Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
31 (2): 163-170, 2025
DOI: 10.9775/kvfd.2024.32812

RESEARCH ARTICLE

A Simple Colorimetric Detection of *Haemophilus parasuis* Based on Aptamer-Functionalized Gold Nanoparticles

Shouping ZHANG [1,†] Mengting ZHANG [1,†] Kunxin LIU [1] Lirong WANG [1] Lei WANG [1] Jianhe HU [1 (*)]

† *These author contributed equally to this research*

[1] College of Animal Science and Veterinary Medicine, Henan Institute of Science and Technology, Xinxiang 453003, CHINA

(*) **Corresponding author:**
Jianhe HU

Phone: +86-0373-3040718
Cellular phone: +86-0373-3040718
Fax: +86-0373-3040718

E-mail: vet_jianhe@sina.com

How to cite this article?

Zhang S, Zhang M, Liu K, Wang L, Wang L, Hu J: A simple colorimetric detection of *Haemophilus parasuis* based on aptamer-functionalized gold nanoparticles. *Kafkas Univ Vet Fak Derg,* 31 (2): 163-170, 2025. DOI: 10.9775/kvfd.2024.32812

Article ID: KVFD-2024-32812
Received: 19.08.2024
Accepted: 04.03.2025
Published Online: 18.03.2025

Abstract

Porcine *Haemophilus parasuis* disease, also known as Graze's disease, is the leading bacterial infectious disease caused by *H. parasuis* in modern pig farms. Due to the high nutritional requirement of *H. parasuis,* it usually gets a false positive result through the traditional methods of plate culture. Therefore, it is necessary to establish a simple and fast detection method for this pathogen identification. This work aimed to develop a colorimetric probe using aptamer-functionalized gold nanoparticles for identifying *H. parasuis*. The detection mechanism is based on the color change of gold nanoparticles (AuNPs) from red to blue-purple through NaCl induction after bacteria incubation and aptamer-target binding. First, aptamer-functionalized gold nanoparticles were synthesized and characterized. Then, the best concentration of aptamers and NaCl for detection was optimized at 80 pM and 0.25 M. Under the above optimized conditions, a good linear relationship between the absorbance ratio A678/521 and *H. parasuis* over the range from 10^3 to 10^9 CFU/mL. The limit of detection (LOD) is 12 CFU/mL. This method is simple and rapid, results in high sensitivity and specificity, and can be used to detect actual samples.

Keywords: Aptamers, Gold nanoparticles, Rapid detection, Colorimetric aptasensor, *Haemophilus parasuis,* Pig

INTRODUCTION

Haemophilus parasuis is a member of the *Pasteurellaceae*, and belongs to the Gram-negative microbiota with diverse forms. *H. parasuis* usually settles on the upper respiratory tract in pigs. Under specific conditions, the virulent strains invade the mucosal layer of the respiratory tract and colonized, destroy epithelial tissue, enter the bloodstream and quickly spread to lung tissue, causing damage and pneumonia symptoms, or invade other organs, leading to multiple fibrous pleurisy, peritonitis, and arthritis. With the worldwide prevalence of *H. parasuis* and the harm and economic losses it brings to the pig industry, early diagnosis and measures to treat *H. parasuis* disease are of great significance [1,2].

In recent years, with the rapid development of molecular biotechnology, various detection methods have been established according to the *H. parasuis* genome sequence, such as conventional PCR, real-time fluorescence quantitative PCR, digital PCR, and loop mediated isothermal amplification technology [3-7]. The application of these rapid molecular detection methods depend on expensive instruments. At the same time, immunoserological detection methods including enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA), and complement binding assay (CF) for *H. parasuis* antibody investigation has been established [8-10]. However, due to the large number of *H. parasuis* serotypes and the lack of specific antibodies, the specificity of currently established serology methods is not high, making them unsuitable for large-scale promotion. These methods based on molecular biology, immune serology and other fields have opened up new ideas for the detection of *H. parasuis* [11,12]. But these above methods have more or less drawbacks, which is not appropriate for routine clinical detection and epidemiological

investigation. Therefore, there is an urgent need to develop simple and fast *H. parasuis* detection methods.

Aptamers, also called chemical antibody are a recognition molecule that has emerged in recent years, and can specifically bind to target molecules. Compared with traditional antibodies, they have advantages such as a wide range of target molecules, vitro preparation, stable performance, and ease of labeling and development of various detection methods [13]. In recent years, various detection methods have been developed based on aptamers, including colorimetry, electrochemistry, chemiluminescence, fluorescence, etc [14-16]. Nanogold (AuNP) is a popularly used nanomaterial for colorimetric detection. AuNP is easy to prepare and to adsorb biomolecules, has a high extinction coefficient and strong particle spacing optical effect. It appears red in a dispersed state and turns blue after condensation. Conversely, it can also change from blue to red. It has been reported that the sensitivity of colorimetric based on AuNPs can reach that of fluorescence method [17-19]. The rapid detection methods of aptamer AuNPs combined colorimetric technology have developed rapidly attributed to its advantages of simple, fast, sensitive, results visible to the naked eye, and ease of on-site use. It was demonstrated that the limit of detection (LOD) with the naked eye was 10^4 CFU/mL for identifying *P. aeruginosa* based on colorimetric biosensor using aptamer-functionalized gold nanoparticles [20].

In the present work, we report a simple visual and sensitive colorimetric method for detection of *H. parasuis* employing aptamer-functionalized gold nanoparticles. In this assay, the aptamers were used as a specific recognition probe, and AuNPs as a signal detector. Determine the presence of a target by observing the color changes of the solution with the naked eye. At the same time, conditions of temperature, incubation time, the concentration of aptamer and NaCl are optimized.

Material and Methods

Ethical Approval

The experimentation with animals was approved by the Experimental Animal Management Methods of Xinxiang Medical University (Approval number: 201206078) and followed Henan Authority's Experimental Animal Regulations.

Bacterial Strains

Staphylococcus aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 19111), *Pasteurella multocida* (ATCC 19427), *Actinobacillus leuropneumoniae* (ATCC27088), *Vibrio parahaemolyticus* (ATCC33847), and *Salmonella enterica* (ATCC 14028) were obtained from the China General Microbiological Culture Collection Center (Beijing, China). *S. aureus, E. coli, S. enterica* and *V. parahaemolyticus* were cultured in nutrient broth medium (V.p containing 3.5% NaCl). *Haemophillus paragallinarum* and *Actinobacillus pleuropneumoniae* was cultured in trypticase soy broth medium containing NAD (10 µg/mL) and 5% (v/v) FCS, while *L. monocytogenes* and *Streptococcus suis* were cultured in brain-heart infusion (BHI) broth respectively at 37°C with 180 rpm shake for 18-24 h. The cultured bacteria were then serially diluted and surface plated on the agar dish containing the appropriate culture medium.

Preparation of AuNPs

The AuNPs was synthesized by citrate reduction of $HAuCl_4$ method according to AuNPs synthesis methods with some modification reported previously [21]. First, 1 mL $HAuCl_4 \cdot 4H_2O$ (1%, w/w) and 99 mL ultrapure water were added. The mixture was heated to boil, 10 mL sodium citrate (1%, w/w) was rapidly injected and reacted for 10 min under magnetic stirring. The solution changed color from pale yellow to purplish red and then to wine red. The obtained red wine solution was AuNPs. The AuNPs were purified for three times with a centrifugation (10.000 rpm, 25 min) and re-dispersed in 40 mL ultrapure and filtered through a 0.22-µm cellulose membrane and stored at 4°C. The AuNPs were analyzed using UV-Vis spectra, TEM and Dynamic Light Scattering (DLS). The AuNPs solution was stored at 4°C for further use.

Preparation of Aptamer-AuNPs probe and Aptamer Concentration Optimization

The *H. parasuis* aptamer discovered following an X-aptamer screening kit (Sangon Biotech, 1004728-0000) (5'-TATGGCGGCGTCACCCGACGGGGACTTGACATTATG ACAG-3') was dissolved in DEPC solution with a series concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 pM) [22]. 100 µL aptamer with different concentration was taken to 500 µL AuNPs solution and mixed it respectively. The above mixture was co-cultured in 37°C shaker for 30 h. Lastly, 60 µL 200 mM NaCl solution was added into the above mixture. The color change of the solution was observed, and characterized using a UV spectrophotometer in the wavelength range at 400 and 700 nm after incubated in a 37°C shaker for 30 min.

After that, 400 µL supernatant were discarded after 10.000 rpm centrifuged for 10 min at 4°C. The aptamer-AuNPs probe was stored at 4°C in dark for further use.

Detection of *H. parasuis*

90 µL aptamer-AuNP probe was added to the 96-well plate, then 100 µL *H. parasuis* solution was added with a concentration of 10^6 CFU/mL to the well. Then 10 µL 200 mM NaCl was added to the well and mixed it. The color change of the solution was observed, and characterized using a UV spectrophotometer in the wavelength range

at 400 and 700 nm after incubated in a 37°C shaker for 30 min.

Optimization of NaCl Concentration

A volume of 20 µL NaCl in different concentrations (0, 1, 2, 2.5, 5, 10, 20, 25, 30, 40 M) was mixed with 180 µL of aptamer-AuNPs in a 96-well microplate. The color change was observed and characterized using a UV spectrophotometer in the wavelength range at 400 nm and 700 nm.

Specificity of the Colorimetric Method

100 µL *H. paragallinarum*, *S. enterica*, *P. multocida*, *A. pleuropneumoniae*, *V. parahaemolyticus*, *L. monocytogenes*, *S. aureus*, *S. suis* and *E. coli* bacterial solutions were added that contain same amount to *H. parasuis* (10^5 CFU/mL) and were tested in accordance with the optimal procedure to detect the specificity of this method.

Results

Principle of the Strategy

Firstly, single stranded DNA of *H. parasuis* specific aptamers can absorb onto the surface of AuNPs forming aptamer-AuNPs probe through electrostatic forces, protecting AuNP from aggregation in high salt concentration solutions; after adding *H. parasuis* to the aptamer-AuNPs probe solution, the aptamer has high affinity to *H. parasuis* that dissociates from the surface of AuNPs and forming a composite structure of aptamer-*H. parasuis*. Finally, adding a certain concentration of salt solution to the reaction system can cause unprotected AuNPs to aggregate under the action of salt ions, turning the solution purple or blue, and the degree of discoloration of the AuNPs solution is positively correlated with the *H. parasuis* concentration *(Fig. 1)*. Thus, a rapid detection technology for *H. parasuis* based on aptamer AuNPs colorimetric probe was established.

Characterization of Aptamer Functionalized AuNPs Probe

A variety of validation methods was conducted to characterize the formation of the aptamer-AuNPs probe complex. The transmission electron microscopy displayed that the AuNPs had a uniform particle size about 18 nm in diameter and with a slight aggregation. After they were coated with aptamer, the AuNPs demonstrated no change in particle size but exhibited better dispersion *(Fig. 2-A)*. The spherical gold nanoparticles were dispersed well attributed to the electrostatic repulsion produced by the negative charges of citrate anions coated on the AuNP surface. The UV-vis absorption spectrum assay demonstrated that the maximum absorption peak of AuNPs is at 520 nm *(Fig. 2-C)*, indicating that the AuNPs were well dispersed in the solution, no change was seen on the absorption peak when the aptamer were added, however, according to the results of dynamic light scattering, the hydrated particle size of the AuNPs increased from 24.62±0.39 nm to 28.92±0.46 nm after they were coated with the aptamer *(Fig. 2-B)*, indicating that aptamer was bound to the surface of the AuNPs.

Optimization of Incubation Time and Reaction Temperature for Aptamer Modified AuNPs

According to Li and Rothberg's research in 2004, single stranded DNA exhibits an irregular curling shape in solution and can be adsorbed on the surface of AuNPs through electrostatic forces, van der Waals forces, and water transport forces [23]. And the degree of adsorption is dependent on temperature. Therefore, this experiment optimized the incubation time and reaction temperature for the adsorption of the aptamer onto AuNPs. By measuring the absorbance values of the centrifuge supernatant at 260 nm at different incubation times under three reaction temperatures (4ºC, 20ºC, and 37ºC), the ratio of the absorbance values to the absorbance values

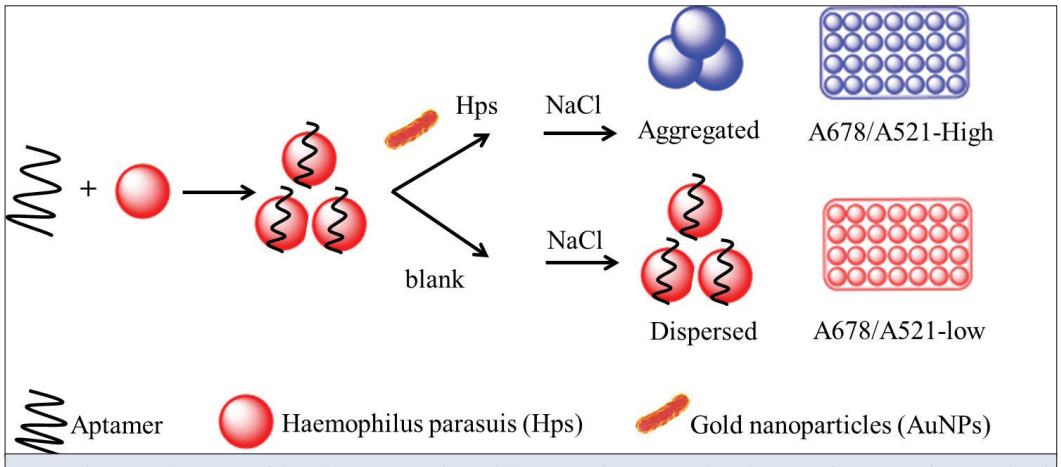

Fig 1. Schematic illustration of the colorimetric and visual detection of *H. parasuis* based on specific aptamer functionalized AuNPs probe

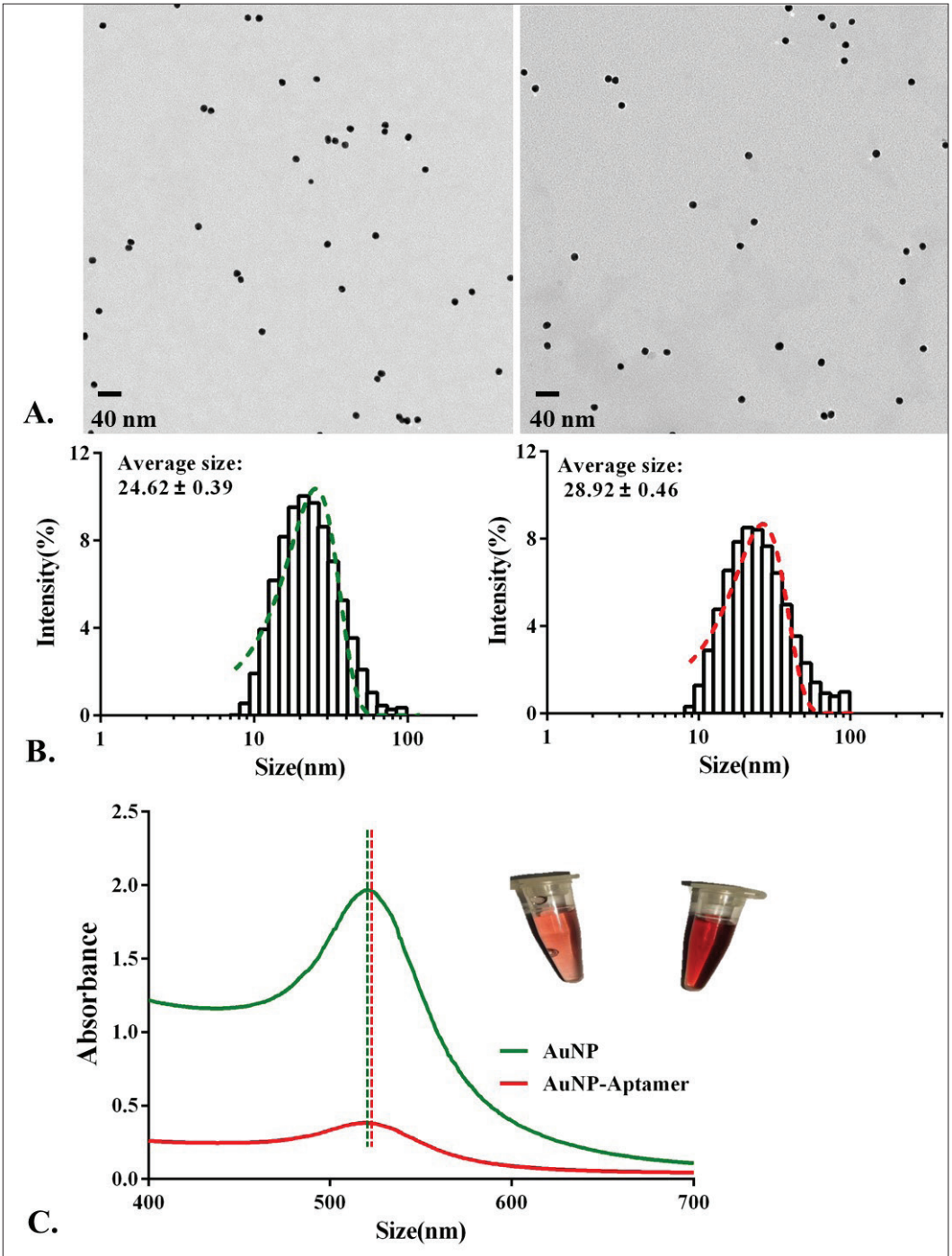

Fig 2. Characterization of AuNPs and aptamer-AuNPs complex. (A) Transmission electron microscopy (TEM) images; (B) Dynamic light scattering (DLS) measurements of the average hydrodynamic diameters; (C) UV-vis spectra

of the initially added adapter solution at 260 nm is the binding rate. The results are shown in *Fig. 3*. At the same temperature, as the incubation time increases, the binding rate continuously increases, reaching its maximum value at 30 h. Afterwards, as time increases, the binding rate no longer changes. As the reaction temperature continues to rise, the binding rate decreases continuously at the same incubation time. When the reaction temperature is 4ºC, the binding rate has reached over 50%. Considering that if the temperature further decreases, the requirements for the reaction equipment will increase, and at the same time, low temperature may accompany with inconvenient operation, the optimal incubation time is selected as 30 h, and the optimal reaction temperature is 4ºC.

Optimization of NaCl Concentration

In general, by adding a high concentration salt solution to an unmodified AuNPs, the originally dispersed AuNPs

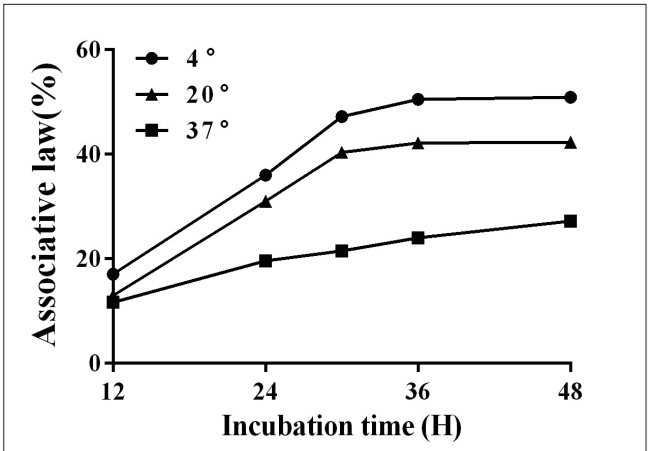

Fig 3. The influence of incubation time and reaction temperature for the binding rate of aptamers coated on AuNPs

will aggregate and forming an irregular network, make the color of the AuNPs change from red to blue. When the aptamer is modified on the surface of AuNPs and the salt concentration is within a certain threshold, due to the electrostatic forces of the aptamer ssDNA on salt ions, its negatively charged phosphate skeleton can effectively protect the AuNPs from aggregation and discoloration due to the presence of salt ions. Therefore, finding the maximum concentration of NaCl solution that can protect the AuNPs from discoloration is a key factor for the success of subsequent experiments. This experiment added 180 µL aptamer-AuNPs complex, then, a gradient increase concentration of 20 µL NaCl solution was added to observe color changes and characterized using a UV spectrophotometer. As shown in *Fig. 4-A*, when the concentration of NaCl solution added increased from 0.1 mol/L to 0.2 mol/L, the absorbance of the solution slightly decreased, and the color of the nanogold system remained red. When further increased to 0.25 mol/L, the color of the nanogold system became purple. The absorption spectrum visible through UV also showed a significant decrease in absorbance at 520 nm, a red shift in peak position, and an increase in absorbance at 678 nm, this indicates that the aggregation of gold nanoparticles occurs, and the concentration of NaCl exceeds the range within which the aptamer can protect the AuNPs from aggregation and discoloration. Therefore, in this experiment, 0.25 mol/L was chosen as the concentration for adding NaCl solution in subsequent experiments.

Optimization of the Ratio Between Aptamers and AuNPs

In order to explore the protective effect of aptamers on AuNPs in high concentration salt solutions, an appropriate amount of aptamers was explored. 100 µL aptamers was added under different dilution concentrations (10 pmol/L, 20 pmol/L, 30 pmol/L, 40 pmol/L, 50 pmol/L, 60 pmol/L, 70 pmol/L, 80 pmol/L) to 500 µL AuNPs reaction system and incubated at 37°C for 30 hours. Then certain amounts of NaCl solution was added to make it final concentration at 25 mM. The color changes were observed visually and characterized by UV spectrophotometer. As shown in *Fig. 4-B*, when the concentration of the aptamer increases from 10 pmol/L to 70 pmol/L, the protective effect of AuNPs becomes stronger, and it is less likely to change under the action of NaCl solution, and it approaches the UV visible

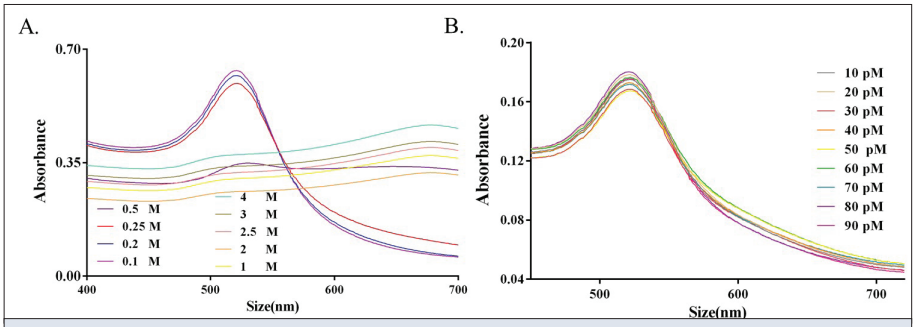

Fig 4. Optimization of the experiment conditions. (A) The UV-visible absorption spectra of AuNPs at different concentrations of NaCl solution (0.1 M, 0.2 M, 0.25 M 0.50 M, 1 M, 2 M, 2.5 M, 3 M, 4 M). (B) The UV-visible absorption spectra of AuNPs at different concentrations of aptamer solution (10 pM, 20 pM, 30 pM, 40 pM, 50 pM, 60 pM, 70 pM, 80 pM, 90 pM)

absorption peak of pure gold nanoparticles solution. When the amount of aptamer added further increased to 80 pmol/L, the UV visible absorption peak did not change significantly and almost overlapped, indicating that the amount of adapter added reached saturation. Therefore, 80 pmol/L was chosen as the optimal addition concentration for the adapter.

Linear Range and Detection Limitation of the Method

In order to explore the linear relationship between *H. parasuis* content and color reaction, the UV-vis absorption spectrum of different concentration of *H. parasuis* in reaction system was detected. When the addition of *H. parasuis* is 10 cfu/mL, no color change was observed and the UV-vis absorption spectrum almost overlapped with the results of the control group without the addition of *H. parasuis* bacteria. Due to low concentration of *H. parasuis*, the amount of AuNPs detached from the ligand protection is very small, and the NaCl solution is not enough to cause the aggregation of AuNPs make obvious color change of the solution. As the concentration of *H. parasuis* solution increases, color changes from red to purple obviously. And the absorption peak of AuNPs shifts red and the peak width gradually increase. The characteristic peak of AuNPs at 521 nm gradually decreases, while the absorption peak near 678 nm gradually increases. The values of A678/A521 also show regular changes. When the concentration of bacterial solution reaches 10^8 CFU/mL, the change is no longer obvious, possibly because all AuNPs have separated from the aptamer and the bacterial solution concentration has reached saturation. The concentration of *H. parasuis* solution ranges from 10^3 CFU/mL to 10^9 CFU/mL *(Fig.5-A)*, and the value of A678/A521 is linearly correlated with the number of colonies. The linear regression equation is Y=0.0817X+0.2075 (R^2=0.9923), with a LOD of 12 CFU/mL.

According to the above method, specificity studies were conducted using the same concentration (10^5 CFU/mL) of *H. paragallinarum*, *S. enterica, P. multocida, A. pleuropneumoniae, V. parahaemolyticus, L. monocytogenes, S. aureus, S. suis* and *E. coli* and *H. parasuis*. The color of these samples remains purple except of *H. parasuis* purple turn to blue. The ratio of A678/A521 in the UV vis absorption spectrum was shown in *Fig. 5-B*, demonstrating the good specificity of this detection method.

Detection of *H. parasuis* in Tissue Effusion from Pig

Several pericardial fluid and articular fluid that collect from different suspected cases was detected using the established detection method and plate counting method. And compare the results to obtain the recovery rate in *Table 1*. It can be concluded that the number of bacterial colonies measured by this method is basically consistent with the results obtained by traditional plate counting methods. The detection recovery rate of *H. parasuis* is good which resides between 91.7% and 129.2%. The results of the *H. parasuis* detection method established in this experiment are consistent with the traditional plate counting method indicating that can be applied to the detection of *H. parasuis* in actual samples.

In this study, a colorimetric strategy based on specific aptamer functionalized AuNPs probe was successfully developed for rapid detection of *H. parasuis*. According

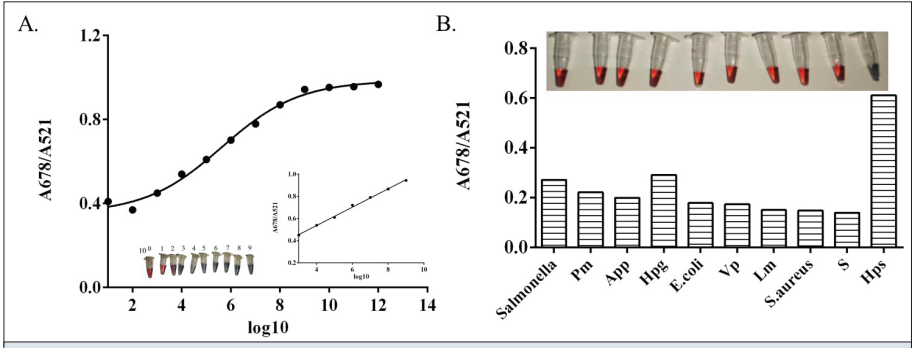

Fig 5. Standard curve and specificity of the detection method. (A) The linear relationship between A678/A521 and *H. parasuis* concentration from 10 to 10^{12} cfu/mL. (B) The specificity result for the detection of *H. parasuis, H. paragallinarum, S. enterica, P. multocida, A. pleuropneumoniae, V. parahaemolyticus, L. monocytogenes, S. aureus, S. suis* and *E. coli*

Table 1. *Comparison of the tissue sample results obtained from the colorimetric detection and classical plate counting method (all results were repeated three times and shown as average ± SD)*

Tissue Sample	Plate Counting	Colorimetric Method	Recovery Ration (%)
1	36±6	33±5	91.7
2	2.4x10^6±213	3.1x10^6±125	129.2
3	2674±89	2801±32	104.7

to the AuNPs probe, the positive sample change in color from red to blue was easily visualized with the naked eye. And, there was good linear relationship between the absorbance variation and *H. parasuis* in the range of 10^3 CFU/mL to 10^9 CFU/mL. Moreover, the aptamer functionalized AuNPs probe exhibited high selectivity to *H. parasuis* other than other bacteria. Therefore, we proposed colorimetric alternative method that offers a simple, rapid, and sensitive tool for *H. parasuis* detection.

Discussion

Recently, largely numbers of colorimetric biosensor for specific detection of pathogens or other molecular markers were developed by using DNA aptamer as recognition element and unmodified gold nanoparticles (AuNPs) as colorimetric indicator [20,24,25]. This research offers a significant breakthrough in the detection of *H. parasuis* by introducing an aptamer-AuNPs based colorimetric probe. This may provide new technical support for pathogen diagnosis according to these hard for culture due to its high nutritional requirement.

The electrostatic forces that shape the formation of the aptamer-AuNPs complex and its subsequent reactions to *H. parasuis* and salt ions form the core of this novel approach. The careful optimization of multiple parameters-incubation time, reaction temperature, NaCl concentration, and the ratio of aptamers to AuNPs is vital [26]. It not only boosts the performance and reliability of the detection system but also provides valuable insights into the underlying thermodynamic and kinetic processes. Transmission electron microscopy, UV-vis absorption spectroscopy, and dynamic light scattering offer an in-depth understanding of the properties of the aptamer-AuNPs probe, validating its formation and modification and reinforcing the detection mechanism. The establishment of a linear relationship between *H. parasuis* concentration and the absorbance ratio, along with a defined limit of detection, showcases the method's potential for sensitive and quantitative analysis. Specificity studies, revealing selective responses for *H. parasuis*, enhance confidence in its practical application. The successful application of the detection method in tissue fluid samples and comparable results to traditional plate counting methods highlights its potential for clinical and diagnostic purposes.

Nevertheless, further studies are necessary to overcome potential challenges such as matrix effects and to evaluate its performance in situations with low *H. parasuis* concentrations [27]. It displayed that the LOD of colorimetric detection method for *Vibrio parahaemolyticus* is 2.4 CFU/mL which higher sensitive than our method [28]. The sandwich structure based on magnetic nanoparticles (MNPs) and gold nanoparticles (AuNPs) proposes a high reference value. Future research could center on integrating the technology with portable devices for on-site detection and delving deeper into the molecular interactions for improved probe design.

Declarations

Availability of Data and Materials: Data and Materials are available from the corresponding author (J. Hu).

Funding Support: This work was supported by the National Key R&D Program of China (No. 2021YFD1301200), Program for Innovative Research Team (in Science and Technology) in University of Henan Province (No. 22IRTSTHN026, 24IRTSTHN035) and the National Natural Science Foundation of China (No. 32202763).

Competing Interests: The authors declare no competing of interest.

Declaration of Generative Artificial Intelligence (AI): The article and tables and figures were not written/created by AI and AI-assisted technologies.

Authors Contributions: Conceptualization, S.Z.; methodology, S.Z. and M.Z.; software, K.L., and L.W.; resources, L.W.; writing - original draft preparation, S.Z.; writing - review and editing, J.H. and S.Z.; funding acquisition, S.Z., L.W. and J.H

References

1. Macedo N, Rovira A, Torremorell M: *Haemophilus parasuis:* Infection, immunity and enrofloxacin. *Vet Res,* 46:128, 2015. DOI: 10.1186/s13567-015-0263-3

2. Ni HB, Gong QL, Zhao Q, Li XY, Zhang XX: Prevalence of *Haemophilus parasuis "Glaesserella parasuis"* in pigs in China: A systematic review and meta-analysis. *Prev Vet Med,* 182:105083, 2020. DOI: 10.1016/j.prevetmed.2020.105083

3. Angen O, Oliveira S, Ahrens P, Svensmark B, Leser TD: Development of an improved species specific PCR test for detection of Haemophilus parasuis. *Vet Microbiol,* 119 (2-4): 266-276, 2007. DOI: 10.1016/j.vetmic.2006.10.008

4. Frandoloso R, Martinez-Martinez S, Rodriguez-Ferri EF, Gutierrez-Martin CB: Comparison of real-time PCR and culture isolation in colostrum-deprived pigs immunized and challenged with *Haemophilus parasuis. Lett Appl Microbiol,* 54 (2): 149-152, 2012. DOI: 10.1111/j.1472-765X.2011.03187.x

5. Howell KJ, Weinert LA, Peters SE, Wang J, Hernandez-Garcia J, Chaudhuri RR, Luan SL, Angen O, Aragon V, Williamson SM, Langford PR, Rycroft AN, Wren BW, Maskell DJ, Tucker AW: "Pathotyping" multiplex PCR assay for *Haemophilus parasuis:* A tool for prediction of virulence. *J Clin Microbiol,* 55 (9): 2617-2628, 2017. DOI: 10.1128/JCM.02464-16

6. Pilchova V, Seinige D, Hennig-Pauka I, Buttner K, Abdulmawjood A, Kehrenberg C: Development and validation of a loop-mediated isothermal amplification (LAMP) assay for rapid detection of *Glaesserella (Haemophilus) parasuis. Microorganisms,* 9 (1):41, 2020. DOI: 10.3390/microorganisms9010041

7. Zhang K, Sun Z, Shi K, Yang D, Bian Z, Li Y, Gou H, Jiang Z, Yang N, Chu P, Zhai S, Wei Z, Li C: RPA-CRISPR/Cas12a-based detection of *Haemophilus parasuis. Animals (Basel),* 13 (21):3317, 2023. DOI: 10.3390/ani13213317

8. Chen S, Chu Y, Zhao P, He Y, Jian Y, Liu Y, Lu Z: Development of a recombinant OppA-based indirect hemagglutination test for the detection of antibodies against *Haemophilus parasuis. Acta Trop,* 148, 8-12, 2015. DOI: 10.1016/j.actatropica.2015.04.009

9. Liu Y, Du Y, Song Y, Tian Y, Qi Y, Zhang Q, He Q, Wang X, Chen H, Cai X, Xu X: Development and application of an antibody detection ELISA for *Haemophilus parasuis* based on a monomeric autotransporter passenger domain. *BMC Vet Res,* 15 (1):436, 2019. DOI: 10.1186/s12917-019-2128-x

10. Nicolet J, Paroz P, Krawinkler M, Baumgartner A: An enzyme-linked immunosorbent assay, using an EDTA-extracted antigen for the serology of *Haemophilus pleuropneumoniae. Am J Vet Res,* 42 (12): 2139-2142, 1981.

11. Oliveira S, Pijoan C: *Haemophilus parasuis:* New trends on diagnosis, epidemiology and control. *Vet Microbiol,* 99 (1): 1-12, 2004. DOI: 10.1016/j.vetmic.2003.12.001

12. Olvera A, Segales J, Aragon V: Update on the diagnosis of *Haemophilus parasuis* infection in pigs and novel genotyping methods. *Vet J,* 174 (3): 522-529, 2007. DOI: 10.1016/j.tvjl.2006.10.017

13. Kinghorn AB, Fraser LA, Lang S, Shiu SCC, Tanner JA: Aptamer bioinformatics. *Int J Mol Sci,* 18 (12):2516, 2017. DOI: 10.3390/ijms18122516

14. Davydova A, Vorobyeva M: Aptamer-based biosensors for the colorimetric detection of blood biomarkers: Paving the way to clinical laboratory testing. *Biomedicines,* 10 (7):1606, 2022. DOI: 10.3390/biomedicines10071606

15. Marrazza G: Aptamer Sensors. *Biosensors (Basel),* 7 (1):5, 2017. DOI: 10.3390/bios7010005

16. Qiao Q, Guo X, Wen F, Chen L, Xu Q, Zheng N, Cheng J, Xue X, Wang J: Aptamer-based fluorescence quenching approach for detection of aflatoxin M_1 in milk. *Front Chem,* 9:653869, 2021. DOI: 10.3389/fchem.2021.653869

17. Aldewachi H, Chalati T, Woodroofe MN, Bricklebank N, Sharrack B, Gardiner P: Gold nanoparticle-based colorimetric biosensors. *Nanoscale,* 10 (1): 18-33, 2017. DOI: 10.1039/c7nr06367a

18. Chang CC, Chen CP, Wu TH, Yang CH, Lin CW, Chen CY: Gold nanoparticle-based colorimetric strategies for chemical and biological sensing applications. *Nanomaterials (Basel),* 9 (6):861, 2019. DOI: 10.3390/nano9060861

19. Eilers A, Witt S, Walter J: Aptamer-modified nanoparticles in medical applications. *Adv Biochem Eng Biotechnol,* 174, 161-193, 2020. DOI: 10.1007/10_2020_124

20. Schmitz FRW, Cesca K, Valerio A, de Oliveira D, Hotza D: Colorimetric detection of *Pseudomonas aeruginosa* by aptamer-functionalized gold nanoparticles. *Appl Microbiol Biotechnol,* 107 (1): 71-80, 2023. DOI: 10.1007/s00253-022-12283-5

21. Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ: A DNA-based method for rationally assembling nanoparticles into macroscopic materials. *Nature,* 382 (6592): 607-609, 1996. DOI: 10.1038/382607a0

22. Wang L, Lyu S, Gu G, Bolten S: Selection of aptamers targeted to food-borne pathogenic bacteria *Vibrio parahaemolyticus. Food Sci Nutr,* 8 (7): 3835-3842, 2020. DOI: 10.1002/fsn3.1677

23. Li H, Rothberg L: Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc Natl Acad Sci U S A,* 101 (39): 14036-14039, 2004. DOI: 10.1073/pnas.0406115101

24. Xiao S, Lu J, Sun L, An S: A simple and sensitive AuNPs-based colorimetric aptasensor for specific detection of azlocillin. *Spectrochim Acta A Mol Biomol Spectrosc,* 271:120924, 2022. DOI: 10.1016/j.saa.2022.120924

25. Zahra QUA, Luo Z, Ali R, Khan MI, Li F, Qiu B: Advances in gold nanoparticles-based colorimetric aptasensors for the detection of antibiotics: An overview of the past decade. *Nanomaterials (Basel),* 11 (4):840, 2021. DOI: 10.3390/nano11040840

26. Nguyen DK, Jang CH: A simple and ultrasensitive colorimetric biosensor for anatoxin-a based on aptamer and gold nanoparticles. *Micromachines (Basel),* 12 (12):1526, 2021. DOI: 10.3390/mi12121526

27. Flores-Contreras EA, Gonzalez-Gonzalez RB, Gonzalez-Gonzalez E, Melchor-Martinez EM, Parra-Saldivar R, Iqbal HMN: Detection of emerging pollutants using aptamer-based biosensors: Recent advances, challenges, and outlook. *Biosensors (Basel),* 12 (12):1078, 2022. DOI: 10.3390/bios12121078

28. Sadsri V, Trakulsujaritchok T, Tangwattanachuleeporn M, Hoven VP, Na Nongkhai P: Simple colorimetric assay for *Vibrio parahaemolyticus* detection using aptamer-functionalized nanoparticles. *ACS Omega,* 5 (34): 21437-21442, 2020. DOI: 10.1021/acsomega.0c01795