaluation in practical applications.

Comments 14: Section 4 should be «Conclusion», not “Discussion” .

Response 14: Thanks for your reminding, we have changed the manuscript and used the Conclusion correctly.

4. Response to Comments on the Quality of English Language

Point 1: The English is fine and does not require any improvement.

Response 1: Thank you for the review.

5. Additional clarifications

We acknowledge the issues identified in the review and reiterate our gratitude to the reviewers and editors.