SI Appendix

Title: Multitarget, quantitative nanoplasmonic electrical field-enhanced resonating device (NE^2RD) for diagnostics

Short title: Quantitative Diagnostic Device for Multitargets

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Classification: Biological Sciences (Major) and Applied Biological Sciences (Minor).

MATERIALS AND METHODS

A. Materials. In the experiments, we used the following reagents and materials, whose product number and manufacturer information were indicated below:

Item	Product Number	Manufacturer
Gold nanoparticle (10 nm)	15703	TedPella, Redding, CA
Polystyrene 96-well plate	3997	Corning Inc., Corning, NY
Polystyrene 96-well plate	CLS3370	Sigma Aldrich Chemical Co., Milwaukee, WI
11-Mercaptoundecanoic acid (MUA)	450561	Sigma Aldrich Chemical Co., Milwaukee, WI
N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC)	E6383	Sigma Aldrich Chemical Co., Milwaukee, WI
N-hydroxysulfosuccinimide (NHS)	56485	Sigma Aldrich Chemical Co., Milwaukee, WI
Bovine serum albumin (BSA, 10%)	A1595	Sigma Aldrich Chemical Co., Milwaukee, WI
Phosphate buffered saline (PBS, pH: 7.4)	10010	Invitrogen Co., Carlsbad, CA
MES	M3671	Sigma Co., St. Louis, MO
Poly-L-lysine (PLL) hydrobromide (MW: 70,000-150,000)	P1274	Sigma Co., St. Louis, MO
Protein G	21193	Thermo Scientific, Rockford, IL
NeutrAvidin	31000	Pierce, Rockford, IL
Recombinant Human interferon-gamma (IFN-γ)	554617	BD Biosciences, Franklin Lakes, NJ
Tween 20	P1379-25ML	Sigma Aldrich Chemical Co., Milwaukee, WI
High Sensitivity Streptavidin-HRP	21130	Fisher Scientific, Waltham, MA
1-Step Ultra TMB - ELISA Substrate	34028	Fisher Scientific, Waltham, MA
Anti-IFN-γ antibody, Clone: NIB42	551221	BD Biosciences, Franklin Lakes, NJ
Rabbit anti-casein polyclonal antibody	bs-0813R	Bioss, Woburn, MA
Carbamazepine (CBZ)	C4024	Sigma Co., St. Louis, MO
Anti-CBZ monoclonal antibody	MCA5730G	AbD Serotec, Raleigh, NC
Anti-lipopolysaccharide binding protein (LBP) polyclonal antibody	AF870	R&D Systems, Minneapolis, MN

Recombinant Human LBP, CF	870-LP-025/CF	R&D Systems, Minneapolis, MN
Luria–Bertani (LB) agar plates containing ampicillin	L5667	Sigma Co., St. Louis, MO
LB Broth (Lennox)	L3022	Sigma Co., St. Louis, MO
Escherichia coli (*E. coli*) strain BL21 Star™	C6000-03	Invitrogen Co., Carlsbad, CA
pRSET/EmGFP plasmid	V353-20	Invitrogen Co., Carlsbad, CA
Staphylococcus aureus (*S. aureus*)	25923	American Type Culture Collection (ATCC), VA
Gram Positive Bacteria anti-LTA Antibody	MA1-7401	Pierce, Rockford, IL
Human anti-EpCAM/TROP‑1 antibody (biotinylated)	BAF960	R&D Systems, Minneapolis, MN
anti-Dengue Virus 1 (DENV-1) antibody [9.F.13]	ab31469	Abcam, Cambridge, MA
anti-DENV-2 antibody, clone 3H5-1	MAB8702	EMD Millipore, Darmstadt, Germany
RNeasy kit	74104	Qiagen, Venlo, Netherlands
iScript kit	1708891	Bio-Rad, Hercules, CA
Q SYBR Green Supermix	1708882	Bio-Rad, Hercules, CA
Leibovitz medium (L-15 medium) supplement	10-045-CV	Corning Inc., Corning, NY
FBS	16000-044	GIBCO, Grand Island, NY
EBSS	SH30029.02	Thermo Scientific, Rockford, IL
Minimum Essential Medium Eagle α (MEM-α)	10-022-CV	Corning Inc., Corning, NY
MEM-α powder	MEP17-10LT	Caisson, North Logan, UT
Carboxymethylcellulose (CMC)	C4888	Sigma Co., St. Louis, MO
Sodium bicarbonate	S5761	Sigma Co., St. Louis, MO
Penicillin/Streptomycin	SV30010	Thermo Scientific, Rockford, IL
Formaldehyde	BDH0500-4LP	BDH
Crystal violet	548-62-9	Acros
anti-HBV-HBsAg antibody	AB-A101621	Novateinbio, Woburn, MA
FBS	100-106	Gemini Bio-Products, West Sacramento, CA
Penicillin/Streptomycin	15140-122	Invitrogen Co., Carlsbad, CA
G-418	400-111P	Gemini Bio-Products, West Sacramento, CA
Bradford protein assay	500-0002	Bio-Rad, Hercules, CA
1-mL Melon gel chromatography cartridge	89932	Pierce, Rockford, IL

Anti-KSHV/HHV-8 ORF K8.1 A monoclonal antibody	13-212-100	Advanced Biotechnologies Inc., Columbia, MD
Goat anti-HIV-1 polyclonal antibody (biotinylated)	ab53937	Abcam, Cambridge, MA
DMEM	11995073	Invitrogen Co., Carlsbad, CA
Bovine growth serum (BGS) HyClone	SH30541.03	Thermo Scientific, Rockford, IL
Gentamicin	400-108	Gemini Bio-Products, West Sacramento, CA
Puromycin	NC9138068	Invivogen, San Diego, CA
G418	400-113	Gemini Bio-Products, West Sacramento, CA
Doxycycline	D9891	Sigma Co., St. Louis, MO
0.45 µm filter	256136	RPI Corp., Mount Prospect, IL
SYBR green	472908	Invitrogen Co., Carlsbad, CA
QIAamp DNA mini kit	51304	Qiagen, Venlo, Netherlands
Ethanol (200 proof)	E7023	Fisher Scientific, Fair Lawn, NJ
Paraformaldehyde	30525-89-4	Electron Microscopy Sciences, Hatfield, PA

B. Device fabrication. We designed two prototypes of Nanoplasmonic Electrical field-Enhanced Resonating Device (NE^{2}RD) platform: (i) plate (96-well) format and (ii) chip format. In plate prototype, polystyrene wells were used as base surfaces (surface area for each well: ~37 mm^2). In chip prototype, we used poly(methyl methacrylate) (PMMA) (50 mm x 25 mm) layer, double-sided adhesive film (DSA, 50 µm in thickness) and polystyrene surface (7 mm x 7 mm of biosensing area in x-y plane). PMMA and DSA layers were cut using a Versa LASER (Universal Laser Systems Inc., Scottsdale, AZ). The DSA film was then used to attach the PMMA and polystyrene layers (0.8 mm in thickness). In both formats, polystyrene surfaces were first cleaned using absolute ethanol (100 µL), rinsed with PBS (100 µL) three times, and dried with nitrogen gas. Polystyrene surfaces were then modified with 0.05 mg/mL of PLL to fabricate the surfaces, and this solution was incubated on the wells overnight. In this step, PLL molecule generated amine-terminated groups, where gold nanoparticles reacted. 50 µL of gold nanoparticle solution was then added to each well, and the surfaces were incubated for overnight. In each fabrication step, using a pipette, we rinsed the surfaces with 100 µL of PBS three times to remove unbound molecules/residues, and incubated them at +4°C.

C. Device surface modification. After the immobilization of gold nanoparticles, the NE^{2}RD surfaces (both plate and fluidic chip prototypes) were modified with MUA (1 mM, 100 µL) to generate carboxyl groups for further chemical modifications. To immobilize recognition elements (antibodies), MUA-modified surfaces were first treated with EDC/NHS crosslinking agents to generate succinimide groups for binding of antibody anchors (Protein G or NeutrAvidin). In this step, EDC and NHS were dissolved in 50 mM MES buffer (pH 5.0), and the final concentrations were adjusted to 100 mM and 50 mM, respectively. To tether antibodies, Protein G (100 µL, 0.1 mg/mL) was then

incubated for 3 hours on succinimide-modified surfaces. For minimization of nonspecific binding on the surfaces, 100 µL of 10% BSA (blocking agent) was incubated at 4°C for an hour. For each set of experiments, specific antibodies were diluted in PBS by adjusting the concentration to 5 µg/mL, and incubated at 4°C for three hours. Following each surface modification and antibody immobilization step, the device surfaces were washed with 100 µL of PBS three times to remove unbound molecules/residues.

D. Durability and response to pH and temperature changes. Existing biosensing platforms in clinical settings are remarkably hindered by properties that are either internal (*e.g.,* pH, ionic content and ionic strength) or external (*e.g.,* temperature) characteristics of the biological specimens, challenging those platforms' capabilities to provide reliable and repeatable results. Hence, we evaluated the performance of the NE^2RD platform (plate prototype) in various conditions, mimicking a broad range of internal and external properties of clinical specimens.

The device surfaces were evaluated in terms of internal (*e.g.,* pH, ionic content and strength) and external (*e.g.,* temperature) factors within 14 days of period. After the immobilization of gold nanoparticles, the surfaces were treated with three different buffers varying pH values. Here, we used MES buffer for pH 5.0, PBS buffer for pH 7.4, and Borax buffer for pH 10.0. These buffers were then incubated in the wells for 1, 2, 5, 7 and 14 days to demonstrate the durability of the platform. Spectral color changes for each well were measured in these different time periods, and compared with the spectral resolution of device (Varioskan Flash spectral scanning multimode readers, Thermo Scientific). Further, a new set of the device surface were treated with PBS buffer in different temperatures (4°C, 25°C, and 37°C) for an hour. As performed in pH experiments, spectral color change measurements were employed, and the changes were again compared with the spectral resolution (1 nm) (**fig. S3**).

E. Biologic sample preparation and application to the NE^2RD surfaces.
Accurate and sensitive biosensing approaches hold key potential roles in medical diagnostics, the pharmaceutical industry, and global and public health. One of the major convincing applications of such platforms is the detection of multiple biotargets. In addition to multiplicity performance, the ability to detect a diverse range of biotargets within clinically relevant sample source types is another key capability of a successful biosensing platform for clinical studies(1).

1) IFN-γ experiments. The NE^2RD surfaces (for plate and chip prototypes) were modified with anti-IFN-γ (Clone: NIB42) antibodies (100 µL, 5 µg/mL) after Protein G modification step by incubating for three hours at +4°C. The surfaces were then washed with PBS to remove unbound antibodies and residues. We then spiked IFN-γ stock concentration with PBS and serum samples (**Figs. 2, 4a, 4c, and 6b**). First, as described in the manufacturer's protocol, recombinant human IFN-γ samples were dissolved in PBS to prepare 0.5 mg/mL of stock solution containing BSA, and kept at -80°C for further use. To dilute the samples, the stock solution was reconstituted with PBS to adjust the concentrations ranging from 1 fg/mL to 1 ng/mL (**Figs. 2, 4a, and 6b**). Second, recombinant human IFN-γ samples were dissolved in serum to prepare 0.5

mg/mL of stock solution containing BSA, and kept at -80°C for further use. Serum samples were purchased from Research Blood Components, LLC (Brighton, MA). To dilute the samples, stock solution was reconstituted with fresh serum sample to adjust the concentrations ranging from 500 fg/mL to 100 pg/mL. After preparing these dilutions, the samples (100 µL) were immediately applied to anti-IFN-γ antibody-tethered surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4c**).

2) Casein experiments. The NE^{2}RD surfaces (plate prototype) were modified until Protein G step, and then, anti-casein polyclonal antibodies (100 µL, 5 µg/mL) were immobilized by incubating for three hours at +4°C. Unbound antibodies and moieties were removed by washing out with PBS. We then prepared 0.1 mg/mL stock solution dissolved in high pure water by heating to 75°C for 10 min with intermittent agitation to dissolve completely. The stock solution was diluted with PBS to adjust the concentrations to 1, 10^2, and 10^4 pg/mL. The samples (100 µL) were added to anti-casein antibody-coated surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

3) CBZ experiments. After Protein G modification, the device surfaces (plate prototype) were first modified with anti-CBZ monoclonal antibodies (100 µL, 5 µg/mL) by incubating for three hours at +4°C. The surfaces were then washed with PBS to remove unbound antibodies and moieties. We prepared 1 mg/mL solution dissolved in fresh serum, and this solution was then diluted in 0.5, 5, and 10 µg/mL with fresh serum samples. After preparing the dilutions, the samples (100 µL) were immediately applied to anti-CBZ antibody immobilized surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

4) *E.* coli experiments. We used genetically modified *E. coli* to selectively grow in agar plates as we reported previously (2). Briefly, *E. coli* strain BL21 Star™ was modified with pRSET/EmGFP plasmid including ampicillin resistance and green fluorescence gene cassettes by incubating at 41°C for 30 seconds, followed by transferring onto ice. The modified strain was incubated for an hour in LB Broth (Lennox) with catabolite repression medium on a shaker adjusted to 250 rpm and 37°C. The strain was plated onto LB agar containing ampicillin (100 mg/mL), and then, incubated for 16 hours at 37°C. An individual colony was chosen to inoculate in 5 mL of LB medium including ampicillin (100 mg/mL) for 16 hours on a shaker adjusted to 250 rpm and 37°C. For quantification, the bacteria solution was diluted in PBS, and plated onto LB-ampicillin plates for overnight incubation at 37°C. Individual colonies were then quantified. The concentration of bacteria was counted as 10^8 colony forming unit (cfu)/mL. This stock concentration was then diluted with PBS to adjust the final concentrations 10^3, 10^4, and 10^5 cfu/mL. To prepare the NE^{2}RD platform (plate prototype) for *E. coli* capture, anti-

LBP polyclonal antibody (100 µL, 5 µg/mL) containing 0.1% BSA was immobilized by incubating for three hours at +4°C on Protein G-coated surfaces. LBP (30 µL, 10 µg/mL) was then tethered on the surfaces by incubating for three hours at +4°C. The bacteria dilutions (100 µL) were applied onto the modified surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

5) *S. aureus* experiments. *S. aureus* (ATCC #25923) were obtained from American Type Culture Collection. The stock culture were first hydrated and streaked onto a Luria Bertani agar (LA) plate. Then, a single isolated colony was chosen and inoculated in 3 mL of Luria broth (LB) media for 18 hours at 37^0C using an incubator shaker (250 rpm) until the stationary phase. *S. aureus* stock concentration was quantified by diluting the overnight cultures nine-fold in PBS. These diluted cultures were again streaked onto LA plates, and then, incubated at 37°C overnight. Individual colonies were counted, and the concentration of stock cultures was calculated as 10^9 CFUs/mL.

To prepare the NE^{2}RD platform (both plate and chip prototypes) for *S. aureus* capture, anti-LTA antibody (100 µL, 5 µg/mL) was immobilized by incubating for three hours at +4°C on Protein G-coated surfaces. The bacteria dilutions (100 µL) were applied onto the modified surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well and fluidic chip to measure spectral color changes (**Fig. 4a**).

6) Lung cancer cell experiments. A549 lung adenocarcinoma cell line was derived from epithelial cells. The frozen stock were first thawed in 37°C in three minutes, followed by centrifugation at 3000 rpm for 90 seconds to sediment cells. After discarding supernatant, the pellet was then re-suspended in 1 mL PBS. The stock sample ($1.5x10^6$ cells/mL) was diluted with PBS to adjust the final concentrations to $1.5x10^2$, $1.5x10^3$, and $1.5x10^5$ cells/mL.

To prepare the NE^{2}RD platform (plate prototype) for lung cancer cells, we modified the surfaces with NeutrAvidin and human anti-EpCAM/TROP-1 antibodies (biotinylated) (100 µL, 5 µg/mL) by incubating for three hours at +4°C. The surfaces were then washed with PBS to remove unbound antibodies or moieties. Upon preparation of sample solutions, the samples were immediately applied antibody-modified surfaces, and incubated for 30 min at +4°C, followed by washing out with PBS three times. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

7) DENV-1 and DENV-2 experiments. DENV-1 was obtained from Aravinda De Silva (University of North Carolina-Chapel Hill School of Medicine), and DENV-2 New Guinea C (NGC) was received from Lee Gehrke (Harvard University and MIT). These two serotypes were propagated as follows. Briefly, dengue viruses (DENV-1 or DENV-2) were adsorbed for an hour to confluent layers of C6/36 cells at 28°C, rocked every 15 minutes, and then, L15 medium supplemented with 2% fetal bovine serum (FBS) was added. DENV-infected cells were incubated for 4 to 5 days at 28°C. Supernatants were

then harvested, clarified by centrifugation for 5 min at 1000 *g*, and the aliquots were stored at -80°C. One aliquot was used to determine the viral titer by viral plaque assay as follows. BHK-21 cells were seeded in a 24-well plate and incubated overnight to form a confluent monolayer. Ten-fold serial dilutions of viral supernatant were prepared in EBSS in triplicates, and each dilution (100 µL) was added to cells. Plates were incubated for an hour at 37°C, and rocked gently every 15 minutes, after which non-adsorbed virus was removed and cells were subject to one wash with PBS. MEM-α medium supplemented to final concentrations of 1.05% carboxymethylcellulose (CMC), 44 mM sodium bicarbonate, 10 units/mL penicillin, 10 µg/mL streptomycin, and 2% FBS was added to each well. Cells were incubated at 37°C for 5 days to allow for viral plaque formation. The CMC overlay was aspirated, and cells were washed twice with PBS, then fixed and stained with a solution of paraformaldehyde and crystal violet. Plaque forming units (PFU) were measured by counting the number of plaques in each well to calculate the number of infectious viral particles. DENV-1 and DENV-2 data are reported as pfu per mL, corresponding to the number of infectious particles per mL sample **(Figs. 4a-b)**.

To prepare the device platform for DENV-1 and DENV-2 capture, anti-DENV-1 and anti-DENV-2 antibodies (100 µL, 5 µg/mL) were incubated for 3 hours at +4°C on Protein G-coated surfaces, respectively. The surfaces were then washed with PBS to remove unbound antibodies. For DENV-1 experiments, this stock was diluted with simulated saliva to adjust the final concentrations ranging 10^3, 10^4, and 10^5 PFU/mL **(Fig. 4b)**. Preparation of simulated saliva was indicated in the following section in **SI Appendix**. For DENV-2 experiments, this stock was diluted with PBS, to adjust the final concentrations ranging from 10^4, 10^5, and 10^6 PFU/mL **(Fig. 4a)**. These dilutions (100 µL for each) were immediately applied to anti-DENV-1 and anti-DENV-2 antibody-coated surfaces, and incubated for 1 hour at 4°C followed by washing out with PBS three times. To fix captured viruses on the surface, we used 6% paraformaldehyde solution in PBS and incubated the surfaces for 10-30 minutes at room temperature. 100 µL of PBS were then added to each well to measure spectral color changes **(Fig. 4a)**.

8) HBV experiments. G2.215 and control HepG2 cell lines were obtained from Dr. Andrea Cuconati, Pennsylvania Biotechnology Center at Institute for Hepatitis and Virus Research, Doylestown, PA. Upon the arrival, the cell lines were quickly thawed at 37°C, and then, seeded in a 75 cm flask with 25 mL of pre-warmed complete media. For G2.215 cell line, RPMI with 10% FBS, Pen/Strep (1 mg/mL of penicillin and 100 units/mL of streptomycin), and 200 mg/mL of G-418 were used as media. For HepG2, RPMI with 10% FBS and Pen/Strep (1 mg/mL Penicillin and 100 units/mL Streptomycin) was used as media. Media were removed and changed to fresh media next day. Cells were grown to confluence before seeding into another 75 cm flask at 1 to 4 ratios. Once cells reached to confluence again, we removed the media and washed cells with PBS before leaving them in PBS for 24 hours. Supernatants were collected and stored at -80°C. The stock HBV DNA concentration was determined to be 189,223 IU/ml.

HBV DNA RT-PCR assay for both the clinical and cell culture supernatant samples was performed at the Molecular Laboratory, Beth Israel Deaconess Medical Center (BIDMC) using the Cobas Ampliprep/Cobas Taqman HBV Test v2.0 (Roche). Linear range of

quantification and limit of detection were calculated as 20 IU/mL - 170 million IU/mL, and 20 IU/mL, respectively.

To prepare the NE^{2}RD platform (plate prototype) for HBV capture, the surfaces were modified with anti-HBV HBs antibodies (100 µL, 5 µg/mL) by incubating for three hours at +4°C. The surfaces were then washed with PBS to remove unbound antibodies. The concentration of virus was counted as international unit (IU)/mL. For sample preparation, we used HBV spiked in PBS and whole blood samples (**Figs. 4a, and 4d**). For PBS spiked experiments, the stock concentration was diluted with PBS to adjust the final concentrations 10^2, 10^3, and 10^5 IU/mL (**Fig. 4a**). For whole blood spiked experiments, the stock concentration was diluted with fresh whole blood samples to adjust the final concentrations 10^2, 10^3, and 10^5 IU/mL (**Fig. 4d**). Whole blood samples were purchased from Research Blood Components, LLC (Brighton, MA). After preparing these dilutions, the samples were immediately applied to anti-HBV HBs antibody-immobilized surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. To fix captured viruses on the surface, we used 6% paraformaldehyde solution in PBS and incubated the surfaces for 10-30 minutes at room temperature. 100 µL of PBS were then added to each well to measure spectral color changes (**Figs. 4a, and 4d**).

9) Trichomonas vaginalis virus-1 (TVV-1) experiments. This work was approved by the Institutional Review Board for Human Subject Research at Brigham and Women's Hospital. TVV-1 virions were purified from cultures of a clinical *Trichomonas vaginalis* isolate obtained upon informed consent from a woman with symptomatic vaginitis as previously described(3). The concentration of purified virions in virions/mL was estimated by using a modified Bradford protein assay with bovine serum albumin as the standard and a conversion factor based on the calculated protein mass of a TVV-1 virion (9.0 MDa). An anti-TVV-1 antiserum that had been raised in rabbits against bacterially expressed TVV-1 capsid protein(4) was used as the source of polyclonal antibodies for this study following purification on a 1 mL Melon gel chromatography cartridge.

To capture intact TVV on the device surfaces (plate prototype), we first produced specific antibodies. After Protein G modification, anti-TVV antibodies (100 µL, 5 µg/mL) were incubated for three hours at +4°C. The surfaces were then washed with PBS to remove unbound antibodies or moieties. For sample preparation, we used TVV spiked in PBS samples, and the stock concentration was diluted with PBS to adjust the final concentrations 10^3, 10^4, and 10^7 virions/mL (**Fig. 4a**). After preparing these dilutions, the samples were immediately applied to anti-TVV antibody immobilized surfaces, and incubated for 1 hour at +4°C, followed by washing out with PBS three times. To fix captured viruses on the surface, we used 6% paraformaldehyde solution in PBS, and incubated the surfaces for 10-30 minutes at room temperature. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

10) Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 (KSHV/HHV-8) experiments. iSLK.219 cells were gifted from Don Ganem(5), and then, cultured in

DMEM medium supplemented with 10% bovine growth serum (BGS), 15 µg/mL gentamicin, puromycin (1 µg/mL) and G418 (250 µg/mL). Recombinant KSHV.BAC219 virus stocks were prepared by inducing iSLK-219 cells with doxycycline (1 µg/mL) in the absence of puromycin and G418. Three days later, supernatant was collected and cleared of cells and debris by filtration (0.45 µm filter). Virus particles were pelleted by centrifugation (13,300 g for 2 hours at 4°C) using a Sorvall SLA-600TC rotor. To quantify stock concentration, virus supernatants from induced iSKL.219 was boiled for 20 minutes, and used as a template for quantitative real-time PCR using SYBR green and primers specific for Lana (forward, 5'-CGCGAATACCGCTATGTACTCATCTCTTCCA-3' and reverse, 5'-GGAACGCGCCTCATACGA -3') (6). Here, we performed qPCR using ABI 7300 Real-Time PCR System (Applied Biosystems). The amplification program was set to 1 cycle of 95°C for 10 minutes, 40 cycles of: 95°C for 15 seconds, and 60°C for 1 minute. Fluorescent product was then detected at the last step of each cycle. Amplified DNA was quantified using a standard curve from pT7Lana plasmid dilution series (7). The concentration of virus was counted as 6.9×10^6 copies/mL.

To prepare the device platform (plate prototype) for HHV-8/KSHV experiments, anti-HHV-8/KHSV monoclonal antibodies (100 µL, 5 µg/mL) were incubated for three hours at +4°C after Protein G modification. The surfaces were then washed with PBS to remove unbound antibodies or moieties. For sample preparation, we used KSHV spiked in PBS samples, and the stock concentration was diluted with PBS to adjust the final concentrations 10^2, 10^4, and 10^6 copies/mL (**Fig. 4a**). After preparing these dilutions, the samples were immediately applied to anti-HHV-8/KHSV monoclonal antibody immobilized surfaces, and incubated for an hour at +4°C, followed by washing out with PBS three times. To fix captured viruses on the surface, we used 6% paraformaldehyde solution in PBS, and incubated the surfaces for 10-30 minutes at room temperature. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 4a**).

11)Patient sample collection (serum and whole saliva) and viral load measurements. The study was reviewed and approved by the Dana Farber Cancer Institute/Harvard Cancer Center Institutional Review Board. Informed consent was obtained from all subjects. In serum experiments, KSHV patient samples were obtained from a subset HIV-infected individuals diagnosed clinically with KS planning on receiving or after systemic anti-tumor chemotherapy. This chemotherapy most commonly used in our patients for KS was liposomal doxorubicin, and the majority of patients had cutaneous or oral mucosal involvement. Subjects were part of a larger cohort of HIV-infected subjects with malignancies enrolled in a multi-clinic study. Briefly, blood samples were first collected longitudinally in EDTA tubes from subjects by venipuncture. Plasma was separated from blood via double centrifugation and stored at -80°C. Viral DNA was extracted from 1 mL of thawed plasma using the QIAamp DNA mini kit (Qiagen) as per manufacturer directions. In saliva experiments, there were no stimulants used on sample collection, and then, the samples were frozen down at -80ºC in aliquots. For DNA isolation, we used a QIAamp DNA Blood Mini Kit from Qiagen. We followed the manufacturer's supplementary protocol for isolation of genomic DNA from

saliva and mouthwash. The same real time-PCR procedure in serum experiments was followed for whole saliva samples.

To quantify HIV-1 viral load levels, the samples were analyzed at affiliated clinical laboratories (Brigham and Women's Hospital/Massachusetts General Hospital/Dana Farber Cancer Institute/Harvard Cancer Center) using the Roche Cobas Taqman v.1 or v.2 HIV-1 reverse-transcription-qPCR assay with a sensitivity of <50 copies/mL.

Plasma and saliva KSHV viral load was quantified based on a previously reported highly sensitive and specific real-time PCR assay(8). Briefly, standards were prepared by serial dilution of a highly conserved 170 base pair segment of the KSHV minor capsid protein genome flanking the forward and reverse real-time primer sequence locations. The DNA standard was amplified by PCR from viral DNA isolated from the KSHV-infected BCBL-1 laboratory cell line(9) using forward primer 5'-CAGTACGTGGATCCGTGTTG and reverse primer 5'-AGAATAGCGTGCCCCAGTT. Patient plasma/saliva DNA was quantified in triplicate real-time PCR using 50 µL reactions incorporating primers TAQ8A (5'-GTCCAGACGATATGTGCGC) and TAQ8B (5'-ACTCCAAAATA TCGGCCGG) and probe 5'-FAM-TTGGTGGTATATAGATCAAGTTC-MGB. BCBL-1 DNA was used as a positive control. The real-time assay had a lower limit of detection of 15 DNA copies/mL of plasma/saliva.

To prepare the NE^{2}RD platform (plate prototype) for KSHV patient samples (serum and whole saliva), we used two sets of surfaces. One set of surface was decorated with anti-HHV-8/KHSV monoclonal antibodies (100 µL, 5 µg/mL) by incubating for three hours at +4°C after Protein G modification. The other set was decorated with anti-HIV-1 polyclonal antibody (biotinylated) (100 µL, 5 µg/mL) by incubating for three hours at +4°C after NeutrAvidin modification. The surfaces were then washed with PBS to remove unbound antibodies or moieties. For sampling, KSHV patient samples (100 µL) were added to two sets of surfaces, followed by an hour incubation at +4°C. Then, we washed out the surfaces with PBS three times. To fix captured viruses on the surface, we used 6% paraformaldehyde solution in PBS, and incubated the surfaces for 10-30 minutes at room temperature. 100 µL of PBS were then added to each well to measure spectral color changes (**Fig. 5a**).

12)Preparation of simulated saliva. Samples were prepared as reported previously(10). Simulated saliva consisted of the following chemicals with indicated concentrations:

Chemical	MW (g/mol)	Molarity (mM=mmol/L)	Volume (L)
K_2HPO_4	174.2	25	0.1
Na_2HPO_4	141.96	24	0.1
$KHCO_3$	100.115	150	0.1
NaCl	58.443	100	0.1
$MgCl_2$	203.31	1.5	0.1
Citric acid	192.124	25	0.006
$CaCl_2$	147.014	5	0.1

The pH of simulated saliva was set to pH 6.7 by either adding NaOH or HCl to the solution, and adjusted volume to 1 L. This solution was stored for 1-2 weeks.

F. ELISA measurements. We performed conventional ELISA for detection of IFN-γ spiked in PBS samples. Briefly, a 96-well plate was first coated with anti-human IFN-γ monoclonal antibodies (100 µL, 1 µg/mL) by incubating at 4 °C overnight. The plate was then blocked with 1% BSA for an hour at ambient temperature, followed by washing out three times using a washing buffer containing PBS and 0.05% Tween 20. 100 µL of IFN-γ spiked PBS samples ranging 7.813 pg/mL to 4 ng/mL were then added to the plate and incubated at ambient temperature for an hour. The plate was then washed three times using the washing buffer. Biotinylated anti-IFN-γ antibody (100 µL, 500 ng/mL) was added to the plate, followed by and incubation for an hour to form a sandwich assay. The plate was again washed three times using the washing buffer. For generating ELISA signal, HRP-conjugated streptavidin (100 µL, 250 ng/mL) was incubated in the plate for an hour, and washed three times with the washing buffer again. 1-Step Ultra TMB was added for color development for 20 minutes. A stop solution (100 µL, 0.2 M sulfuric acid) was added before ELISA measurements. The absorbance data was measured at 450 nm using a Tecan Infinite M1000 Reader (Mannedorf, Switzerland).

G. Portable set-up. Tungsten halogen light (OceanOptics HL-2000-CA) was used as light source, whose spectrum ranges are from 360 nm to 2400 nm. For measurements, we used the spectral range between 450 nm to 700 nm with a 0.3 nm spectral resolution with a fixed slit setting. The optical resolution of Spectrometer Ocean Optics HR2000+ can be as fine as 0.035 nm (FWHM). Spectrometer was able to capture full spectrum on a linear CCD with 1 ms data acquisition rate. Bright light was transmitted through samples using 0.4 mm diameter of fiber optic cables (Ocean Optics, Premium Fiber, UV VIS, 2 m). The sample holder mounted on x-y-z positioner was designed to move and scan the chips manually.

H. Spectral measurements and data analysis. In plate format of the NE^{2}RD, each surface modification and biotarget binding events were measured using Varioskan® Flash Spectral Scanning Multimode Readers (Thermo Scientific) and Tecan Spectrometer, having 1 nm spectral resolution and 0.003 a.u. intensity accuracy with a fixed slit setting. The NE^{2}RD surfaces were scanned ranging from 450 nm to 700 nm in terms of the extinction intensity changes per wavelength of spectral color. In chip prototype of the NE^{2}RD, a custom spectral measurement tool was used, and the surfaces were measured from 450 nm to 700 nm with a 0.3 nm spectral resolution with a fixed slit setting. In both prototypes, a detectable spectral color shift of the maximum extinction point of gold nanoparticles were collected for data analysis.

In both 96-well and chip format of the NE^{2}RD, we employed a MATLAB code to demonstrate the peak point of each nanoplasmonic (spectral color) spectra in terms of wavelength and extinction intensity values. Raw data was analyzed by a curve fitting method utilizing a Fourier type expansion with 8 harmonics calculated with:

$$f(x) = a_0 + \sum_{n=1}^{8} (a_n \cos(n\omega x) + b_n \sin(n\omega x))$$

where ω represented the fundamental frequency of recorded data, and a_n and b_n were expansion coefficients (11). The peak points were then rounded to the first decimal digit considering the finite resolution of the experiment. The peak points at the maximum extinction value for each spectra were then rounded to the first decimal digit considering the finite resolution of the experiment. To understand how well data points fit a statistical model, R^2 values were calculated, and we observed them to be greater than 0.99 with the MATLAB fit code. All results were subtracted from previous individual spectra data, and presented as the mean of wavelength measurements ± standard error of the mean (SEM).

I. Repeatability analysis. Evaluating the repeatability of the NE^2RD platform, we defined an equation indicated below (11-13):

$$Repeatability = \frac{Mean\ of\ WS}{Mean\ of\ WS\ + SEM}\ x\ 100$$

where WS represented wavelength shift, and SEM was the standard error of the mean.

J. Limit of detection and linear dynamic range. To translate laboratory methods into clinical practice, the biosensing platforms are first evaluated to reliably measure the amount of a target analyte in terms of performance characteristics such as limit of detection and linear dynamic range. The first characteristics, the limit of detection parameter, is defined as the lowest actual amount of an analyte that the method can reliably detect to determine as statistically different from an analytical blank (absence of a target) (14, 15). This parameter is critical for clinical and laboratory applications since detecting extremely low concentrations of a target analyte can be necessary to monitor a specific disease, determine disease status of patient, or distinguish the presence or absence of a target analyte in clinical and laboratory examinations such as tumor biomarkers, bioagents for infectious diseases, drug and allergen monitoring. The latter parameter, linear dynamic range, is indicated as the response of a method has a mathematically linear relationship with the concentrations of a target analyte (14, 15). The linearity parameter of a laboratory method presents a significant information for clinical practice by demonstrating minimum and maximum analytical concentrations in the linear range and providing a variety of medical decision limits.

K. Statistical Analysis. To evaluate each surface modification and biotarget binding events, we employed one-way analysis of variance (ANOVA) with Tukey's *posthoc* test followed with Bonferroni's Multiple Comparison Test for equal variances for multiple comparisons with statistical significance threshold set at 0.05 ($p<0.05$). Error bars in the

plots represented SEM. For clinical samples, we performed Bland-Altman analysis to assess the repeatability of the NE^2RD count by comparing real time-PCR count. The coefficient of repeatability was used as 1.96 times the standard deviation of the difference between two different measurements (the NE^2RD and real time-PCR counts for the same sample). Here, clinically acceptable range was determined as 95% confidence interval within the difference between the measurements. Minitab software (Release 14, Minitab Inc., State College, PA) was used in all statistical analyses.

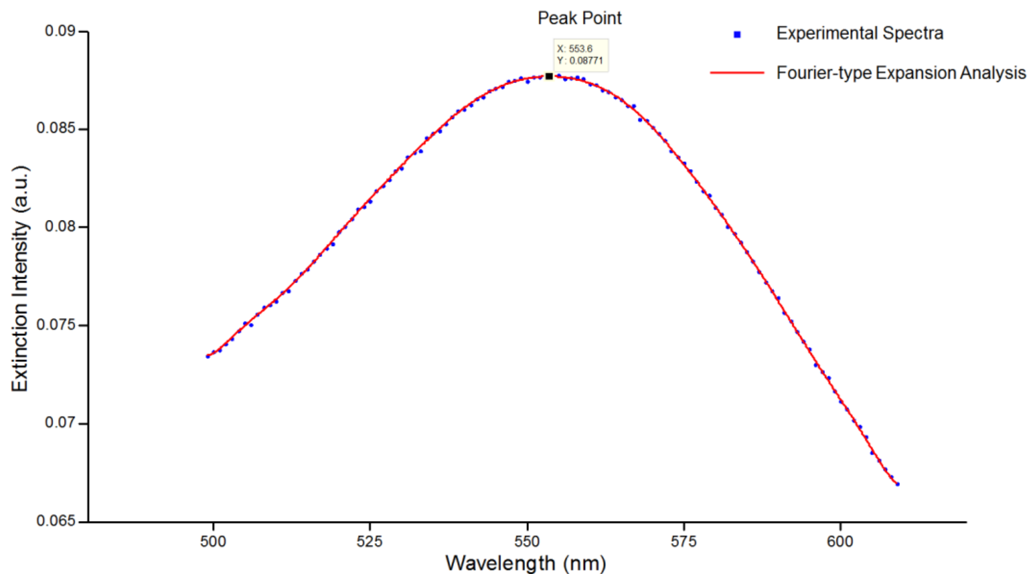


Figure S1. Fourier-type expansion analysis for binding and capture events. Spectral color change was monitored in terms of wavelength and extinction intensity values, and the raw data was then analyzed to demonstrate the peak point of spectral color value using a curve fitting method described in **SI Appendix**. Corresponding finite resolution of the experiment, the peak points in wavelength value for each recorded curve were rounded to the first decimal digit.

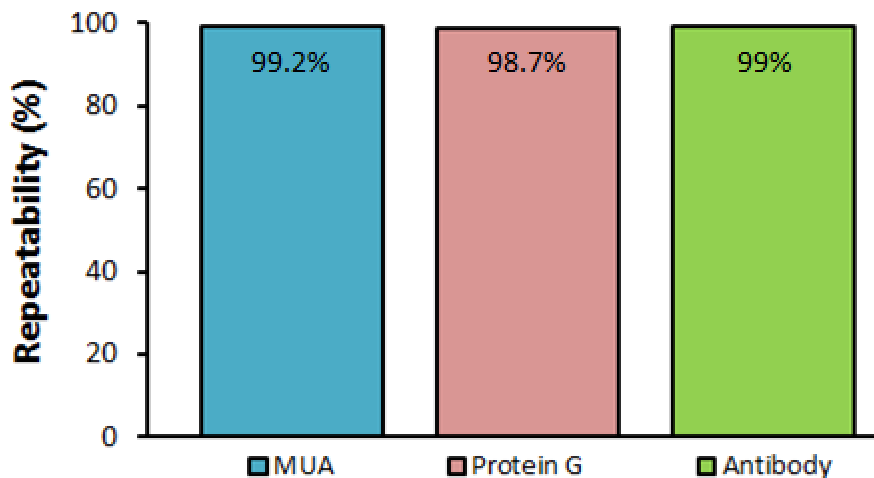


Figure S2. Repeatability analysis for surface modification. The surfaces of the NE^2RD (plate prototype) were modified to tether antibodies using surface activators (*i.e.,* 11-Mercaptoundecanoic acid (MUA), N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (NHS)), and antibody supporters (Protein G). Wavelength shift data was used to calculate repeatability of each surface modification as described in **SI Appendix** and **Fig. 2a**. Each wavelength data was subtracted from the baseline (*i.e.,* the data obtained from gold nanoparticle (519 nm)) (n=96). Layer-by-layer surface modifications on the surface provided more than 98% of repeatability.

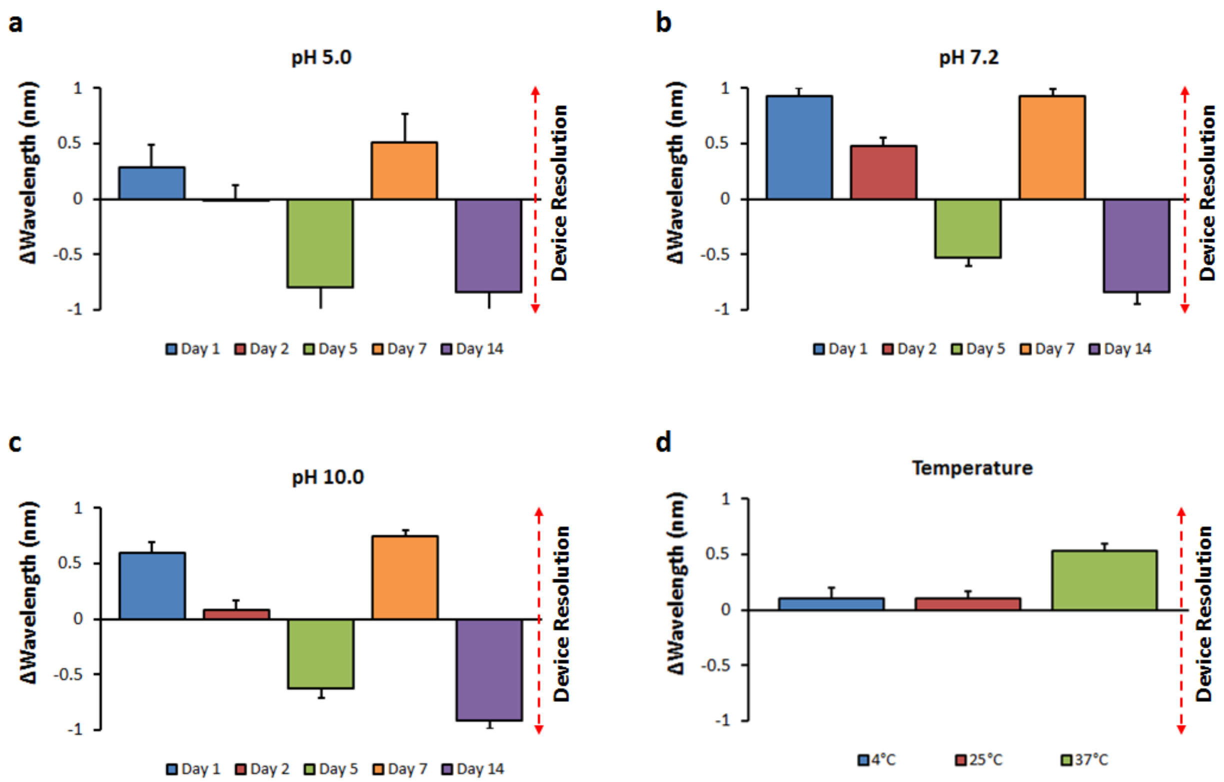


Figure S3. Evaluation the effect of internal and external factors over the surfaces of NE²RD. The unmodified device surfaces (plate prototype) were evaluated in terms of different buffer solutions having various pH and ionic content as well as diverse temperature ranges. One-way analysis of variance (ANOVA) with Tukey's *posthoc* test followed by Bonferroni's Multiple Comparison Test was used for equal variances for multiple comparisons, and statistical significance threshold was set at 0.05 ($p<0.05$). Spectral resolution was 1 nm, and the results for both pH and temperature changes did not resulted in a significant signal change in spectral color of wavelength over time ($n=16$, $p>0.05$).

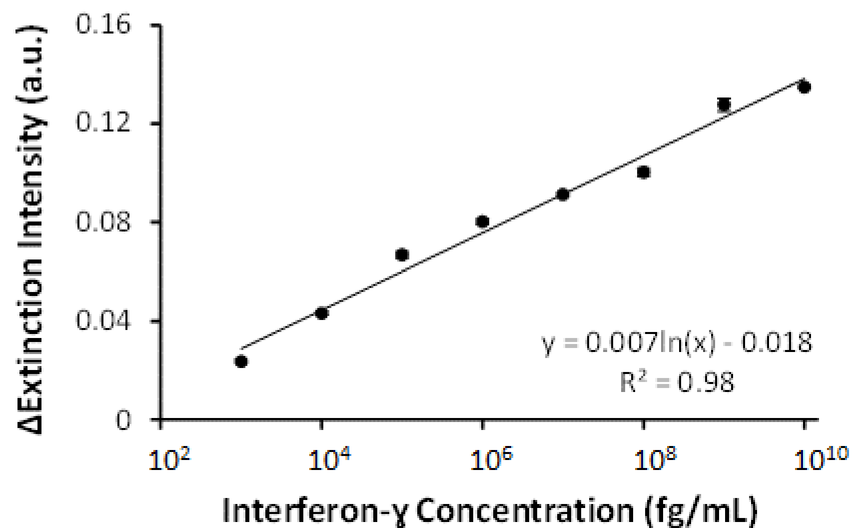


Figure S4. Analyzing extinction intensity data for linear dynamic range. Various interferon-gamma (IFN-γ) concentrations ranging from 1 pg/mL to 10 µg/mL are re-plotted to evaluate the linear dynamic range of the NE^{2}RD platform. The platform provides a broad clinical linear dynamic detection range up to 8 orders of magnitude.

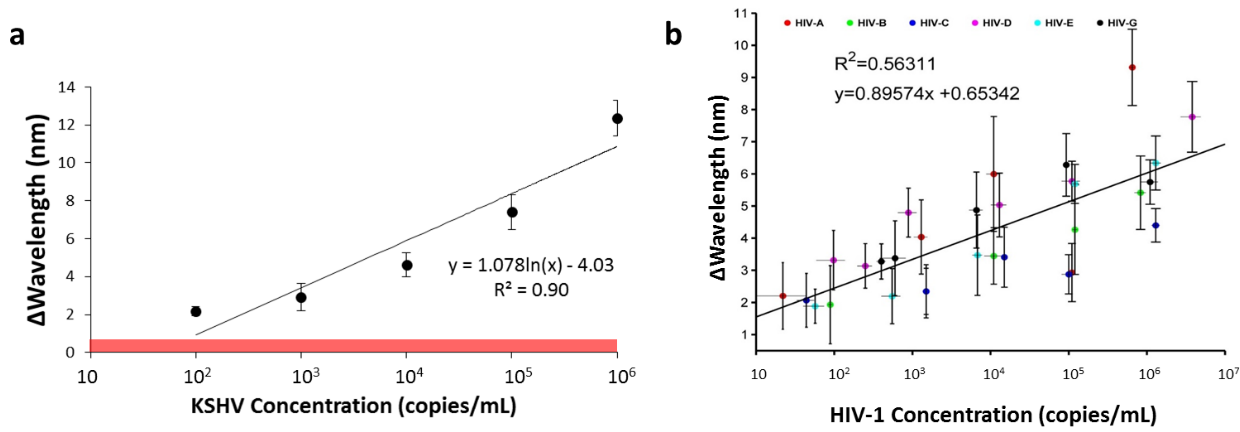
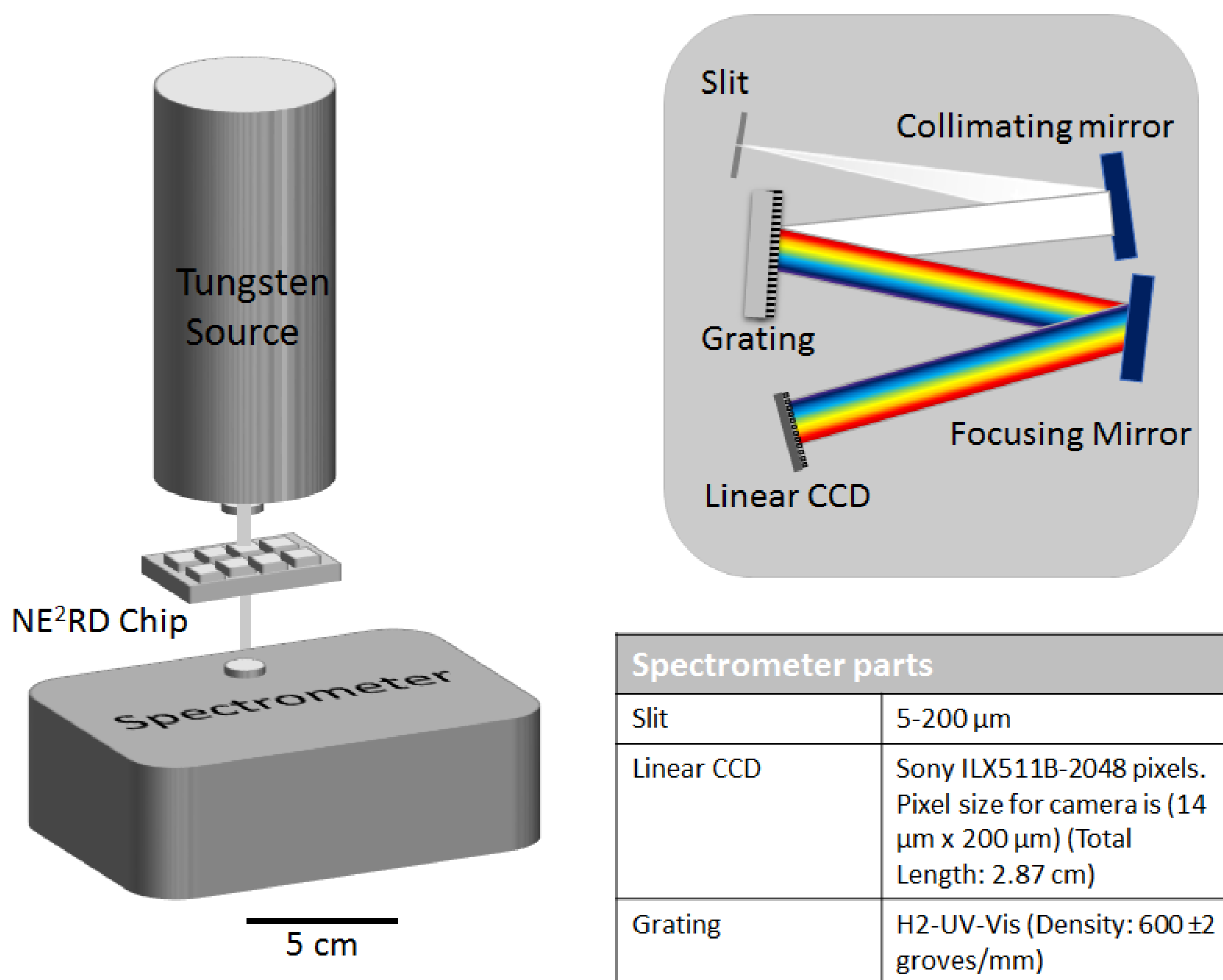


Figure S5. Standard curves of KSHV and HIV-1 samples. (a) Various concentrations of KSHV samples ranging from 10 to 10^6 copies/mL were evaluated. Samples without KSHV were used as control sample that was indicated as red line in the plot. This standard curve was then used to quantify KSHV viral load in Kaposi sarcoma (KS) patient samples. (b) Various concentrations of multiple HIV-1 subtypes (A, B, C, D, E, and G) ranging from ~50 to (3.8±1.2) x 10^6 copies/mL were evaluated (11). This standard curve was then used to qualitatively determine HIV status of KS patient samples.



Spectrometer parts	
Slit	5-200 µm
Linear CCD	Sony ILX511B-2048 pixels. Pixel size for camera is (14 µm x 200 µm) (Total Length: 2.87 cm)
Grating	H2-UV-Vis (Density: 600 ±2 groves/mm)

Figure S6. Illustration of portable set-up for NE^{2}RD chip prototype and light path. The set-up consists of a tungsten light source and spectrometer. The NE^{2}RD chip is placed between the light source and spectrometer using a chip holder. Scale bar demonstrates the size scale for light source, spectrometer and the chip. Detailed diagram of spectrometer is demonstrated on the right side of the figure and spectrometer parts are shown on the table. The slit size can vary for different applications. After light is collimated on the grating, it splits into wide spectrum. Using focusing beams, full spectra was detected on a linear CCD with 2048 pixels. Size of the aperture controls the amount of light that enters to the optical system and regulates the spectral resolution.

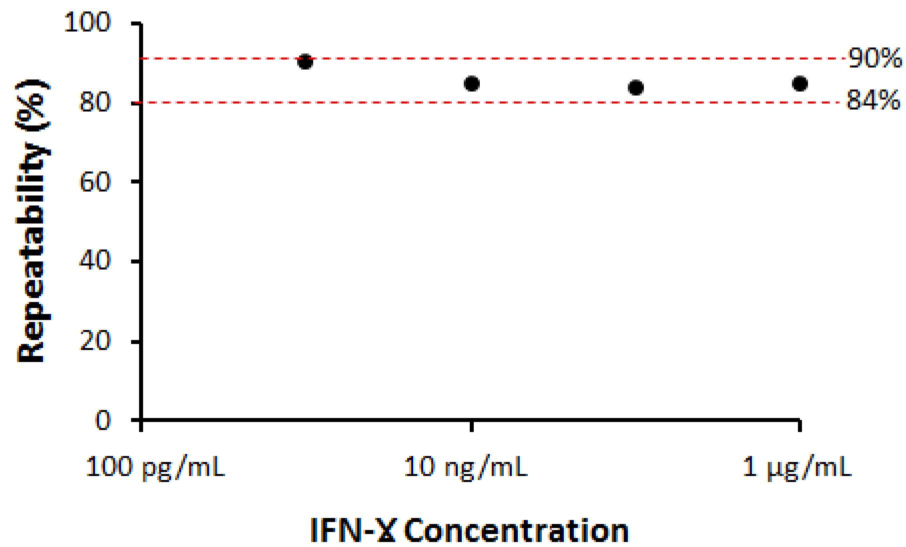


Figure S7. Repeatability analysis of IFN-γ experiments on the chip format of NE²RD. Data of IFN-γ measurements were further analyzed to demonstrate repeatability of the chip prototype. More than 84% of repeatable measurements were observed.

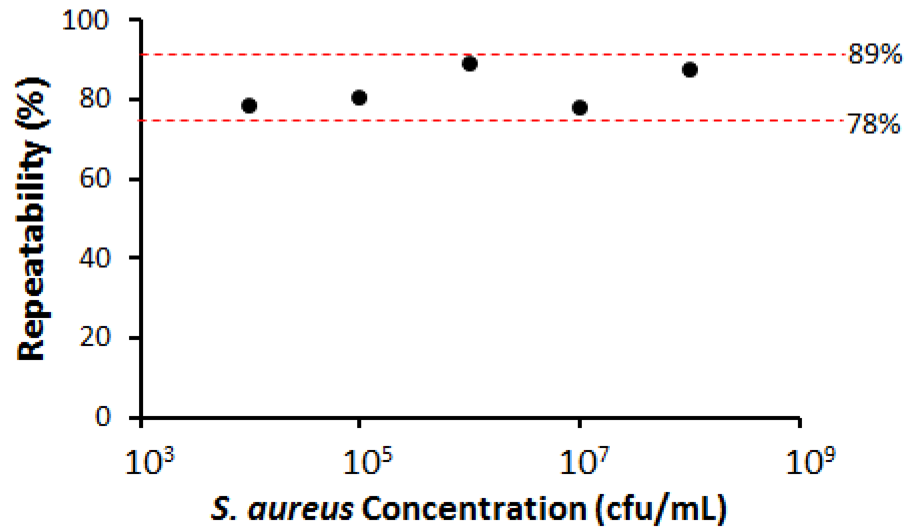


Figure S8. Repeatability analysis of *S. aureus* experiments on the chip format of NE²RD. Data of *S. aureus* measurements were further analyzed to demonstrate repeatability of the chip prototype. More than 78% of repeatable measurements were observed.

Table S1. Comparison of the NE^{2}RD with conventional platform technologies for biotarget detection.

Parameters	Protein Detection	Nucleic Acid Detection	Multiple Biotarget detection
	ELISA	**RT-qPCR(16)**	**NE^{2}RD**
Ease-of-operation	Requires multiple washing steps, sampling, secondary antibody labeling, and color generation	Requires multiple extraction and amplification steps	Only sampling/incubation and a single step wash
Readout	Optical (Absorbance)	Optical (Fluorescence)	Plasmonic (Wavelength and extinction intensity)
Assay Time	4 – 6 h	~6 h	1 h for incubation and 10 min for analysis
Linear Dynamic Range	Two – three orders of magnitude	Five orders of magnitude	Eight orders of magnitude
Limit of Detection	> 250 pg/mL	~48 copies/mL	400 fg/mL or 100 copies/mL
Multiple biotarget detection	Only for proteins and antibodies	Only for nucleic acids	Protein biomarkers, protein allergens, drugs, bacteria, eukaryotic cells, and viruses
Material Cost *(excluding cost for the instrument)*	~ $500 per test	~ $200-600 per test	$1.25 per test
Accessibility	No	No	Portable and potentially integrated with mobile platforms such as tablet and cell phones

Table S2. Comparison of the NE²RD with the most sensitive platform technologies for biotarget detection.

PARAMETERS	Magneto-nanosensor chip (17)	SMC™ (Singulex) (18)	Simoa™ (Quanterix) (18)	Bio-barcode assay (19)	Chip–NMR biosensor (20)	Advanced SPR biosensor (21)	Plasmonic ELISA (22)	Plasmonic gold chip (23)	NE²RD
Target	Lactoferrin, survivin, CEA, VEGF, EpCAM, G-CSF, TNF-α, and eotaxin	cTnI, cytokines, amyloid-beta, IL-22	PSA, TNF-alpha, Tau	PSA	*S. aureus*, mouse macrophages, breast cancer cells, and multiple protein biomarkers	*E. coli* O157:H7	PSA and HIV-1 capsid antigen p24	Insulin Ab, GAD65 Ab, and IA2 Ab	IFN-γ, carbamazepine, casein, *E. coli*, Human lung adenocarcinoma epithelial cells, HBV, DENV-1, DENV-2, TVV-1, KSHV and HIV-1
Assay Time	> 2 h and 20 min	4×96-well plates per day (for single assay, not multiplex)	5×96-well plates per day (for single assay, not multiplex)	> 3h and 30 min	30 min for incubation and 10 min for analysis	30 min	> 5h and 30 min	< 2 h	1 h for incubation and 10 min for analysis
Multiple biotarget detection	Only protein biomarkers	Not available	10-plex	Not available	Bacteria, mammalian cells, and protein biomarkers	Not available	Only two protein biomarkers	Only autoimmune antibodies	Protein biomarkers, protein allergens, drugs, bacteria, eukaryotic cells, and viruses
Readout	Magnetic field–based detection	Fluorescent-based system (Digital and analog at high concentrations)	Fluorescent-based system (Digital and analog at high concentrations)	Scanometric detection for DNA	NMR signal	Long range surface plasmons enhanced by magnetic nanoparticles	Optical-based detection	Near-infrared fluorescence–enhanced spectroscopy (NIR-FE)	Plasmonic signal on a 3-D oriented substrate
Limit of detection	1 pg/mL or 5 fM (with mono labelling) 10 fg/mL or 50 aM (with dual labelling)	fg-pg/mL	fg-pg/mL	330 fg/mL	5 pg/mL (~1 ng in 5 μL)	50 cfus/mL	1 ag/mL	0.1 KU/mL	400 fg/mL and ~100 copies/mL
Linear Dynamic Range	Six orders of magnitude	Four orders of magnitude	Four orders of magnitude	Two – three orders of magnitude	Four orders of magnitude	Three – four orders of magnitude	Three – four orders of magnitude	Four – five orders of magnitude	Eights order of magnitude

Sample type	PBS, serum, urine, and saliva	Plasma, serum, cerebrospinal fluid, cell lysate, urine, human brain tissue homogenate	Serum	PBS and serum	PBS and serum	PBS	PBS	Whole serum or blood	PBS, whole saliva, serum and whole blood
Internal Factors (e.g., pH, ionic strength and ionic content)	Stable in different pH values	Data not available.	Data not available. pH potentially interferes with the enzymatic readout.	Data not available	Data not available. pH potentially interferes with the readout.	Data not available	Data not available	Data not available	Stable in different pH values between pH 5.0 to pH 10.0
External Factors (e.g., temperature)	Stable in different temperature values	Data not available.	Data not available. Temperature potentially interferes with the enzymatic readout.	Data not available	Data not available. Requires a fixed temperature (20ºC) setting.	Data not available	Data not available	Data not available	Stable in different temperature values between 4ºC and 37ºC
Complexity	Requires multi-step standard sandwich ELISA procedures	Requires multi-step processing, *i.e.,* fluorescent labelling, washing out, elution, and molecule counting	Requires multi-step digital ELISA procedures	Requires multi-step processing, *i.e.,* gold binding, magnetic particle capture, separation, DNA cleavage, and barcode assay	Requires multi-step signal enhancement (*i.e.,* magnetic particles-coated with detection antibodies) and measurement procedures	Requires mutli-step signal enhancement procedures (*i.e.,* binding of magnetic particles-coated with detection antibodies) and controlled sampling (*i.e.,* specified pumping system)	Requires multi-step standard sandwich ELISA procedures	Requires multi-step sample preparations such as centrifuging	Only incubation and a single step washing
Labeling	Requires labeling	Alexo Fluor Label	β-Galactosidase enzyme	Requires dual labeling	Requires labeling	Requires labeling	Requires labeling	Requires labeling	No labeling
Cost	Not available	Not available	Not available	Requires Perkin-Elmer liquid handling system modified with	Requires a customized device with sophisticated instruments,	Requires a bench-top device integrated with optics, magnetic	Requires a bench-top device, *i.e.,* SpectraMax M5 plate reader	Requires two bench-top devices, *i.e.,* Cary 300UV–Vis–	$1.25 per test Requires only a portable spectral measuring tool

				a magnetic separation device and Scanometric device	*i.e.,* permanent dipole magnets, radio frequency generation and NMR signal amplification	setup, and flow cell unit		NIR and Licor Odyssey scanner	

Abbreviations: CEA: Carcinoembryonic antigen, VEGF: Vascular endothelial growth factor, EpCAM: Epithelial cell adhesion molecule, G-CSF: Granulocyte-colony stimulating factor, TNF-α: Tumor necrosis factor-alpha, PSA: Prostate-specific antigen, *S. aureus: Staphylococcus aureus*, *E. coli: Escherichia coli*, GAD65: glutamic acid decarboxylase-65, IA2: Islet antigen-2, and Ab: Antibody, cfu: colony forming unit.

Table S3. Material cost per well on the NE^{2}RD.

Reagents/Items	Cost
Well	¢5
Poly-$_L$-lysine	¢1
Gold	¢16
Chemical Activators and Antibody Anchors	¢23
Antibody	¢80
TOTAL	$1.25

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