Supporting information for the article: **"Genetic Analysis of H1N1 Influenza Virus from Throat Swab Samples in a Microfluidic System for Point-of-Care Diagnostics"** by B. Scott Ferguson, Steven F. Buchsbaum, Ting-Ting Wu, Kuangwen Hsieh, Yi Xiao, Ren Sun, and H. Tom Soh*

Device fabrication: Two borofloat glass wafers were sputter-coated with a 100-nm layer of SiO_2. A CNC mill (Flashcut CNC, San Carlos, CA) with 0.75 mm diamond bit (Triple Ripple, Abrasive Technology, Lewis Center, OH) was used to drill fluidic vias in the top wafer. Gold working electrodes and platinum counter and reference electrodes were patterned onto the bottom wafer photolithographically and electron-beam evaporated (VES 2550, Temescal, Livermore, CA) to a thickness of 200 nm on 20 nm titanium adhesion layers. The channel was cut from a 250-μm-thick PDMS sheet (BISCO Silicones, Rogers Corporation, Carol Stream, IL) using a cutting plotter (CE5000-60, Graphtec, Santa Ana, CA) and bonded to the diced glass substrates via 300 s UV-ozone treatment. Fluidic port connectors (Labsmith, Livermore, CA) were glued onto the device with thermal-resistant epoxy (Loctite, Henkel, Düsseldorf, Germany), readying the chip for probe immobilization.

E-DNA probe immobilization: The gold working electrode surfaces were first cleaned via cyclic voltammetry, with twelve potential sweeps ranging from -0.8 to 0.9 V (with respect to the platinum reference electrode) applied at 0.1 V s^{-1} with a sample interval of 0.001 V and 10 μA sensitivity in 50 mM H_2SO_4. Subsequently, the chamber was flushed with deionized water (DI), followed by the addition of probe solution. Probe solution was prepared by adding 2 μL of 100 mM tris(2-carboxyethyl)phosphine (TCEP, Invitrogen, Carlsbad, CA) to 2 μL of 1.2 μM probe stock and incubating at room temperature (RT) for 40 minutes to enable cleavage of disulphide bonds, after which 46 μL of high-salt incubation E-DNA buffer (1x HSIEB, 1.5 M NaCl, 100 mM phosphate, 1 mM Mg^{2+}) was added to aid in subsequent self-assembly. The chip was incubated for 1 hour at room temperature, permitting the thiolated stem-loop structure to form a self-assembled monolayer on the gold electrode surface. The chamber was then flushed with 1 mL DI water and the remaining electrode area was incubated for two hours in passivation solution, which was prepared at 8 mM by adding 1 μL stock 6-mercaptohexanol (Sigma-Aldrich, St. Louis, MO) to 915 μL 1x HSIEB. The chamber was flushed again with DI and filled with HSIEB, after which the device was ready for use in trials. To prevent carry-over contamination, probe preparation was conducted in a laboratory in a separate building.

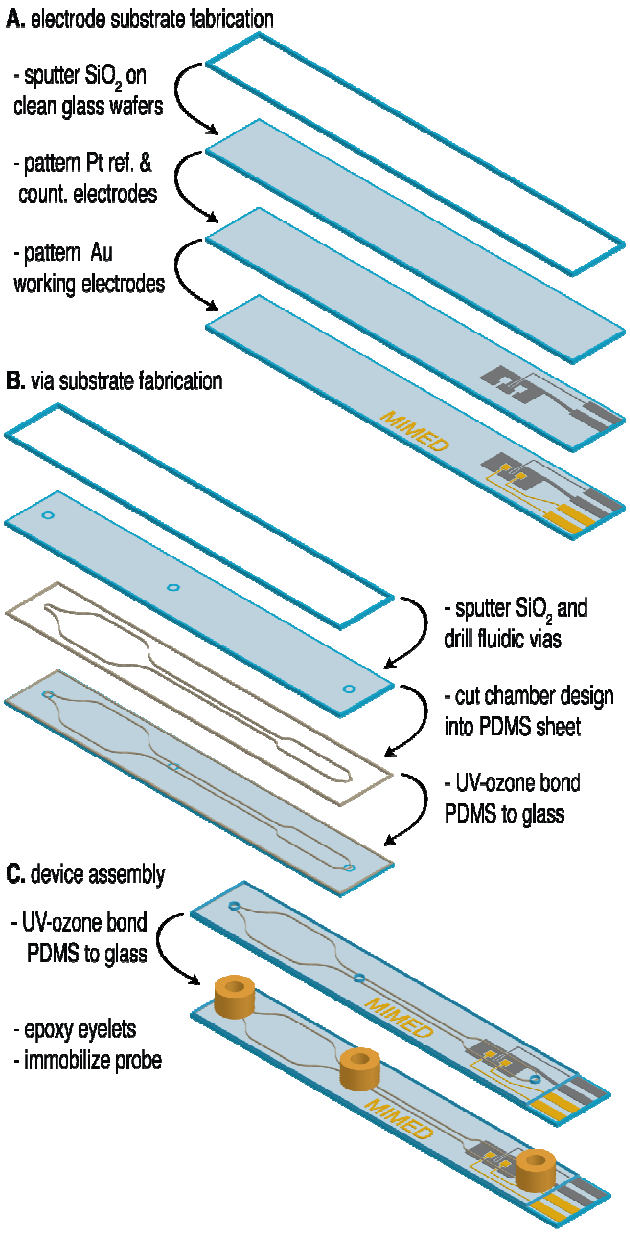
DNA sequences: The following sequence was chosen for the detection of influenza A/PR/8/34 H1N1, targeting a highly conserved region of segment 7 of the viral genome encoding the matrix protein M1: 5'- cca gct cta tgc tga caa aat gac cat cgt cag cat cca cag cac tct gct gtt cct ttc ga-3'. For E-DNA sensor calibration, we used a synthetic derivative of this target sequence from Integrated DNA Technologies (Coralville, IA). To generate the target sequence *in vitro*, we selected the following sense and antisense primer sequences (also from Integrated DNA Technologies): 5'-/5Phos/-tcg aaa gga aca gca gag tg-3' and 5'-cca gct cta tgc tga caa aat g-3'. Note that the forward primer is phosphorylated at the 5' end to permit lambda exonuclease digestion during ssDNA generation. The E-DNA stem-loop probe was synthesized by Biosearch Technologies (Novato, CA) with the following sequence: 5'- HS-$(CH_2)_{11}$-gtg cac gaa agg aac agc aga gtg cac- NH_2-MB 3'. An 18-nucleotide sequence complementary to the underlined region was used for detection.

Bead preparation: Using a magnetic particle concentrator (Invitrogen, Carlsbad, CA), we washed 10 μL of streptavidin-coated magnetic beads (Dynabeads MyOne C1 Streptavidin, diameter = 1 μm, Invitrogen, Carlsbad, CA) with 1 mL of 1x PBS, and then resuspended them in 10 μL 1x PBS. We then added 2 μL of biotinylated anti-influenza A nucleoprotein antibody (Bioscience Research Reagents, Temecula, CA) to the beads and incubated the mixture for 30 minutes at 4 ºC to conjugate the antibody with beads. The mixture was washed twice with 1 mL and resuspended in 50 μL 1x PBS.

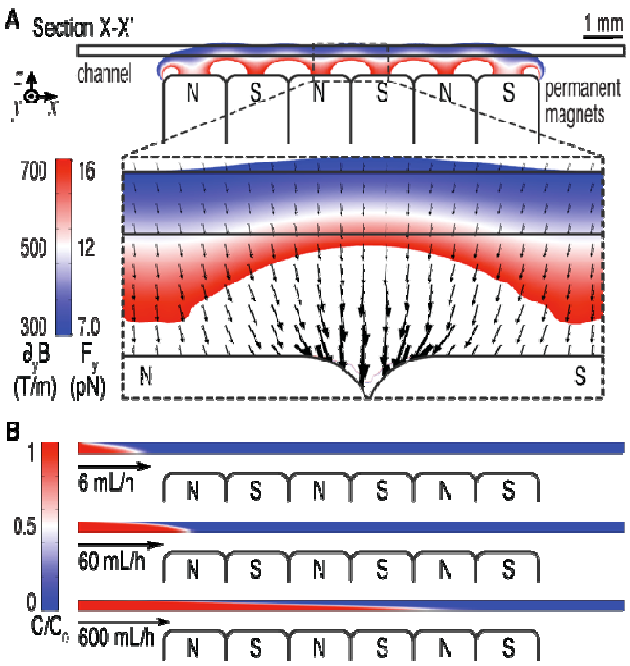
RNA Stabilization: RNA degradation is a significant problem for samples containing nucleases, such as throat, buccal or nasopharyngeal swabs, and we observed that swab samples could rapidly degrade during incubation. This problem was circumvented by incubating samples in RNA-stabilization medium to suppress nuclease activity. We observed that high reagent concentration appears to reduce virus-antibody affinity, and determined empirically that the most effective concentration of RNAlater was 10%.

Voltammetry: All electrochemical measurements were conducted with an electrochemical analyzer (30B, CH Instruments, Inc., Austin, TX). The chip was connected via a standard five-pin card-edge connector and subjected to ACV for end-point analysis or SWV for continuous monitoring. ACV, which offers superior signal-to-noise ratio, was performed between -0.7 V and -0.2 V at a frequency of 10 Hz, amplitude of 25 mV, period of 0.5 s and sensitivity of 200 nA. To verify all signals, ACV scans were performed on both working electrodes, each in duplicate. ACV curves were aligned by peak potential to correct for drift due to the on-chip platinum pseudo-reference electrodes. SWV, which offers high temporal resolution, was performed between -0.7 V and -0.2 V at a frequency of 100 Hz, amplitude of 10 mV and sensitivity of 500 nA. To correct for baseline drift due to temporal SWV measurements, a linear fit was applied to target-negative signals and subtracted from target-positive signals.

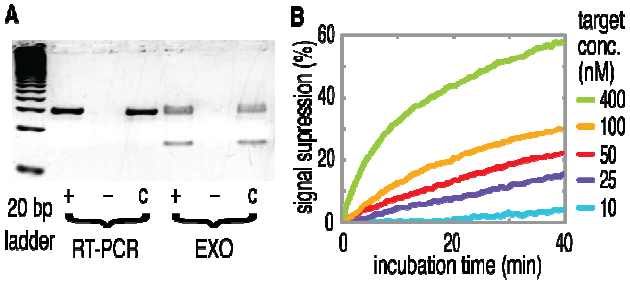
MIMED performance in buffer: It is known that saliva contains nucleases that degrade the RNP complex of influenza viruses.[10] To measure MIMED performance without the effects of sample degradation, we first challenged the chip with viral samples spiked into PBS (Supporting Information, Fig. S4). For each sample, we conducted ACV scans prior to hybridization, yielding a baseline of peak faradic current at the methylene blue redox potential (red curves). After 30-minute hybridizations, we repeated the scans, yielding changes in peak currents as a function of virus concentration (purple curves). We detected current changes of 35 ± 0.6 %, 29 ± 0.3 % and 21 ± 0.4 % for viral loads of 1000, 100 and 10 $TCID_{50}$ mL^{-1}, respectively (Fig. S4 B-D). We note that these current signals are significantly greater than the zero-template negative control, which generates 0.7 ± 0.5 % signal (Fig. S4 A). To validate that these responses were indeed the result of DNA hybridization, we regenerated the sensor with 50 mM sodium hydroxide and deionized water, which dehybridized the target from the probe and returned it to its initial state (dashed blue curves). Each sensor signal could be regenerated to within 96% of initial peak faradic current.



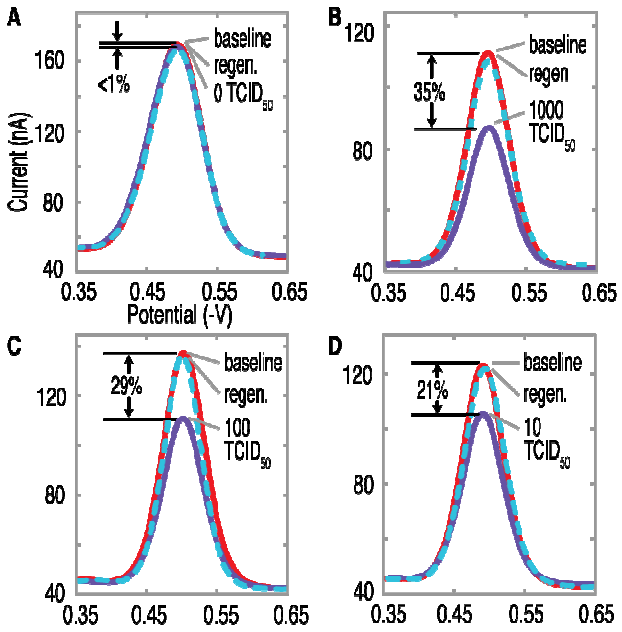
Supplementary Figure S1. Device Fabrication. The disposable MIMED device is designed for simple manufacture. The device is comprised of two borofloat glass substrates and a PDMS channel. The wafer substrate (A) is first cleaned and sputter-coated with a 100-nm layer of SiO_2 to improve PCR efficiency over the bare-glass surface. 200-nm-thick platinum counter and reference electrodes and 300 nm-thick gold working electrodes are photolithographically patterned and deposited via electron-beam evaporation. A 20 nm-thick titanium adhesion layer is used for each deposition. The via substrate (B) is also sputter-coated with SiO_2, after which fluidic vias are drilled into the wafer with a CNC mill with a 0.75-mm-diameter diamond bit. The channel pattern is cut into 0.25 mm-thick PDMS sheet via a cutting-plotter, and bonded to the glass substrate via 300s UV-ozone treatment. To assemble the device (C), UV-ozone is applied to the opposite face of the PDMS channel, which is then bonded to the electrode substrate. Fluidic connectors are then affixed to the device with thermal-resistant epoxy. The probe immobilization protocol is then applied to functionalize the E-DNA sensor surface.



Supplementary Figure S2. Magnetic force and simulation of bead pull-down. (A) Section view inset of the MIMED trapping chamber illustrates the magnetic gradient, which ranges from 300-600 T m^{-1}. This gradient exerts a magnetic pull-down force of ~10 pN on the beads directed towards the trapping surface. (B) Magnetic pull-down efficiency at 6, 60 and 600 mL h^{-1}. Magnetic bead concentration (C) is calculated by balancing magnetic force with Stokes drag across the velocity field, and normalized against the input concentration C_0. This ratio represents the fraction of beads remaining suspended in the solution at a given point in the channel.



Supplementary Figure S3. MIMED module performance. (A) Efficiency of on-chip RT-PCR and ssDNA generation. RT-PCR on-chip (c) and off-chip (+) show similar output of specific 62-bp product, while zero-template controls (-) produce no product (left lanes). ssDNA is likewise generated (EXO) with > 90% efficiency (right lanes). (B) E-DNA sensor response curve. We calibrated the E-DNA signal as a function of time and target concentration, and resolved the expected RT-PCR product concentrations (10-300 nM) within 30 min. All curves are normalized with respect to baseline-corrected signal.



Supplementary Figure S4. The integrated MIMED system readily detects viral loads as low as 10 $TCID_{50}$ in PBS. MIMED full system performance is assessed in the absence of swab-sample interferents. Prior to target incubation (A), the sensor reports a baseline peak faradic current (red), which is suppressed in the presence of ssDNA product (B-D; purple). The MIMED system unambiguously detects viral loads of 1000 (B), 100 (C) or 10 (D) $TCID_{50}$ mL^{-1}, as demonstrated by respective current suppression of 21 ± 0.4%, 29 ± 0.3%, 35 ± 0.6% compared to the zero-virus control (0.7 ± 0.5%). The sensor can be regenerated (via NaOH and DI water) to within 96% of the baseline signal (dashed blue), validating the specificity of the detection.