Electronic Supplementary Information

Nanoparticle-enhanced electrical detection of Zika virus on paper microchip

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Supplementary Methods

Synthesis of PtNPs
Briefly, 36 ml of a 0.2 % solution of chloroplatinic acid hexahydrate was mixed with 464 ml of boiling deionized water. 11 ml of a solution containing 1 % sodium citrate and 0.05 % citric acid was added followed by a quick injection of 5.5 ml of a freshly prepared 0.08 % sodium borohydrate solution, containing 1 % sodium citrate and 0.05 % citric acid. The reaction continued for 10 min and the formed nanoparticles solution was gradually cooled down to room temperature.

Virus culture and isolation
Aedes albopictus cells (C6/36) were prepared and infected with ZIKV and harvested. 4 flasks (225 cm$^3$) of the cells were prepared and infected with the virus. Infection was carried out by adding serum sample to the culture flask and incubating at an angle of 20° at 33 °C for 6 days. After incubation, the virus was harvested by treating with 23 % v/v FBS. The supernatant from the flasks was collected and centrifuged at 4000 g for 30 min. The supernatant after centrifugation was collected without disturbing the cell pellet and 500 μL aliquots were prepared and stored.

Biotinylation of Anti-Zika antibodies
Anti-Zika antibodies were biotinylated using the Biotin type A fast conjugation kit (Abcam, ab201795). 1 μL of Biotin Modifier reagent was added to every 10 μL of antibody to be labeled and was mixed gently. This was added to a vial containing lyophilized Biotin and was left for incubation at room temperature (20 °C to 25 °C) for 15 min. 1 μL of Quencher reagent was then added to every 10 μl of the antibody used. The mixture was incubated at room temperature for 4 min and stored at 4 °C.

Magnetic bead modification
200 μl of streptavidin-coated magnetic beads (Thermo Fisher Scientific- PierceTM Streptavidin magnetic beads; 88816) of 1 μm diameter was washed thrice using PBS, during which the beads were isolated using a MagnaGRIP™ (MilliPore) magnetic stand. 10 μL of the biotinylated target was added to the magnetic bead solution and left for incubation overnight on a shaker at 4 °C. Antibody conjugated beads were then washed twice using PBS and suspended in 2 mL PBS.
Supplementary Results:

Figure S1. Detailed preparation protocol of Pt-nanoprobe. The surface of PtNPs of the prepared platinum nanoparticles (PtNPs) was modified with 3-(2-Pyridyldithio)propionyl hydrazide (PDPH) through the thiol-metal interaction. The hydrazide terminal of PDPH then allowed to couple to the free aldehyde group (CHO) in the oxidized FC region of Zika virus monoclonal antibody (anti-ZIKV mAb), forming the Pt-nanoprobes used in labeling Zika virus.
Figure S2. Dynamic light scattering (DLS) analysis of the size distribution of the prepared PtNPs used in the preparation of platinum nanoprobe (Pt-nanoprobe).
Figure S3. Zeta potential of the prepared citrate capped PtNPs used in the preparation of platinum nanoprobe (Pt-nanoprobe).
Figure S4. FTIR analysis of anti-Zika monoclonal antibody showing different peaks at 1643.40 cm\(^{-1}\), 1537.31 cm\(^{-1}\) and 1111.03 cm\(^{-1}\) that are characteristic to the amide I and amide II bands of protein.
Figure S5. The detection of ZIKV on the developed paper microchip without post-capture labeling step with Pt-nanoprobes. Different concentrations of ZIKV ($10^1$ particle/μl to $10^5$ particle/μl) were prepared in 1x PBS and captured with magnetic beads, lysed and tested on-chip. Error bars are standard deviations of mean from a total of three independent measurements.
Table S1. Summary of common types of paper-based systems recently reported for human viruses detection

<table>
<thead>
<tr>
<th>Paper systema</th>
<th>Target virusb</th>
<th>Assay type</th>
<th>Detection Method</th>
<th>Detection limit</th>
<th>Ref.</th>
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<tbody>
<tr>
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<td>HBV</td>
<td>Immunoassay</td>
<td>Electro-chemiluminescence</td>
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<td>ZIKV</td>
<td>Intact virus assay</td>
<td>Electrical</td>
<td>$10^2$ particle/μl</td>
<td>Current study</td>
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<tr>
<td>Wax-printed cellulose paper</td>
<td>NoV</td>
<td>Immunoassay</td>
<td>Scattering</td>
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<td></td>
<td>EBOV</td>
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<td>HIV-1</td>
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<td>LFA strip</td>
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<td>HIV-1</td>
<td>Intact virus assay</td>
<td>Electrochemical</td>
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<td>Barcode LFA</td>
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<td>Electrochemical paper system</td>
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<td>Nucleic acid assay</td>
<td>Electrochemical</td>
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<td>Silica sprayed cellulose paper</td>
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</table>

aLFA: lateral flow assay; NC: nitrocellulose membrane; ELISA: enzyme-linked immunosorbent assay
bDENV: dengue virus; EBOV: Ebola virus; HBV: hepatitis B virus; HCV: hepatitis C virus; HHV-5: herpes virus-5; HIV-1: human immunodeficiency virus-1; HPV: human papillomavirus; IAV: influenza A virus; NoV: norovirus; RSV: respiratory syndrome Virus; ZIKV: Zika virus
References