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Paper-based detection of Epstein-Barr virus using asymmetric polymerase chain reaction and gold silicon particles

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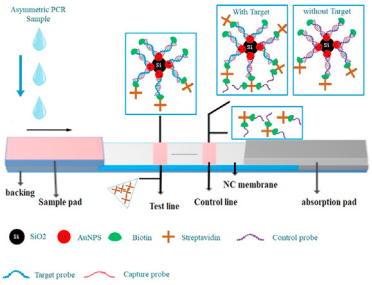
HIGHLIGHTS

- This visual detection system is suitable in low-resource settings.
- This new method allowed a visual detection of EBV nucleic acids with high specificity and low cost.
- Au@SiO2 can load more capture probes due to its large specific surface area and good biological activity.
- Asymmetric PCR using biotinylated primers produced a large number of biotinylated target sequences.
- Compared with antibody detection, the nucleic acid detection in this paper has higher specificity.

GRAPHICAL ABSTRACT



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ABSTRACT

A new paper-based lateral flow nucleic acid (LFNA) test platform was established in this study using asymmetric polymerase chain ceaction (A-PCR) for signal amplification. This new method allowed a visual detection of Epstein-Barr virus (EBV) nucleic acids with high specificity and low cost. In addition, as part of our strategy we employed a sandwich system of capture probe (CP)/gold nanoparticles (AuNPS) and silicon dioxide (SiO2) (AuNPS@SiO2) nanospheres/target DNA/avidin complexes as the sensing platform. Biotin-labeled target DNA was obtained by A-PCR and later introduced in the LF device. The CP/target DNA/AuNPS@SiO2 complexes were captured on the test zone by the specific reaction between biotin and avidin, and the remaining CP/AuNPS@SiO2 particles were captured on the quality control zone by the hybridization between CP and a quality control probe. Au@SiO2 accumulation in the test and quality control zones of the device enabled a visual detection of the specific target sequences. The method detection limit was 50 nM of the target DNA, which was lower than that of the LFNA biosensor (LFNAB) without PCR amplification and Au@SiO2 particles. In conclusion, the novel paper-based platform

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described here is a low cost, efficient and fast visual detection method that offers high sensitivity and other benefits compared to alternative methods in use.

1. Introduction

Epstein Barr virus (EBV) is a ubiquitous oncogenic virus that belongs to the Herpesviridae family. EBV is often associated with lymphoma, especially in immunosuppressed populations, such as HIV-infected and transplanted patients [1,2]. Nasopharyngeal carcinoma (NPC) is a malignant tumor of the mucosal epithelium of the nasopharynx, also linked to EBV infection. EBV latent infection is closely associated with nasopharyngeal non-keratinized carcinoma and poorly differentiated keratinized squamous cell carcinoma [3]. In the clinic, EBV detection in patients is very important for treatment monitoring and prognosis of related diseases [4,5].

Currently, the techniques available for EBV detection include immunofluorescence, viral culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). Challenges associated with the ELISA include its numerous detection steps, long periods of incubation, and the possibility of false positive results because of antigen-antibody cross-reactions. On the other hand, PCR assays represent a high sensitivity detection strategy, but they also require more analytical steps and are susceptible to cross-contamination.

The traditional paper nucleic acid detection platform is a kind of sensor using lateral chromatography based on capillary principle [6]. When the single-stranded nucleic acid is captured by the probe wrapped in the test line, a large number of colloidal gold is gathered in the test zone and the color is displayed. The remaining colloidal gold-labeled probe continues to travel to the control zone, where the probe is captured, and the color is displayed. However, the specificity and sensitivity of this method are poor, and further PCR confirmation is needed. Specifically, this approach can only detect single-stranded RNA or DNA, and cannot directly detect double-stranded DNA. Therefore, an improved method for detection of EBV, which is faster and more convenient, is needed.

Nucleic acid molecular diagnosis technology plays an important role in medical diagnosis, food safety analysis and environmental monitoring [7–9]. In recent years , the application of lateral flow nucleic acid biosensor (LFNAB) has developed rapidly, and it is considered to be the most promising nucleic acid molecular diagnostic technology for point of care (POC) [10,11]. LFNAB has the advantages of short detection time, long-term stable storage, low cost and portability [12,13]. In addition, LFNAB is easy to operate, does not require professional personnel, complex analysis, or expensive instruments. The color intensity can be directly observed by naked eye, and the detection result can be easily judged. Therefore, LFNAB is also suitable for use outside of the laboratory [14].

Composite nanomaterials, which are composed of metal shell and dielectric core, are a new type of composite material. By controlling the ratio of the core to the thickness of the shell, the optical properties can be adjusted in a wide range of wavebands. Therefore, this kind of composite is widely used in catalysis, sensors, surface-enhanced stretch-scattering substrates, photonic band gap materials, multifunctional composites and biomedical fields [15–19]. Silica is an important oxide; silica particles are strong and not easy to deform, as well as a high degree of monodispersion properties. They are characterized by a smooth surface, which easily undergoes physical and chemical modification and has other advantages. Taken together, its properties make it suitable to be used as the core of core shell materials.

In this study, we developed a paper-based platform based on gold magnetic nanoparticles to detect EBV DNA. In brief, a large number of gold nanoparticles (AuNPS) were assembled on the surface of silicon dioxide (SiO2) particles to prepare the AuNPS@-SiO2 nanospheres. The SiO2 surface has an amino group that can interact with the negatively charged surface of the colloidal gold particles, forming shell core gold NPS. These NPS have a high specific surface area, which can enhance the adsorption capacity. They can quickly and stably adsorb nucleic acid without substantially changing their biological activity. Asymmetric polymerase chain reaction (A-PCR) technology is proposed in this study. A large amount of biotin-labeled EBV single-stranded DNA was obtained by A-PCR with different concentrations of primers using genomic DNA as a template. Single-stranded DNA produced by A-PCR has been shown to have high hybridization efficiency, with the aim of maximizing the concentration of the target sequence and thus improving the sensitivity [20,21]. Later, A-PCR products were used on the paper-based platform based on gold magnetic nanoparticles.

A-PCR can produce a large number of highly efficient hybridization, biotin-labeled target sequences. The core-shell material is a silica core and colloidal gold shell. The composite particles have a certain strength and high dispersion, which can enrich a large number of trapping probes. Target sequences generated by A-PCR can be combined with capture probes enriched at AuNPS@SiO2 to greatly improve the sensitivity of detection. In this paper, the advantages of A-PCR and AuNPS@SiO2 particles are combined on a paper-based platform to obtain a visual detection system with efficient, high sensitivity and low cost.

2. Materials and Methods

2.1. Experimental materials

2.1.1. Laboratory reagents

All the chemicals used in this study were of analytical grade. Moreover, deionized distilled water was used to prepare all of the solutions for the experiments. Tetrachloroauric acid trihydrate (HAuCl4·3H2O, 99.99%), trisodium citrate dihydrate (Na3C6H5O7·2H2O), sodium chloride (NaCl), potassium chloride (KCl), Tween 20, streptavidin, 2 × Taq PCR Mix, Tris (2-carboxyethyl) phosphine (C9H16ClO6P, TCEP), plastic plates, sample pads, and water-absorbing pads were purchased from Labkoda Technology Co., Ltd. (hainan, china) Monodisperse amino silica microspheres (0.1 μm, 0.3 μm) were purchased from Tianjin Junyijia Technology Co., Ltd. (Tianjin, China). Nitrocellulose membranes were purchased from Sartorius Co., Ltd. (Gottingen, Germany). The virus DNA extraction kit was obtained from Biomiga Co., Ltd. (San Diego, California, USA). The virus samples came from the Second Affiliated Hospital of Hainan Medical University.

2.1.2. Primers and probes

All oligonucleotides, including EBV-DNA-specific target sequences, were synthesized by Sangon Biotech (Shanghai, China) (Table 1). The samples were transported at 4 °C and preserved at −20 °C until further use. The specificity of the designed primers

Table 1
Primers and probes.

Sequence name	Sequence (5′-3′)
capture probe	5′−SH−AGCCACACGGGCCTCT-3′
Quality Control Probe	5′-Biotin-AGAGGCCCGTGTGGCT-3′
Target DNA	5′-Bio-GCCAGCCTCAACTACGACTCGTGGCCCCTGTTTGCCGACTGTGAGGGTCCAGAGG CCCGTGTGGCTGCGTTACACCGATATAATGCCAGCCTGGCCCCCCACGTGTCCACGCAGATCTTTGCCA-3′
Upstream primer	5′-TGGCAAAGATCTGCGTGGACA-3′
Downstream primer	5′ -Bio-GCCAGCCTCAACTACGACT-3′

was confirmed by performing an *in-silico* analysis using the Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). All blood samples were collected from the Second Affiliated Hospital of Hainan Medical College (Hainan Province, China).

2.1.3. Experimental equipment

In this experiment, the characterization of colloidal gold and AuNPS@SiO2 microspheres was performed using an ultraviolet spectrophotometer (model N50 TOUCH) and a transmission electron microscope (TEM, model JEM 2100). The equipment used in this experiment include a centrifuge (model S1010e), an electronic balance (model PL203), a magnetic stirrer (model 85-1), a PCR thermal cycler (model Biometra TOne 96), a pure water meter (model RCT) −2100A) and an ultrasonic cleaner (model JP-010T).

2.2. Experimental methods and procedures

Positive control samples were tested 20X. Negative and VZV (varicellA-zoster virus)-postitve samples)-postitve samples controls were tested 6X. All other studies were repeated 5X.

2.2.1. AuNPS preparation

The preparation of AuNPS and AuNPS connection capture probe was carried out as previously described, with some changes [22,23]. The trisodium citrate reduction method was carried out for synthesis. After heating 100 mL of 0.1% $HAuCl_4$ solution to boiling, 4.8 mL of 1% sodium citrate solution was added, followed by stirring. When the color of the solution changed from light yellow to transparent wine red, the heating stopped. The prepared solution was subjected to UV−Vis analysis and stored at 4 °C, and protected from light.

2.2.2. Preparation and optimization of AuNPS@SiO2 composites

Amine silica microspheres of 300 nm and 13 nm diameter AuNPS were selected to prepare the AuNPS@SiO2 particles. Briefly, 0.1 mL amine silica microspheres was mixed with 20 mL AuNPS and stirred at a low speed for 2 h in a magnetic agitator. After standing for 1h, 10 mL of deionized water was added. Following ultrasonic dispersion, the precipitate was centrifuged for 5 min at 3300 g. This process was repeated 3X. Next, for the precipitate to reach a peak absorbance of 1, the appropriate amount of deionized water was added. Lastly, 0.2 g of polyvinyl pyrrolidone were added, and the solution was left shaking overnight. Finally, the solution was stored at 4 °C away from light until used.

2.3. Preparation AuNPS@SiO2-capture probe

The capture probe (200 μL, 15 μM) modified with mercapto was mixed with 1 M tris(2-carboxyethyl)phosphine (TCEP) (5 μL, 1 M) for 1h at room temperature. Following this, 50 μL of the probe was added to 200 μL of the AuNPS@SiO2 solution and diluted to a final volume of 400 μL. The mix was incubated at room temperature for 24 h under gentle stirring. Every 4 h, 0.8 μL, 2 μL, 2.5 μL, 3 μL of 2 M NaCl, and 10 μL of 0.1 M KCl were successively added. After 24h, the solution was stored at 4 °C in a dark place until use. UV−Vis analysis and transmission electron microscopy (TEM) were employed to characterize the AuNPS@SiO2 particles.

2.4. Test preparation

The main body of the strip included four parts (plastic plate, sample pad, nitrocellulose (NC) film, and water-absorbing pad). Each component was first pretreated as follows: the sample pad was pretreated with 5% BSA containing 0.5% Tween-20 prepared with ultrapure water. Following this, the treated NC film was naturally dried for 10 min. The pores on the surface of the NC membrane treated with BSA and TW-20 were closed and reduced to non-specific reactions. Next, the NC film was pasted on the plastic plate, and different concentrations of avidin were embedded on the NC film to mark the detection line. Streptavidin was mixed with the quality control probes at a 1:1 ratio, and then the prepared control probes were dropped on the NC membrane. Finally, all the components were dried thoroughly, and the paper-based detection platform was assembled. For the test validation, 10 μL of sample solution, containing different concentrations of target sequence, was added dropwise to 90 μL of AuNPS@SiO2-Capture Probe solution to make a 100 μL mixed solution. The reaction was carried out for 20 min. The prepared sample end of the NC membrane was inserted into the solution and the results were observed after 10min.

2.5. Epstein-Barr virus nucleic acid extraction

EBV and VZV were extracted from blood samples following the instructions from a nucleic acid extraction kit (Biomiga, America).

2.6. PCR amplification and agarose gel electrophoresis

The extracted genomic DNA of EBV and primers were evenly mixed with 2* Taq Mix and amplified in the PCR thermal cycler. The reaction conditions were as follows: Pre-denaturation at 95 °C for 5 min, followed by 30 cycles of: 30s denaturation at 95 °C, 30s annealing at 53 °C, 30s extension at 72 °C, and a final cycle at 72 °C for 10 min. The PCR products were analyzed by electrophoresis in a 4.5% agarose gel.

Viral DNA was extracted from serum samples for A-PCR amplification, and the product was added to AuNPS@SiO2@capture probe solution for 10 min. Finally, the test strip was inserted into the reaction solution for 10 min to observe the color.

3. Results

3.1. Detection principle

The methodology employed uses a shell core structure, SiO2 as the core, and AuNPS as the shell, forming AuNPS@SiO2 composite material. The capture probe is modified on AuNPS@SiO2 particles by an Au−S bond. Avidin and Control Probe were pre-immobilized

on the test and control zones of the paper-based platform (Scheme 1), respectively.

The biotin-labeled single-stranded EBV DNA product (10 μL) obtained by A-PCR amplification was incubated with the AuNPS@SiO2- Capture Probe complexes (90 μL) for 20 min at room temperature. Following this, the sample pad was embedded into the sample solution containing AuNPS@SiO2- Capture Probe-target complexes. At this point, the solution traveled by capillarity, and the target complexes were captured by the modified avidin in the test zone. The remaining AuNPS@SiO2- capture probe passed and reacted with the control probe. The control area utilizes the high affinity between biotin and avidin to capture the target molecule of AuNPS@SiO2- Capture probe and make the area display the color.

3.2. Characterization of AuNPS

Visual observation: the color of AuNPS was wine-red, clear, and transparent, with no particle precipitation (Fig. 1A). UV—Vis spectrophotometer characterization: The absorbance of the newly synthesized AuNPS was detected with deionized water as the control, and a characteristic absorption peak appeared at 520 nm (Fig. 2A). TEM characterization: 10 μL of the pre-prepared AuNPS solution was used to evaluate the particle size, morphology, and dispersion. The diameter of the particles was observed to be 13 ± 2 nm, the particle size was uniform, and the dispersion was optimal (Fig. 1B).

3.3. AuNPS@SiO2 characterization of the materials and stability

In this experiment, it was essential to optimize the size of SiO2 used to prepare the Au@SiO2 complexes to achieve optimal detection. Thus, we directly compared 100 nm and 300 nm SiO2@AuNPS in terms of preparation of complexes. The 300 nm SiO2@AuNPS particle size was uniform, the dispersion was adequate, the colloidal gold particles were uniformly attached to the surrounding SiO2, the background was clean, and there were no colloidal gold particles residues. In contrast, the 100nmSiO2@AuNPS complexes had poor dispersion, less colloidal gold attached to the SiO2, and we observed a single residue of colloidal gold. Therefore, the 300 nm SiO2 particle was selected for use in our study.

UV—Vis spectrophotometer characterization: Colloidal gold particles and Au@SiO2 particles were characterized using a UV—Vis spectrophotometer. Pure water was used as a control. AuNPS produces a UV absorption peak at 519. As SiO2 combines with the colloidal gold particles, a characteristic absorption peak at 528 nm is generated (Fig. 2A). TEM characterization: AuNPS@SiO2 (100 nm SiO2) particle diameter was observed to be 110 ± 5 nm (Fig. 2B). Au@SiO2 particles prepared with 300 nm amino silica microspheres had a diameter of 310 ± 5 nm (Fig. 2C, D, and Fig.2E). 300 nm SiO2@AuNPS was evenly distributed, firmly combined and uniform in size.

In order to test the stability of AuNPS@SiO2, we tested the change of its ULTRAVIOLET absorbance after three months (2A). The wavelength and peak value of its ultraviolet absorbance peak did not change significantly, proving that the material had good stability.

3.4. Agarose gel electrophoresis after A-PCR

A-PCR products were electrophoretically characterized using 4.5% agarose. The result is shown in Fig. 3. The first lane is a synthetic 125 nucleotide (nt) single-stranded DNA, the second lane is a 500 bp DNA marker, and the third to fifth lanes are 1:5, 1:10, and 1:25 upstream and downstream primers. The ratio of upstream and downstream primers in lanes 7 to 10 was 1:50, 1:75, 1:100, and 1:200. PCR results showed that a 125 nt fragment was generated when the ratio of upstream and downstream primers was controlled, and the maximum amount of target DNA was obtained with a 1:50 upstream and downstream primers ratio. Images were recorded with a GelDoc UV gel documentation system.

3.5. Optimization of detection conditions

3.5.1. The amount of capture probe

According to the concentration of AuNPS, 60 μL 310 nm AuNPS@SiO2 particles were combined with 15 μM to capture probe. The resulting AuNPS@SiO2-capture probe mixture is red in color. The properties of the composite were stable and did not change with the amount of capture probe added.

3.5.2. Optimization of avidin concentration

For optimizing the avidin concentration, 1 μL of different avidin solutions (0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL) were added to the NC membrane detection area. Then, 1 μL of Quality Control Probe (1 μM) was added in the Quality Control area. After 5 min, the results were registered. Interestingly, a steric hindrance effect was observed when avidin was used at 30—100 ng/mL on the NC membrane, with weak positive results observed without addition of the target sequence. However, 0.1—10 ng/mL of avidin is able

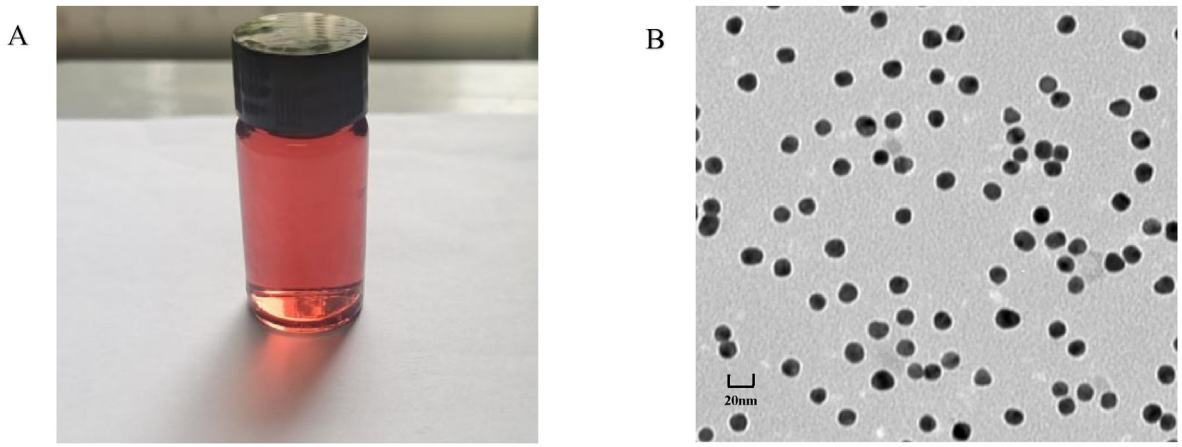
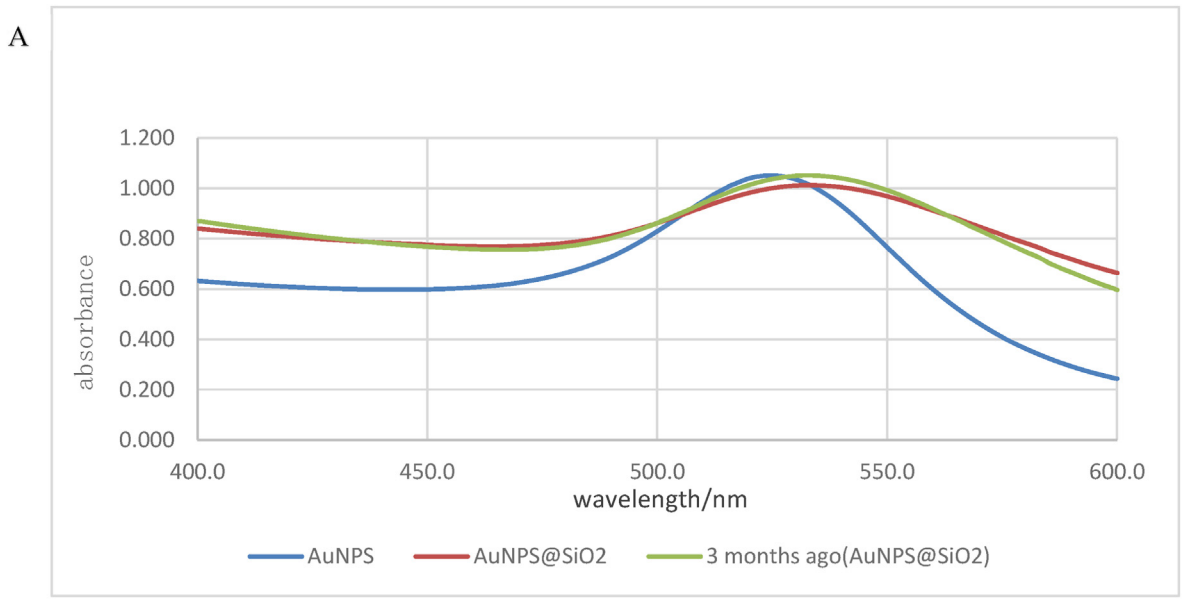


Fig. 1. (1A)Visual observation of AuNPS. (1B)TEM oF AuNPS.



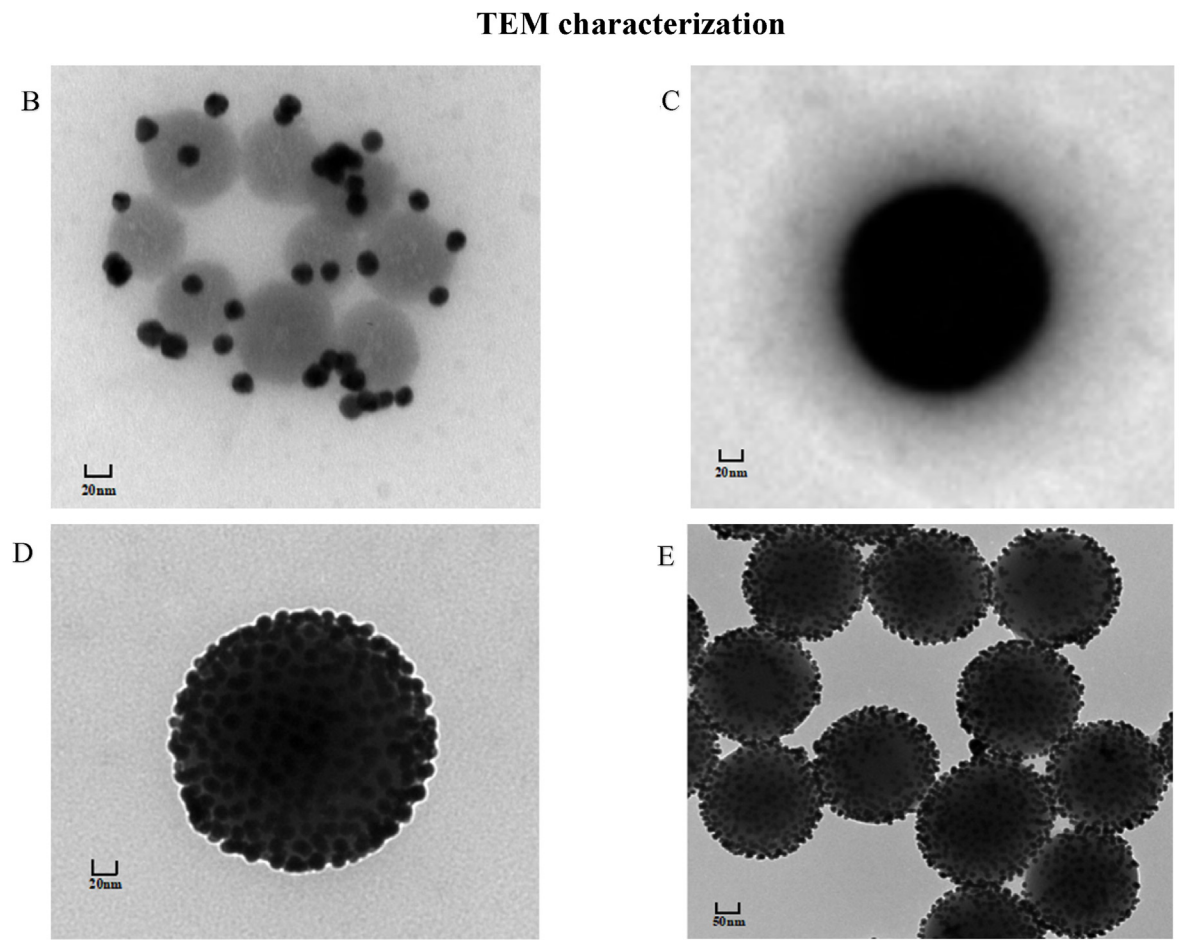


Fig. 2. Photograph of UV–Vis spectrum and TEM. (2A) AuNPS, Au@SiO2 and Au@SiO2 (3 months ago). (2B) 100nmSiO2 connect with AuNPS (TEM). (2C) CP@Au@SiO2 (TEM). (2D/E) 300nmSiO2 connect with AuNPS (TEM).

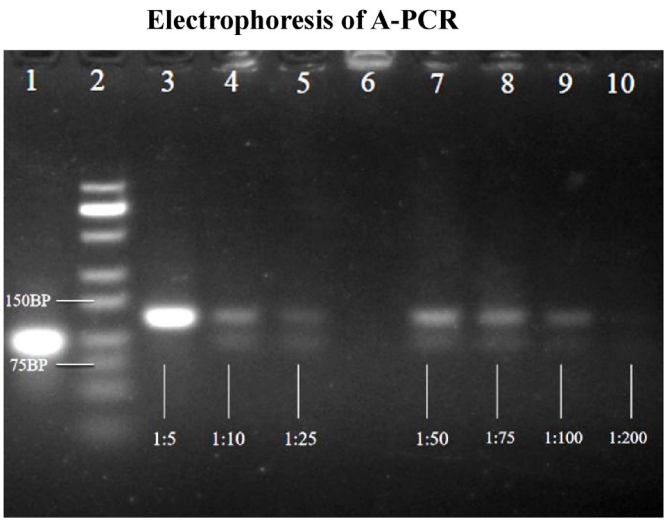


Fig. 3. Lane 1: 125 nt single-stranded DNA. Lane 2: DNA maker (500bp,400bp,300bp,200bp,150bp,100bp,75bp,50bp,25bp). Lanes 310: Different upstream and downstream primer concentrations.

to detect the target sequence on the strip without an evident steric hindrance effect. We found that 1 ng/mL of avidin was superior to the other concentrations (Fig. 4A). Therefore, 1 ng/mL of avidin and 1 μM Quality Control Probe were selected for our studies.

3.6. Assay performance of the paper-based platform detection method

3.6.1. Specific detection

We used healthy blood samples as negative controls and VZV-infected patients as specific controls. As shown in Figure (4B-1/2/3), red bands appeared on the T line and the C line in the EBV-positive group, while red bands appeared only on the C line in the negative control groups. Thus, results suggest that the nucleic acid immunocolloidal gold strip prepared in this study can be used to detect EBV with good specificity.

3.7. Sensitivity detection

Different target concentrations (10 μM, 1 μM, 0.5 μM, 0.1 μM, 0.05 μM, 0.01 μM and 0 μM) were chosen to determine the detection limit of the AuNPS@SiO_2@ probe-based platform. There was no detection of 0.01 μM EBV DNA (Fig. 4C), and the detection limit for this method was determined to be 0.05 μM.

3.7.1. Reproducibility test

We next investigated the reproducibility of this method by repeating the 10 μM target sequence 5X. We calculated the coefficient of variation of the T/C line at the same concentration. The T/C values of the 10 μM target sequences were collected, and the coefficient of variation was calculated to be 0.087. These result suggest that there is good reproducibility of this method (Fig. 4D).

4. Discussion

Epstein-Barr virus infection is associated with the occurrence and development of a variety of tumors. Our study presents a fast, accurate, convenient, and low-cost Epstein-Barr virus diagnostic method. Our approach included an asymmetric interleaved PCR technique to obtain the biotin-labeled single-stranded EBV DNA. The amplified biotin-labeled EBV-ssDNA was added to the prepared AuNPS@SiO2@ capture probe. In the probe gold label solution, the red circle can be observed by the naked eye on the chromatographic test strip through the specific binding of avidin and biotin to detect the presence of a trace amount of Epstein-Barr virus.

Although the combination of A-PCR and LF assay have been reported multiple times, previous reported studies used single colloidal gold nanoparticles for sandwich probe hybridization experiments [24,25]; the number of probes supported by a single colloidal gold is limited. When the concentration of the target sequence is too high, one part of the target sequence may bind to the quality control probe on the membrane and the other part to the capture probe on the colloidal gold, resulting in a false negative. The application of AuNPS@SiO2 to immunodetection methods has also been reported [26], but the combination of antigen and antibody is prone to cross reactivity, and nucleic acid detection has higher specificity than immunodetection approaches. Here, we combined the amination modified nano-sized SiO2 microspheres with nano-gold particles to form the shell-core AuNPS@SiO2 microspheres. The prepared nano-gold particles and AuNPS@SiO2 microspheres were characterized via UV spectrophotometer and TEM. We observed the binding of 13 nm AuNPS with SiO2 at different sizes. When the size of SiO2 was 300 nm, AuNPS@SiO2 formed by the binding was uniform in size, evenly dispersed and firmly bonded. AuNPS@SiO2 microspheres were connected to capture probes, and this was followed by a series of centrifugations, sealing, and resuspension steps to obtain AuNPS@SiO2@capture probe gold labels (proved by lateral chromatography blank control experiments). During the preparation of the Au@SiO2-capture probe, we optimized the salt concentration and incubation time (following testing of 6h, 12h, 18h and 24h shaking times). The 24h preparation was observed to be superior to the other time points.

The roles of NaCl and KCl are to incubate the probe and maintain a certain salt concentration in the solution. In this way, ssDNA can maintain better chain rigidity on the colloidal gold through electrostatic interaction and can more quickly complement the target sequence base. The proper salt concentration can also ensure the monodispersity of the composite material.

The sudden addition of a high concentration salt solution will destroy the electrostatic system of the solution and cause the composite material to coagulate. The salt solution was thus gradually added from low concentration to high concentration every 4h in our study.

We optimized A-PCR for EBV positive samples, controlling different ratios of upstream and downstream primer concentrations. The maximum amount of target DNA was obtained with a 1:50 upstream and downstream primers ratio. We tested a total of twenty EBV-positive samples, including one false negative, and we tested five EBV-negative samples, with no false positives. The positive detection rate was determined to be 95%. The T/C values of the 10 μM target sequences of 5 cases were collected, and the coefficient of variation was calculated to be 0.087. This suggests that this system is reliable with good reproducibility.

With the above methodology, we successfully prepared a solution loaded with a large number of capture probes and no non-specific sites. The A-PCR-LFA sensor developed in this study is able to detect at least 50 nM EBV-ssDNA. We used this sensor to successfully detect the presence of the Epstein-Barr virus in patient samples. In conclusion, according to our results, EBV detection with A-PCR-LFA is a simple, rapid, and sensitive diagnostic method.

5. Conclusion

We developed a visual enzyme-free EBV detection method combining A-PCR and a side flow biosensor. After the target sequence is amplified by A-PCR, colorimetric detection is carried

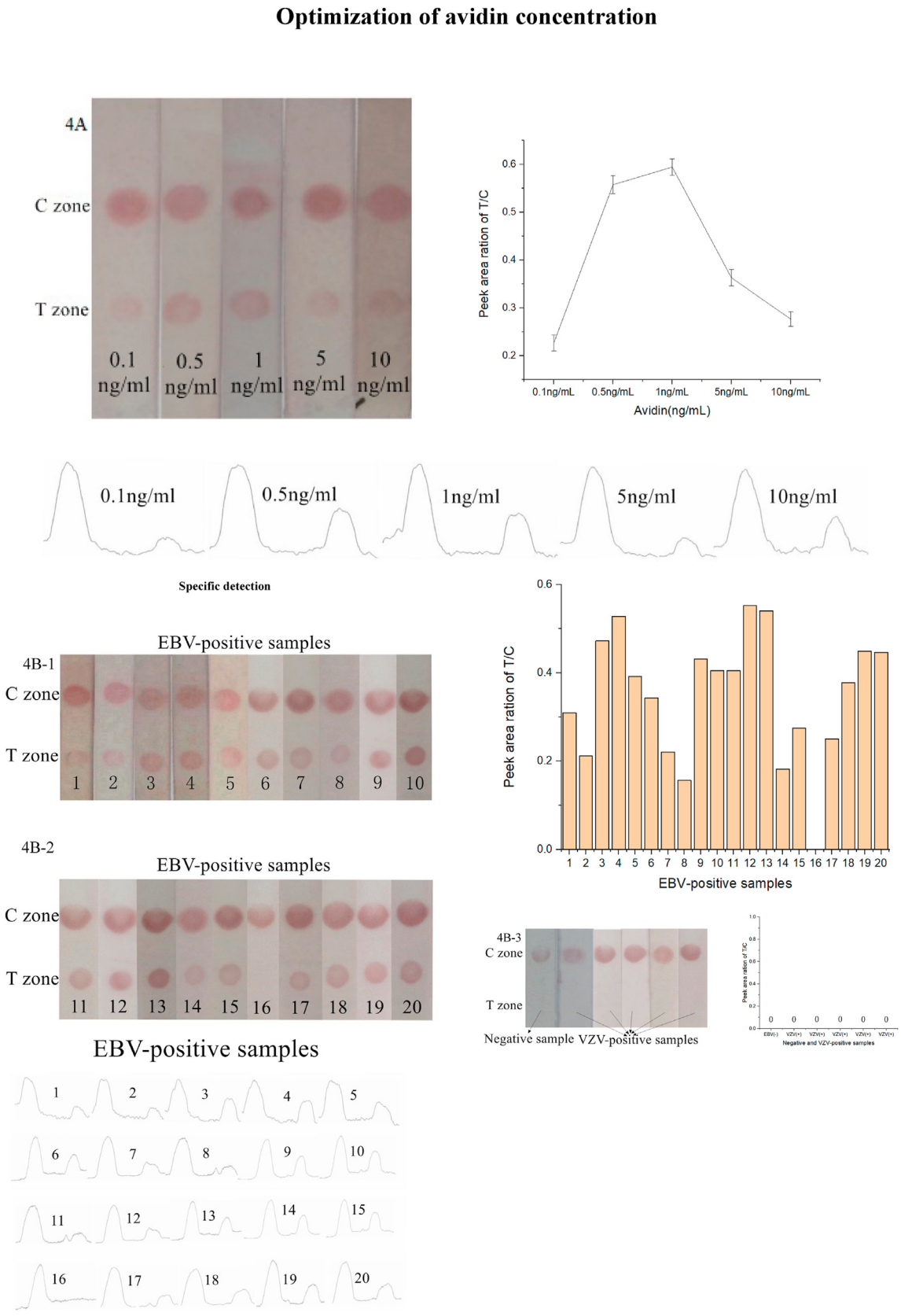


Fig. 4. Paper-based platform. (4A) Avidin concentration. (4B) Specific test. (4C) Sensitivity test. (4D) Reproducibility test.

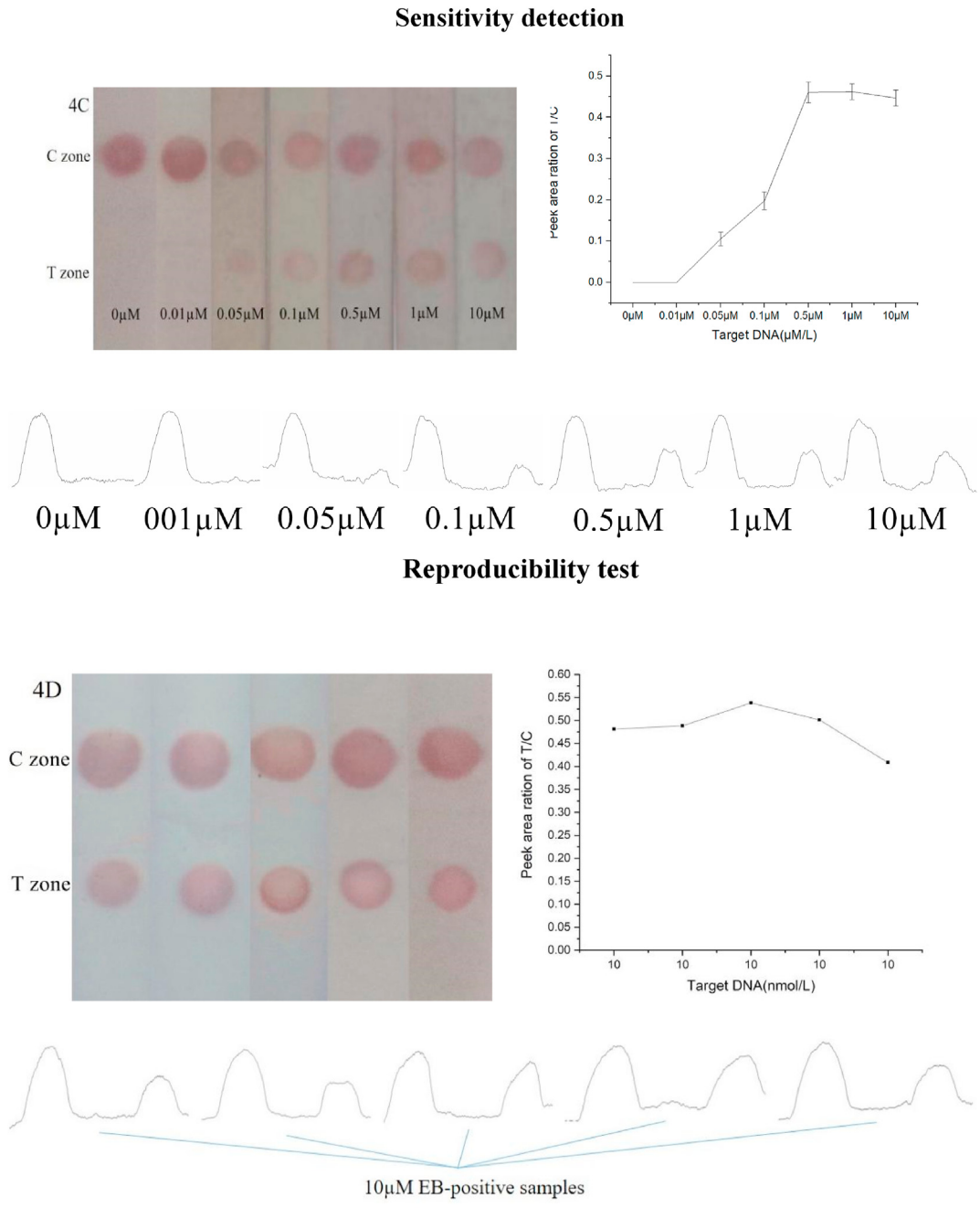


Fig. 4. (*continued*).

out through a simple paper-based platform, and semi-quantitative detection of Epstein-Barr virus can be carried out without a large signal output instrument. Composite materials enable multiple AuNPS to be combined on a single SiO2, increasing the binding sites of the capture probe, thereby increasing the detection limit. At this time, after a small amount of target sequence is recognized by the capture probe, a large amount of AuNPS can be carried for subsequent paper-based color development. In general, this method has a detection limit of 0.05 μM viral DNA in serum and does not require enzymes, professional operators, or equipment. In addition, the new technology can also be used to detect other viruses. Thus, this technique provides a simple, rapid, low-cost, and convenient diagnostic method for detecting the virus in the field.

CRediT authorship contribution statement

Haoze Chen: Conceptualization, Methodology, Software, Investigation, Data curation, Writing – original draft. **Shoujia Lin:** Conceptualization, Methodology, Software, Writing – review & editing. **Yongxia Wang:** Methodology, Formal analysis. **Shuqi Fu:**

Resources, Investigation. **Yueting Ma:** Conceptualization, Resources. **Qianfeng Xia:** Resources, Validation, Data curation, Project administration, Funding acquisition. **Yingzi Lin:** Supervision, Methodology, Writing – review & editing, Funding acquisition, Project administration, Data curation.

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.

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