INTRODUCTION

Mobile health diagnostics are changing the face of modern medicine and health care (1, 2). The advances in consumer electronics and portable communication systems, particularly mobile phones, have led to rapid and cheaper approaches for developing point-of-care (POC) diagnostics (3–8). The global unique mobile subscribers in 2019 was approximately 5.18 billion and is estimated to reach more than 5.7 billion by 2025, and more than 10% of this number will be in sub-Saharan Africa alone where most of infection outbreaks occur (9). Such global access to mobile phones combined with its powerful computing ability and built-in sensors present a promising potential to develop digital diagnostics that may help in large-scale and efficient management of infectious diseases (3, 10).

Smartphone systems can also benefit from the recent unprecedented advancements in nanotechnology to develop diagnostic approaches. Catalysis can be considered as one of the popular applications of nanoparticles because of their large surface-to-volume ratio and high surface energy (11–16). So far, numerous diagnostic platforms for cancer and infectious diseases have been developed by substituting enzymes, such as catalase, oxidase, and peroxidase with nanoparticle structures (17–20). Here, we adopted the intrinsic catalytic properties of platinum nanoparticles (PtNPs) for gas bubble formation to detect viruses on-chip using a convolutional neural network (CNN)–enabled smartphone system. Our unique approach of visual signal amplification through on-chip bubble formation combined with a CNN algorithm allows simple and rapid virus detection using a smartphone camera without the need for any external smartphone optical attachment for image magnification and read-out or any target amplification. The virus is captured on the surface of a microchip using monoclonal antibody (mAb) against the virus envelope protein and labeled with PtNPs, forming Pt-virus immunocomplexes on the surface of the microchip (Fig. 1, A and B). The addition of a catalyzer solution containing hydrogen peroxide (H$_2$O$_2$) to the formed Pt-virus complexes results in the formation of gas bubbles due to the catalase-like activity of PtNPs that drives the decomposition of H$_2$O$_2$ into water and O$_2$ (Fig. 1B). The on-chip gas bubble formation was controlled by optimizing the density of the reaction medium to form stable bubbles that can be detected by the CNN-enabled smartphone system (Fig. 1, C and D). Although using metal nanoparticles for target labeling and detection has been previously reported, unlike all other previous work in mobile health technologies for protein/virus detection, our system is simple in sample processing, sensitive, and adaptable to different smartphone models, mainly because our CNN–nanoparticle-enabled smartphone (NES) system does not need any hardware optical smartphone attachment for signal detection (21–30).

RESULTS

Virus capture and labeling using Pt-nanoprobes

Specifically designed Pt-nanoprobes were prepared with PtNPs and mAb against the envelope protein of ZIKV as illustrated in fig. S1. The preparation reaction involves the coupling of the antibody molecules to the surface of 4.5-nm PtNPs through the carbohydrate residues in the fragment crystallizable (FC) region of the immunoglobulin G (IgG) antibody using a short pyridyldithiol-and-hydrazide cross-linker of 3-(2-pyridyldithio)propionyl hydrazide (PDPH). The prepared PtNPs and their coupling to PDPH and antibody molecules were assessed by transmission electron microscopy (TEM), ultraviolet-visible (UV-vis) spectroscopy, dynamic light scattering (DLS), gel electrophoresis, and Fourier transform infrared (FT-IR) spectroscopy...
techniques (Fig. 2 and figs. S2 to S7). For antibody coupling to the surface of PtNPs, agarose gel electrophoresis showed that the migration of PtNPs in gel is slightly retarded after conjugation reaction, suggesting the addition of antibodies to their surface, which increases the size of PtNPs and partially interferes with their electrostatic behavior (fig. S6). FT-IR spectroscopy spectra of PtNPs and antibody-modified PtNPs are shown in fig. S7. The conjugation of mAb to the surface of PtNPs resulted into the following peaks: C─OH stretch around 1087.8 cm⁻¹, the absorption bands of ammonium ions at 2316.5 cm⁻¹, amide I groups at 1766.8 cm⁻¹, and amide II groups at 1579.7 cm⁻¹ (31). Bicinchoninic acid (BCA) protein assay showed an average antibody concentration of 348.32 ± 13.14 μg/ml (n = 2) in the formed Pt-nanoprobe preparations, which confirms the conjugation of the antibody to the surface of PtNPs (Materials and Methods).

The microchip surface is functionalized with anti-ZIKV mAb to allow efficient capture and labeling of on-chip ZIKV particles. Anti-ZIKV mAbs were conjugated to the surface of the chips using a surface chemistry protocol specifically designed to allow an efficient directional conjugation of antibodies using polyethylene glycol (PEG) molecules bifunctionalized with a terminal thiol and silane group (fig. S8). The glass surface of the microchip was initially silanized with PEG, and then oxidized antibodies activated with PDPH were incubated on the surface of the PEG-modified chip to allow the interaction of pyridyldithiol group of PDPH with the free on-chip –SH groups. The functionalization of the chip with anti-ZIKV mAb was confirmed using SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and FT-IR spectroscopy techniques. For SDS-PAGE, the immobilized antibody molecules on the surface of the chip were digested using an SDS-PAGE sample preparation buffer and eluted for testing on a 4 to 20% precast polyacrylamide gel. The results showed bands around 50 and 25 kDa that are characteristic to IgG heavy and light chains, respectively (fig. S9). FT-IR spectroscopy analysis spectra of antibody and antibody-modified chips were similar in displaying many bands that are characteristic to antibody. Specifically, the absorption bands of amide I groups at 1779.17 cm⁻¹ and amide II groups around 1568.01 cm⁻¹ confirmed the antibody immobilization on the surface of the chip. In addition, sharp peaks at 922.8, 724.03, 669.45, and 560.9 cm⁻¹ are attributable to Si─O─Si stretching, H─Si─O vibrations, and C─H and reflect the efficient salinization and PEGylation of the surface of the chip (fig. S10) (31).

The capture and labeling of the virus on the microchip were evaluated using SDS-PAGE, enzyme-linked immunosorbent assay (ELISA), reverse transcription polymerase chain reaction (RT-PCR),
and electron microscopy techniques. SDS-PAGE showed a high degree of protein similarity with samples eluted from the surface of the chip and ZIKV samples, confirming on-chip virus capture as the major virus structural proteins including envelope, precursor M protein, and capsid were clearly identified (Fig. 2C and fig. S9) (32). ELISA and RT-PCR results indicated that the virus capture efficiency on-chip were 89.08 ± 1.07 and 82.7 ± 5.66%, respectively (Fig. 2D).

Under scanning electron microscopy (SEM), the number of captured viral particles increased with increased virus concentrations of the samples loaded into the chips (fig. S11). We observed one titered viral particle on-chip were 89.08 ± 1.07 and 82.7 ± 5.66%, respectively (Fig. 2D). The ELISA and RT-PCR results indicated that the virus capture efficiency on-chip were 89.08 ± 1.07 and 82.7 ± 5.66%, respectively (Fig. 2D). Under scanning electron microscopy (SEM), the number of captured viral particles increased with increased virus concentrations of the samples loaded into the chips (fig. S11). We observed one viral particle per 3.7 μm² of the microchip surface when a sample viral concentration was incubated on-chip. The average size of the formed virus-PtNP complex was 84.3 ± 26.48 nm, which is substantially larger than ZIKV with 40 to 50 nm in diameter (Fig. 2E and fig. S12) (33, 34).

**Development and evaluation of the CNN-NES for virus detection**

To develop attachment-free smartphone-based virus detection, we integrated the nanoparticle bubble approach with a smartphone system modified with a trained CNN algorithm running on an Android smartphone (XT1575, Motorola) that can image and analyze bubbles on a microchip with a straight microchannel. The CNN algorithm uses the Inception v3 architecture, which was transfer learned using Google’s TensorFlow deep learning framework, with images of microchips containing bubbles analogous to virus samples (figs. S13 to S15). To train our system, we performed five different trials with ZIKV-spiked phosphate-buffered saline (PBS) samples (n = 25) at different virus concentrations around a threshold value of 250 copies/ml, which is clinically relevant to the target viruses in this study (HBV, HCV, and ZIKV) (35–42). The tested virus concentrations were 0, 250, 10³, 10⁴, and 10⁵ copies/ml, and the corresponding microchip images recorded with a smartphone were used for training the algorithm. On the basis of the data associated with virus-spiked samples, we also prepared a set of PtNP-spiked samples that simulated bubbles formed with virus samples for training the CNN-NES. The training and validation dataset included 15,057 images from 38 videos of virus-spiked PBS samples (n = 5) and PtNP-spiked samples analogous to different virus concentrations of 0, 250, 10³, 10⁴, and 10⁵ particles/ml (n = 33) (Materials and Methods). Thus, the developed NES can qualitatively identify samples as positive (viral load above or equal to the threshold) and negative (viral load below the threshold) on the basis of a virus concentration threshold of 250 particles/ml, which is clinically relevant to the target viruses in this study (HBV, HCV, and ZIKV) (35–42).

To evaluate the performance of the NES system for virus detection, we used HBV-spiked serum samples (n = 22), ZIKV-spiked blood samples (n = 60), HCV-infected patient plasma/serum samples (n = 27), and ZIKV-infected patient serum samples (n = 25) (Fig. 3, figs. S16 and S17, and tables S1 and S2). Each virus sample was tested using the single-channel microchip modified with mAbs against the target virus surface protein (i.e., anti-ZIKV envelope antibody for ZIKV, anti-HBV surface antigen antibody for HBV, and anti-HCV core antigen antibody for HCV) following the aforementioned bubble protocol using the catalysis solution containing 5% H₂O₂ and 20% glycerol and incubated for 10 min. We performed receiver operating characteristic (ROC) curve analysis to evaluate the performance of the CNN-NES in qualitatively detecting viral-spiked and viral-infected samples for ZIKV, HBV, and HCV.
samples with a virus concentration threshold of 250 copies/ml (Fig. 3, A and B). The areas under the ROC curve (AUCs) for ZIKV-spiked and HBV-spiked PBS samples, ZIKV-infected patient serum, and HCV-infected patient serum/plasma samples were 0.984 [confidence interval (CI), 0.911 to 1.000], 0.992 (CI, 0.831 to 1.000), 1 (CI, 0.863 to 1.000), and 1 (CI, 0.872 to 1.000), respectively. The AUC when all spiked and patient samples were included was 0.979 (CI, 0.939 to 0.996). Vertical scatterplots between CNN-NES probability value results and PCR qualitative-testing results for spiked samples (Fig. 3C, i) and patient samples (Fig. 3C, ii) showed one

![Fig. 3. Virus detection using the CNN-NES. (A) Digital images of the developed CNN-NES (i) and the actual microchips (ii) used to qualitatively test a virus-spiked sample. The system can measure the probability value of sample for qualitative testing (positive or negative) without the need for any optical smartphone attachment. Photo credit: Mohamed S. Draz, Brigham and Women's Hospital. (B) ROC analysis of the developed CNN-NES compared to the standard RT-PCR using (i) ZIKV-spiked samples \((n = 60)\), (ii) HBV-spiked samples \((n = 22)\), (iii) ZIKV-infected patient serum samples \((n = 25)\), and (iv) HCV-infected patient serum samples \((n = 27)\). (C) Vertical scatterplots representing the CNN-NES probability values of the samples versus qualitative PCR results: (i) ZIKV-spiked samples \((n = 60)\), HBV-spiked samples \((n = 22)\), (ii) ZIKV-infected patient serum samples \((n = 25)\), and HCV-infected patient serum samples \((n = 27)\). (D) Vertical scatterplots representing the CNN-NES probability values of the samples versus quantitative PCR results: (i) ZIKV-spiked samples \((n = 60)\), HBV-spiked samples \((n = 22)\), (ii) ZIKV-infected patient serum samples \((n = 25)\), and HCV-infected patient serum samples \((n = 27)\).]
false positive and one false negative for ZIKV-spiked samples and two false positives for HBV-spiked samples. Vertical scatterplots between CNN-NES probability value results and PCR quantitative-testing results for spiked and patient samples are also shown in Fig. 3D (i and ii, respectively). These results showed that the system was confused for one ZIKV-spiked sample with a viral load of 250 copies/ml, two HBV-spiked samples with viral loads of 200 copies/ml, and one virus-free control sample [Fig. 3, C (i) and D (ii)]. The system was able to accurately identify HCV-infected patient serum/plasma samples without any error [Fig. 3, C (ii) and D (ii)].

Using a panel of 25 ZIKV-infected patient serum samples and 27 HCV-infected serum/plasma samples, the performance of the CNN-NES in qualitatively (virus concentration threshold of 250 particles/ml) identifying the infected serum samples was evaluated and compared to the results obtained by the nucleic acid and immunoassay techniques currently approved by the U.S. Food and Drug Administration and recommended by the U.S. Centers for Disease Control and Prevention (CDC) for virus detection [Fig. 3, B (iii and iv), C (ii), and D (ii); figs. S16 and S17; and table S2]. The CNN-NES showed a concordance of 100% in identifying HCV-infected patient samples compared to PCR and 100% in identifying ZIKV-infected patient samples compared to the nucleic acid assay of Aptima ZIKV Assay and 80% with both the tested immunoassays of ZIKV Detect IgM Capture ELISA (ZIKV IgM detection) and CDC Zika IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), which can be attributed to the false-positive ELISA results usually caused by cross-reactivity with the immune response generated by previous flavivirus infections [Fig. 3, B (iii and iv), C (ii), and D (ii), and table S2] (1, 4). We also evaluated the specificity of the system by testing nontarget viral-infected patient samples (HCV- and HIV-infected patient serum samples) on target microchips (microchips functionalized with anti-HBV antibodies) (table S3). These qualitative test results show that the CNN-NES was able to qualitatively identify target images of target microchips tested with target virus (HBV), even when target HBV-infected patient serum samples with viral loads much lower than the virus concentration of nontarget HCV- and HIV-infected patient serum samples were used. Although our system does not calculate bubble count for its analysis, we have also reported the bubble counts associated with the target and nontarget patient serum samples, as this is the simplest way of quantitatively presenting the data.

**Evaluation of the CNN-NES using a sample-processing cartridge**

To further simplify sample testing using the CNN-NES system, we developed a custom-built microfluidic cartridge for sample processing (Fig. 4, A and B, and fig. S18). The developed cartridge is preloaded with all the reagents and materials required for sample testing (i.e., washing buffer, Pt-nanoprobe, and H$_2$O$_2$ solution), eliminating manual sample preparation and pipetting of multiple reagents and reducing potential user error (fig. S18). The microfluidic cartridge comprises four layers of poly(methyl methacrylate) (PMMA) assembled together using double-sided adhesive (DSA) film that includes a single microfluidic channel with a total volume of 240 μl (fig. S19). The sample is loaded into the sample microchip, which is then inserted into the microchip slot on the cartridge for sample processing. The microchannel on the cartridge is terminally connected to the sample and enabled with a rubber bulb to allow easy and efficient loading and removing of on-chip reagents. The cartridge is also modified with a cellulose paper pad (7 cm in length and 1.5 cm in width) placed in a waste reservoir to absorb the reagents loaded in the sample channel during testing.

To test the performance of the developed microfluidic cartridge in sample testing, we used 1× PBS samples spiked with HBV (n = 42) and ZIKV (n = 36) (Fig. 4 and table S4). ROC analysis showed that the sensitivity and specificity of the CNN-NES when the cartridge was used for sample processing were 100.0% (CI, 93.5 to 100.0%) and 100.0% (CI, 85.2 to 100.0%), respectively. The AUC was 1.0 with a binomial exact CI ranging from 0.954 to 1.0 (Fig. 4C and figs. S20 and S21). Vertical scatterplot showed that the 78 tested virus-spiked PBS samples were correctly classified as positive and negative, indicating a 100.0% accuracy compared with PCR (Fig. 4D). We also evaluated the performance of the CNN-NES in terms of detection reliability and repeatability when different smartphones, sample microchips, and cartridges were used for testing. For device to device, we tested 25 HBV-spiked PBS samples with a virus concentration of 10⁵ copies/ml and virus-free control PBS samples using different sets of cartridges (n = 17). The results showed no difference in identifying samples of the same group (i.e., virus-free control group and HBV-spiked sample group) tested with different cartridges (Fig. 4E). In this part of the study, we reused a set of cartridges (n = 7) for sample testing (cartridge nos. 10, 18, 21, 22, 36, 37, and 38; Fig. 4E). These results showed no difference in the qualitative detection results when the CNN-NES was used to test the same HBV-spiked sample (10⁵ copies/ml) on different microchips (Fig. 4E). To evaluate the user error in sample processing with the developed cartridge, a set of 36 1× PBS samples spiked with ZIKV (n = 18) and HBV (n = 18) were tested by different trained and untrained users (n = 8). The trained and untrained users recruited for the system evaluation had different backgrounds and included a physician and non-PhD graduate and undergraduate students (Fig. 4F and table S5). The untrained users performed the tests using a simple instruction sheet presented in fig. S22. The results indicated 100% agreement in correctly classifying the samples that were tested by both the trained and untrained users. To evaluate the effect of smartphone model on the performance of the CNN-NES, we used Moto X, Redmi Note 4, and Google Pixel 2 smartphones for bubble on-chip detection. The different smartphone systems were used to test the ZIKV-spiked PBS samples (n = 36). We did not observe any significant difference [P > 0.05, one-way analysis of variance (ANOVA) with Kruskal-Wallis test] in qualitatively classifying the tested samples at the same virus concentration in a range of 0 to 10⁷ particles/ml (Fig. 4G and table S6).

**DISCUSSION**

Rapid and sensitive POC diagnostics with the ability to be seamlessly integrated with appropriate and effective surveillance mechanisms can shift the paradigm in outbreak control. The growing advances in consumer electronics and portable communication systems, particularly smartphones, have led to a significant growth in smartphone subscribers worldwide, particularly in the developing countries, and faster and cheaper approaches of data acquisition. Previous mobile health technologies for target virus/protein detection have lacked broad technical applicability, including adaptability to different smartphone models, because of their dependency on smartphone-specific hardware optical attachments (21–30). We overcome this challenge by integrating our unique target labeling and signal
amplification using catalytic properties of nanoparticles to generate bubbles on-chip with an on-phone CNN-based signal detection algorithm, which allowed us to develop a smartphone attachment-free optical sensor for virus detection. In addition, we used a microfluidic cartridge to enable simple sample handling that can be performed by a lay user. The catalytic property of PtNPs allows the formation of gas bubbles creating distinct patterns on-chip, which are easy to be recognized using a smartphone camera. The developed amplification-free and sensitive (250 particles/ml) CNN-NES can qualitatively detect the target virus in 50 min and is not dependent on the model.

**Fig. 4. Virus detection using the CNN-NES and a sample-processing microfluidic cartridge.** (A) Custom-built microfluidic cartridge used for simple sample processing. (B) Exploded three-dimensional schematic of the developed cartridge indicating the detailed layer structure and the configuration of main components of the cartridge. (C) ROC curve analysis of CNN-NES enabled with the developed cartridge compared to the standard RT-PCR using PBS samples spiked with viruses, including HBV \((n = 42)\) and ZIKV \((n = 36)\). The samples with viral loads are classified as positive \((\geq 250 \text{ particles/ml})\) and negative \((<250 \text{ particles/ml})\). (D) Vertical scatterplot 1× PBS-spiked samples. The threshold for the probability values measured was 0.5. Samples with probability values above and below 0.5 were classified as positive \((1)\) and negative \((0)\), respectively, compared to the RT-PCR at a threshold of 250 particles/ml. (E) Evaluation of the repeatability of qualitative detection of samples when different cartridges were used for testing the same HBV-spiked PBS samples \((n = 25)\). The results are expressed as the average values of at least three measurements from one sample. (F) Evaluation of the performance of the system when operated by different users for sample testing using PBS samples spiked with ZIKV \((n = 18)\) and HBV \((n = 18)\). The results are expressed as the average values of three experiments from one sample. (G) Evaluation of the performance of the system when different smartphones were used for sample testing. The results are expressed as the average values of three independent experiments. The probability value is presented for each sample being positive. Error bars represent SDs. ns, not significant.
of the smartphone used for detection, which is a major advancement in the development of mobile health technologies for virus detection. The developed CNN-NES is a powerful universal modality that can be adapted to a wide number of smartphone models without the need for extensive modifications and can be used for the detection of wide range of infections. In this system, we relied on the enhanced catalytic properties and stability of PtNPs over traditional peer enzymes to allow stable and rapid gas bubble formation \((43, 44)\). The catalytic activity of PtNPs for gas formation is directly related to the size of the PtNPs, and larger nanoparticles are expected to have higher activity \((45)\). However, relatively small size PtNPs (4.5 nm in diameter) were used in this study to avoid surface particle load limitation during virus labeling and to minimize the effect of hydrodynamic shear forces on the formed Pt-virus complexes during washing and labeling steps that may release the captured viruses on-chip \((46)\). In addition, high catalytic activity of PtNPs in high concentration of \(H_2O_2\) leads to rapid merging of the generated bubbles and forming irregular bubble shapes, which can make accurate signal detection difficult \((fig.\ S23)\). To avoid rapid bubble merging and to control the stability of the visual patterns on-chip after virus capture and signal amplification, we used glycerol (20% of the solution) to increase the density of the catalyzer solution to \(\sim 0.73 \text{ g/cm}^3\). The formed bubbles reached to macroscopic sizes (up to 90 \(\mu\text{m}\)) after 300 s of incubation at 10% \(H_2O_2\) solution without visible merging, which is even higher than the 5% \(H_2O_2\) concentration used in the study \((figs.\ S24\ and \ S25)\). One additional parameter that played an important role to overcome the limitation on controlling the gas bubble formation process was the well-distributed virus capture on the surface of the chips. Our surface chemistry scheme that included the addition of a bifunctional PEG cross-linker (2 kDa, 10 times smaller than antibody) allowed a controlled indirect linking of antibody to the microchip surface. PEGylation increases the conformational stability of proteins and resistance to degradation. Therefore, we used PEG on the surface of the microchips to help in stabilizing the antibody activity and avoiding nonspecific interactions, for easy washing, and to promote the stability of conjugated biomolecules \((47–50)\). PEG acts as a flexible arm that provides maximum accessibility of antibody and a higher chance for avid interaction with virus. The directional conjugation of antibody through the carbohydrate residue in the FC region of the antibody, other than amine or carboxyl groups that are usually involved in interaction with the target antigen, helps in preserving the full activity and specificity of the conjugated antibodies \((51, 52)\). All these factors together with using highly specific mAb (table S7) led to a detection sensitivity of 98.97% and specificity of 91.89% \((n = 134 \text{ of ZIKV, HCV, and HBV})\) for the reported CNN-NES virus detection approach \((fig.\ S26)\). The developed bubble approach was stable under different environmental conditions including different temperatures and relative humidity. The change in the incubation temperature and relative humidity did not have any significant effect \((P \geq 0.05, \text{ unpaired } t\text{ test})\) on the number of bubbles generated on-chip \((figs.\ S27\ to \ S30)\). Likewise, the change in the \(H_2O_2\) concentration showed insignificant effect \((P \geq 0.05, \text{ unpaired } t\text{ test})\) on bubble formation using a single-channel microchip \((figs.\ S31\ and \ S32)\). We further tested the shelf life time of the antibody-functionalized chips and the results indicated the stability of the prepared chips when lyophilized and stored at room temperature on shelf for 45 days \((figs.\ S33\ and \ S34)\). The current prototype of the developed bubble technology has a great potential for low-cost POC diagnostic applications and has several advantages over the standard detection techniques currently used in virus detections such as RT-PCR and ELISA techniques \((tables\ S8\ and \ S9)\). However, its integration in a fully automated quantitative system is important and can be addressed in future work using an advanced microfluidic platform \((8)\). As a nanoparticle-based technology, the stability and long-term storage can be also improved by the use of specific surface chemistries in the preparation process and the addition of stabilizing agents to the nanoparticle solution \((8, 53)\). In summary, we reported the development and evaluation of a diagnostic assay with the potential for POC viral load testing and demonstrated a unique integration of advancements in nanotechnologies, microfluidics, and consumer electronics to solve an unmet clinical barrier in the management of infectious diseases. The developed platform is simple and cost effective and can be adapted to a wide variety of other pathogens and has the potential for various clinical applications.

**MATERIALS AND METHODS**

**Biosafety and human participant statement**

The research work reported was approved and performed in adherence to guidelines and procedures approved by the Institutional Biosafety Committee of Partners HealthCare (parent organization of Massachusetts General Hospital and Brigham and Women’s Hospital) and under appropriate institutional review boards (IRB nos. 2019P002209 and 2019P001489). All the experiments with ZIKV, HBV, and HCV were performed in BSL2. Deidentified ZIKV-infected patient serum samples were obtained from certified biorepositories of consented patients’ samples at Boca Biologics LLC. Deidentified HCV-infected and HBV-infected patient plasma/serum samples were obtained from Discovery Life Sciences Inc.

**Virus stock samples and viral load measurements**

ZIKV, virus strain PRVABC59 isolated by the CDC from a ZIKV-infected patient who travelled to Puerto Rico in 2015 (National Center for Biotechnology Information accession no. KU501215) was provided by X. G. Yu at the Ragon Institute. HBV-cultured samples were provided by R. T. Chung at Massachusetts General Hospital. HBV stock virus was obtained from HepG2 AD38 cells \((54, 55)\) as described previously. HIV stock–cultured samples were received from our colleagues at the division of engineering in medicine at Brigham and Women’s Hospital. HCV-infected and HBV-infected patient serum/plasma samples were purchased from Discovery Life Sciences Inc. The viral loads of HBV-infected and HCV-infected patient serum samples were measured using a standard RT-PCR system and reported by the vendor (Discovery Life Sciences Inc.). The viral load of ZIKV-infected patient samples received from Boca Biologics LLC was not known, as the PCR test results of those samples were reported qualitatively by the vendor. The viral loads of all ZIKV-spiked samples were measured through RT-PCR by our collaborator at the Ragon Institute. The viral loads of all other samples were either reported by the provider or measured with a RT-PCR system by Viracor Eurofins Inc. The spiked samples that were used in system training or testing throughout the study were prepared by serial dilutions of stock virus samples with known viral loads measured by standard PCR approaches. In these cases, the control samples were virus-free samples that were processed for the detection assay with the same protocol as all other viral samples, with the exception of the addition of viral-stock dilutions.
Microchip design and fabrication

Single-channel microchips functionalized with antibodies against the target viruses were used for virus capturing. The microchip was designed using the vector graphics editor CorelDRAW X7 software (Autodesk Inc., San Rafael, CA, USA). The microchips were fabricated from PMMA (3.1 mm thick; McMaster-Carr, Atlanta, GA, USA), DSA film (80 µm thick; iTapeStore, Scotch Plains, NJ, USA), and glass slides (25 mm by 75 mm; Globe Scientific, NJ, USA). The fabrication of the chip starts by cutting PMMA and DSA film using a laser cutter (Versa Laser, Scottsdale, AZ, USA). The PMMA was prepared to contain the inlets and outlets, while DSA film included the main testing channels. All materials were precleaned with 5% ethanol, 30% H₂O₂, and deionized water using lint-free tissue. The surface of the cleaned glass slides was activated using PE-25 oxygen plasma (100 mW, 15% oxygen; Plasma Etch Inc., Carson City, NV, USA) for 2 to 3 min and modified using silane-functionalized PEG thiol (Nanocs Inc., New York, NY, USA) and mAb for capture of the target virus: anti-ZIKV envelope antibody (catalog no. HM325, EastCoast Bio Inc., North Berwick, ME, USA) for ZIKV, anti-HBV surface antigen (Ad/Ay) antibody (catalog no. ab54247, Abcam, Cambridge, MA, USA) for HBV, and anti-HCV core antigen antibody (catalog no. ab2582, Abcam, Cambridge, MA, USA) for HCV. Then, PMMA and DSA film were assembled on the modified glass slides to form the complete microchip. The entire process of cleaning and assembling was done in a clean hood to prevent contamination due to stray dust and airborne particles.

 Nanoprobe preparation and characterization

Different sets of platinum nanoprobes were prepared of spherical PtNPs modified with mAb against the target virus: anti-ZIKV (ZIKV-Env) antibody (EastCoast Bio Inc. North Berwick, ME, USA) for ZIKV, anti-HBV surface antigen (Ad/Ay) antibody (HB24, catalog no. ab54247, Abcam, Cambridge, MA, USA) for HBV, and anti-HCV core antigen antibody (catalog no. ab2582, Abcam, Cambridge, MA, USA) for HCV. The synthesis protocol starts by modifying the surface of PtNPs with PDPH freshly reduced by 20 mM tris-(2-carboxyethyl)phosphine (TCEP). The prepared PtNP-PDPH was then coupled to antibody oxidized using 10 mM sodium metaperiodate for 1 hour at room temperature. The prepared PtNPs, PDPH-PtNPs, and Pt-nanoprobes were characterized using TEM, UV-vis spectroscopy, FT-IR spectroscopy, and DLS techniques.

PtNP synthesis and characterization

PtNPs were synthesized using a modified method from literature (56). All glassware used was cleaned with aqua regia and ultrapure water. Thirty-six milliliters of a 0.2% solution of chloroplatinic acid hexahydrate was mixed with 464 ml of boiling deionized water. Eleven milliliters 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 borohydride solution, containing 1% sodium citrate and 0.05% citric acid. The reaction continued for 10 min, and the formed nanoparticle solution was gradually cooled down to room temperature. The formed PtNPs were modified with PDPH freshly reduced by 20 mM TCEP. The prepared PtNPs were characterized using TEM, UV-vis spectroscopy, z potential, and DLS.

Antibody oxidation and activation with PDPH cross-linker

The antibody was oxidized using the standard protocol in which antibody was mixed with 10 mM sodium metaperiodate and 0.1 M sodium acetate (pH 5.5) and incubated at 4°C in the dark for 20 min. Then, PDPH-activated PtNPs are allowed to react with the oxidized antibody for 1 hour at room temperature. The prepared PDPH-modified antibodies are then washed three times using 1x PBS to remove the excess PDPH using Amicon Ultra Centrifugal filter units Ultra-4; molecular weight cutoff, 100 kDa (Millipore, MA, USA).

Quantification of the number of anti-ZIKV mAb molecules per Pt-nanoprobe

The concentration of antibody was estimated using the bicinchoninic acid (BCA) protein assay according to the manufacturer’s protocol (Pierce). Absorbance of aliquots of Pt-nanoprobe solution (PtNP + antibody) and PtNPs solution (no antibody) values was measured at 562 nm using a BioTek microplate reader, and the concentration of the antibodies was estimated after subtracting the background absorbance of nanoparticles. The number of antibody molecules on each PtNP was calculated by dividing the antibody concentration by the concentration of the PtNPs in the Pt-nanoprobe solution as follows:

The concentration of Pt-nanoprobes was adjusted to 2.39 μM, and the concentration of protein was estimated to be 348.32 ± 13.14 μg/ml. Considering that the molecular weight of an antibody molecule is 150 KDa, the molar concentration of antibody was calculated to be 2.32 ± 0.085 μM, which implies the presence of 0.971 ± 0.035 antibody molecule per Pt-nanoprobe.

Quantification of virus captured on-chip

Aliquots of ZIKV were loaded on a single-channel chip modified with anti-ZIKV (ZIKV-Env) antibody, incubated for 20 min at room temperature for virus capture, and then washed using phosphate buffer (PB) three times. The captured virus was digested and eluted for testing using ELISA and RT-PCR techniques. The concentration of the eluted virus was calculated from a standard curve prepared from known concentrations of virus.

Bubble on-chip assay

The bubble assay relies on capture of target virus on the surface of microchips and labeled with PtNPs and the formation of gas bubbles in the presence of H₂O₂. The bubble assay working protocol includes three main steps. (i) Virus capture on the surface of microchip. Around 20 μl of the target virus sample was loaded on the microchip and incubated for 20 min at room temperature followed by a washing step using 10 mM PB (pH 7.4). (ii) Virus labeling with Pt-nanoprobes. Twenty microliters of Pt-nanoprobe solution against the target virus was loaded on the surface of the microchip with the captured virus particles. The microchip was incubated for 20 min at room temperature, and the excess Pt-nanoprobes were washed using 10 mM PB (pH 7.4) three times. (iii) Bubble formation. A 5% hydrogen peroxide solution containing 20% glycerol was added to the microchip and incubated for 10 min. This protocol can be performed by manually adding and removing the reagents to the chip or using a microfluidic cartridge preloaded with all the reagents needed for sample testing including PB for washing, PtNP solution, and the 5% H₂O₂ solution. The formed bubbles were then imaged and detected using optical microscopy and the NES system. The capture of virus particles and formation of virus-PtNP complexes on the surface of microchip was confirmed using ELISA, RT-PCR, SDS gel electrophoresis, and SEM techniques.

Cartridge assembly and operation

The cartridge is a core-shell microfluidic unit that is 175 mm in length and 75.5 mm in width and prepared of a total four layers of PMMA and three layers of DSA film. The cartridge core comprises
a set of two PMMA layers (1.5 mm in thickness) assembled together with a DSA film layer to form a single multilane microfluidic channel (260 cm in length, 80 μm in depth, and 1 mm in width). The inside surface of each PMMA layer forming the cartridge core is pretreated with Rain-X 620036 Plastic Water Repellent to be more hydrophobic, allowing easy flow through the microfluidic channel. The PMMA shell set comprises two PMMA layers with 3.2-mm thickness. The top PMMA layer contains a sample housing cavity for sample insertion, while the lower PMMA layer is engraved and modified with a cellulose paper absorbing pad (1 mm in thickness, 71 mm in length, and 22 mm in width). The core set is first loaded with the sample testing reagents through a loading well on the top layer in the following order: 40 μl of PB solution (first washing step), 35 μl of nanoprobe solution (for virus labeling), 60 μl of PB solution (second washing step), 35 μl of 5% H2O2 solution, and 5 μl of indicator dye, separated by 18-μl equivalent space of air. Afterward, the PMMA layers are added, and a rubber bulb (14.4 mm in diameter and 7.2 mm in height) is sealed under the top PMMA layer to allow easy and controlled manipulation of reagents preloaded on the cartridge. The virus sample was loaded on a single-channel sample microchip using 20-μl Non-Sterile Exact Volume Transfer Pipettes (Thermo Fisher Scientific Samco 783NL). The sample microchip was then inserted in the sample housing cavity and connected to the core microfluidic channel with two plastic tips in the core set. The sample processing was started by compressing the rubber bulb until the dye indicator solution reached to position 1 (arrowhead 1 at the left side of the cartridge) to complete the virus sample washing and to add the labeling Pt-nanoprobe solution on the microchip. The cartridge and the loaded microchip were then incubated for 20 min at room temperature, following which the bulb reagent loading was continued until the dye indicator reached position 2 (arrowhead 2 at the left side of the cartridge). The microchip was then detached from the device and incubated for 10 min for bubble formation, and the bubbles were imaged and evaluated using the CNN-NES.

**Smartphone system and CNN algorithm**

We used a CNN model performing supervised learning to automatically recognize differences between two classes of positive (infected) and negative (noninfected) samples. We used Google’s Inception v3 CNN architecture pretrained to 93.33% top-five accuracy on a dataset of 1000 object classes containing 1.28 million images of the 2014 ImageNet challenge. We then performed transfer learning by removing the final classification layer from the network and retrained it with our dataset. Our dataset is composed of prelabeled images organized in two different classes generated from 38 videos of ZIKV-spiked PBS samples and bubble-simulated samples prepared with different dilutions of PtNPs on a single-channel microfluidic cartridge and recorded using Moto G4, Moto X, and iPhone 6 smartphones. The videos were recorded under different orientations (0° to 360°), backgrounds (black, white, brown, and random outdoor backgrounds), and illuminations (150 to 700 lux). The images were created from video files using a small Python script that is run on Linux. Frames were extracted from these videos and organized into two different classes (negative and positive) for training. We used 15,057 images for training and validation and 1502 images for testing in the development of our algorithm. We did not use any of the patient samples or spiked samples used during the prospective testing of the system for training of the network. In other words, none of the test samples were ever seen by the network during the training phase of the neural network development. In this dataset, each image was resized to 299 x 299 pixels to be compatible with the original dimensions of the Inception v3 network architecture. This procedure, known as transfer learning, leverages the natural image features learned by the ImageNet pretrained network. The CNN algorithm was trained using back propagation. All layers of the network were fine-tuned using the same global learning rate of 0.001.

In addition, Google’s TensorFlow deep learning framework was modified with the prepared dataset to develop an android application for testing of different virus samples. The smartphone application reports the probability value of the tested sample as being positive or negative.

**Sample preparation and testing using the smartphone system**

Virus-spiked samples were prepared by diluting stock virus sample (Materials and Methods) in aliquots of 1× PBS (pH 7.4). A panel of ZIKV-infected patient serum samples (n = 25) was purchased from Boka Biolistics LLC (Pompano Beach, FL, USA). HCV- and HBV-infected patient plasma/serum samples were purchased from Discovery Life Sciences Inc. Around 20 μl of each sample was loaded into the single-channel microchip and tested following bubble assay protocol either using manual pipetting or the reported sample-processing microfluidic cartridge. After bubble formation, the microchips were imaged using the smartphone system. The performance of the CNN-NES system in testing virus-spiked samples was evaluated compared to the standard RT-PCR technique. For patient samples, the CNN-NES system was evaluated against commercially available assay of Aptima ZIKV Assay (Hologic, Marlboro, MA, USA), ZIKV Detect IgM Capture ELISA (InBios International Inc., Seattle, WA, USA), and Zika MAC-ELISA (CDC, USA) for nucleic acid and immunological testing of ZIKV.

**Effect of temperature and humidity on bubble assay**

The effect of temperature and humidity conditions on the developed bubble on-chip approach was tested using ZIKV-spiked samples in PBS (pH 7.2) at concentration of 10⁵ particles/ml. Before sample testing, virus sample, functionalized microchip, and all the testing reagents including Pt-nanoprobe solution, washing buffer, and H2O2 solution were equilibrated to specific temperature and humidity. For temperature testing, virus sample was loaded on a sample microchip, and the bubble protocol including virus capture, labeling, and bubble formation was performed at different temperatures of 25°C, 35°C, and 55°C at a relative humidity of 45%. Similarly, virus samples were tested at different relative humidity values of 45, 60, 75, and 95% and a temperature of 25°C for testing the effect of humidity on bubble assay. The tested microchips and the generated bubbles were imaged and evaluated using the CNN-NES.

**Shelf life testing of antibody-functionalized microchips**

The stability of antibody-functionalized microchips was evaluated using ZIKV-spiked samples in PBS (pH 7.2) at a virus concentration of 10⁵ particles/ml. Microchips functionalized with anti-ZIKV antibody were treated with 1.5 mM trehalose solution and then freeze-dried using Eyela FD-1000 Freeze Dryer for 12 hours. The dried microchips were packed using a FoodSaver Vacuum Storage unit, divided into four groups (n = 8 chips), and stored on a shelf at room temperature. Each group of stored microchips was tested using the bubble assay for virus detection at different time points of 0, 2, 4, and 6 weeks, and the microchips and the generated bubbles were imaged and evaluated using the CNN-NES.
Characterization techniques and analyses

**UV-vis spectroscopy**
Absorption spectra were measured on Beckman Coulter DU 800 UV-Vis spectrophotometer. The samples were loaded in 10-mm path length quartz cuvettes and scanned at room temperature.

**Transmission electron microscopy**
TEM images were obtained using a JEOL 2100 TEM microscope at an acceleration voltage of 300 kV. The samples were prepared by dropping 2 μl of the sample onto ultrathin Formvar-coated 200-mesh copper grids and then dried in air. The mean diameter and size distribution histogram of particles were obtained by averaging more than 100 particles from the TEM images using ImageJ software.

**Dynamic light scattering**
DLS experiments were performed using Malvern Zetasizer (Malvern Instruments, Malvern, UK). The samples were initially filtered through a 0.22-μm filter membrane and allowed to settle overnight for DLS measurements. Ultrapure water (>18 megohms) from a PURELAB Ultra water system (ELGA Ltd) was used as a diluent, and three measurements were recorded for each sample at room temperature. The average values were calculated from the reported results for three different batches.

**FT-IR spectroscopy**
FT-IR spectroscopy spectra in the region of 2000 to 500 cm⁻¹ were collected in absorbance mode with a FT-135 Bio-Rad FT-IR spectroscope spectrometer.

**Enzyme-linked immunosorbent assay**
We used ELISA to quantify the captured ZIKV on-chip using a sandwich immunoassay kit (RV-403001-ENV-48 ELISA Kit) that was specifically developed to detect ZIKV envelope protein. ZIKV captured on-chip was eluted from the surface of the chip and digested using the sample preparation buffer provided in the kit, and the virus concentration was estimated using a standard curve prepared from known virus concentrations. An ELISA 96-well plate coated with Anti-Zika envelope Ab was used to capture the target antigen in the samples for 2 hours at 37°C. After incubation, the plate was washed four times using washing buffer, and the plate was dried using paper towels. Diluted biotin detection antibody (100 μl) was added and incubated for 60 min. Then, streptavidin–horseradish peroxidase conjugate solution (100 μl) was added and incubated for 60 min. The plate was washed four times with washing solution after the incubation. Trimethylboron substrate (100 μl) was added and incubated for 15 min. To stop the reaction, 100 μl of stop solution was added, tapped, and mixed gently. When the solution in the wells begins to turn yellow, absorbance of the plate was read at 450 nm using a BioTek plate reader.

**Agarose gel electrophoresis**
The electrophoretic mobility pattern of the prepared Pt-nanoprobes compared with nonmodified PtNPs was evaluated with a horizontal submersed gel electrophoresis apparatus (Mini-Sub Cell GT, Bio-Rad) using a 0.7% (w/v) agarose gel in tris-acetate-EDTA buffer (pH 8.5). Ten microliters of sample aliquots were loaded into wells. The gel was subjected to a typical voltage of 100 V for 30 min and imaged with a digital camera.

**SDS–polyacrylamide gel electrophoresis**
Protein testing was performed using a Mini-PROTEAN Tetra System and 4 to 20% Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad, Hercules, CA). The samples were digested in a sample preparation buffer (2.5 ml of 1 M tris-HCl (pH 6.8), 0.5 ml of double-distilled water (ddH₂O), 1.0 g SDS, 0.8 ml of 0.1% bromophenol blue, 4 ml of 100% glycerol, and 2 ml of 14.3 M β-mercaptoethanol completed to 10 ml with ddH₂O) and heated for 5 min at 95°C on a heat block. Then, 15 μl of a protein standard and 20 μl of the samples were loaded on the gel. The voltage was turned up to 90 V, and the electrophoresis was continued for 50 min. After electrophoresis was done, the gel was rinsed in water for 3 min and stained in Bio-Safe Coomassie blue stain for about 1 hour. Last, the gel was destained and photographed using a gel documentation system.

**Statistical analyses**
Statistical analyses were performed using GraphPad Prism software version 5.01 (GraphPad Software Inc. La Jolla, CA, USA) and MedCalc 14.8.1. (MedCalc Software bvba, Ostend, Belgium). All viral load values were converted into log₁₀ copies/ml. The mean and SDs were calculated for each data point from at least a total of two to three independent experiments unless indicated in the text. The statistical significance of differences between readings under control and experimental conditions for each bubble assay was assessed using a two-tailed unpaired t test or one-way ANOVA with Kruskal-Wallis test. The level of significance was set at P > 0.05. The strength of the linear relationship was evaluated using Pearson’s correlation, and the regression coefficient was calculated. The degree of agreement was assessed by Bland-Altman method. ROC and scattering plots analysis were used to define performance of bubble assay to the standard analytical technique.

**SUPPLEMENTARY MATERIALS**
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/51/eabd5354/DC1

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have no other competing interests. **Data materials and availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Deidentified patient data used in this study may be available through the authors upon request. All reasonable requests for materials sharing will be considered. The patient samples were purchased from commercial vendors, and the exact same patient sample materials may not be available for sharing by the authors or by the commercial vendors because of the limited sample volume available from each participant. The codes and algorithms developed for this study may be obtained by data transfer agreement with Partners HealthCare and Brigham and Women’s Hospital upon request.

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Virus detection using nanoparticles and deep neural network–enabled smartphone system

Mohamed S. Draz, Anish Vasan, Aradana Muthupandian, Manoj Kumar Kanakasabapathy, Prudhvī Thinumalaraju, Aparna Sreeram, Sanchana Krishnakumar, Vinish Yogesh, Wenyu Lin, Xu G. Yu, Raymond T. Chung and Hadi Shafiee

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