Electronic Supplementary Information (ESI)

Clade-Specific, Amplification-Free Molecular Lateral Flow Platform for On-Site Detection of Mpox

Satheesh Natarajan [a,d], Ketan Dighe [b], David Skrodzki [c], Nandhakumar Ponnusamy [a], Dipanjan Pan [a,b,c,d,*]

[a] Department of Nuclear Engineering, The Pennsylvania State University, University Park, PA 16802, USA

[b] Department of Biomedical Engineering, The Pennsylvania State University, University Park, PA 16802, USA

[c] Department of Materials Science and Engineering, The Pennsylvania State University, University Park, PA 16802, USA

[d] Huck Institutes of the Life Sciences, University Park, PA 16802, USA

*Email of the corresponding author: dipanjan@psu.edu

Table of Contents

1. Experimental Sections **S3**
2. Supporting Figures (S1-S12) **S10**
3. Supporting Tables (S1-S5) **S24**
4. Notes and references **S29**

Experimental Sections

Materials. All the chemicals were purchased from commercial vendors. Custom-made oligonucleotides (ssDNAs) with biotin and FAM/DIG for the LFIA and RT-PCR primers and reagents were bought from Sigma–Aldrich (Saint Louis, MO). A viral Swab DNA isolation kit was ordered from Sigma–Aldrich (Saint Louis, MO)

Mpox Lesion Swab Samples for LFA Strip Validation. Lesion swab specimens were purchased from Boca Biolistics, LLC. These samples were originally collected in Peru under the protocol titled *"Collection of Clinical Samples of Tropical, Infectious and Autoimmune Diseases and Healthy Donors"* (BB-ID-061 / CRSPTL-00001), a medium-risk clinical study approved by the Institutional Committee of Research Ethics (CIEI) in accordance with the standards of the Peruvian National Institute of Health (INS) (Hospital Certificate No. 014-2022).

Boca Biolistics obtained written informed consent from all participants (or their legally authorized representatives) using an ethics-committee-approved consent form and subsequently provided de-identified lesion swab specimens to our laboratory. All experiments with these clinical specimens were performed at The Pennsylvania State University in compliance with relevant national and international laws and guidelines, following Penn State institutional policies, and under the oversight and approval of the appropriate institutional review and biosafety committees. Control genomic DNA for Mpox Clade I and Clade II from Twist Bioscience (San Francisco, CA 94080), and the isolated DNA for Clade Ia from the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) repository under a Material Transfer Agreement (MTA). For Clade Ia, a CDC-validated DNA sample originally isolated from a clinical specimen (stock concentration

10^5 copies/μL) was obtained through authorized sources. This DNA was serially diluted in nuclease-free water, and its concentration was independently verified by quantitative PCR prior to use in the lateral flow assay. Due to biosafety level-3 (BSL-3) requirements and regulatory restrictions, no clinical samples for Clade Ib were accessible at our institution. Therefore, sequence-verified synthetic DNA corresponding to the Mpox D14L gene (Clade Ib) was designed based on reference genomic sequences and procured from Twist Bioscience. All synthetic targets were resuspended in nuclease-free water and stored at −20 °C until use. All experiments involving clinical derived DNA were conducted in accordance with institutional biosafety guidelines and applicable regulatory approvals.

Preparation of Citrate-Stabilized Gold Nanoparticles (AuNPs). Gold nanoparticles (AuNPs) were synthesized using a literature protocol [1]. Briefly, 1 mM chloroauric acid (20 mL) was boiled while stirring, followed by adding one percent of sodium citrate solution. After the addition, the solution turned from yellow to red. At this point, stop boiling and allow it to cool for 10 minutes in a shaded area. The red wine solution of the AuNPs was centrifuged and resuspended in Milli-Q water.

Design of Single-Stranded Oligonucleotides (ssDNA). Mpox virus genome sequences were analyzed to design ssDNAs for Clades Ia, Ib, and II using S.Oligo software [2]. Selected ssDNAs sequences were functionalized with thiol moieties at the 5' or 3' end for enhanced agglomeration with gold nanoparticles for the UV-vis Spectroscopy studies.

Functionalization of AuNPs with ssDNA. Citrate-stabilized AuNPs (2 mL) were treated with ssDNAs (0.25–2 μM) and 0.5 mM TCEP, stirred for 2 hours, and kept at 4°C for further use [3].

Limit of Detection (LOD) Calculation. Control genomic DNA for Clades Ia, Ib, and II was titrated in nanocomposite suspensions. The intensity change was measured using an ESI Quant Instrument, and LOD was determined using the formula LOD = 3.3 $(Sy/S)^4$. Mpox DNA standards (10^5 and 10^1 copies/µL) were prepared in nuclease-free buffer. LFIA test strips were fabricated in-house and consisted of a sample pad, conjugate pad, nitrocellulose membrane with immobilized test and control antibodies, and an absorbent pad. Gold nanoparticle–antibody conjugates were prepared and subsequently lyophilized for reagent-stability assessments. All buffers and consumables were of analytical grade.

Cross-Reactivity Assessment. Selectivity was tested by introducing 0.25 $ng.mL^{-1}$ of genomic DNA obtained from SARS-CoV-2, Neisseria gonorrhoeae, and Chlamydia trachomatis into the nanocomposite formulation.

RT-PCR Protocol. The RT-PCR was performed according to the CDC guidelines. In summary, 0.25 µl of each 10 µM forward primer and 0.25 µl of each 10 µM reverse primer, 1 µl of a 10 µM Probe, 5 µl of isolated or extracted DNA, 10 µl of Taq 2 x master mix, and 4.5 µl of DI water were combined in a PCR 96-well plate. A quick spin was applied following a gentle mix to ensure all liquids were at the bottom of the tube. The PCR plate was sealed and placed in an Applied Biosystems™ MiniAmp™ Thermal Cycler, which had been preheated to 95°C. The specified thermocycling parameters were utilized to amplify the DNA: the resulting amplified products were stored at 4°C until further use.

Determination of Cross-Reactivity. To determine the selectivity of the optimized oligonucleotide strands (1^{st} ssDNA and 2^{nd} ssDNA) were mixed in 10 µM concentration with the AuNps, and then the nanocomposite formulation was added to the genomic Mpox

DNA at the concentration of 0.25 ng mL^{-1} to test against the other pathogenic DNA at the same concentration, Chlamydia trachomatis, Neisseria gonorrhoeae bacteria, and RNA from the SARS-CoV-2 virus. The change in absorbance at 540 nm was observed with the use of UV-Vis spectroscopy.

Transmission Electron Microscopy. Twenty microliters of each suspension of the nanocomposite were applied to a formvar-coated copper grid. After ten minutes, the surplus solution was wiped off the grid and air dried. Transmission electron microscopy (TEM) data was acquired from Thermo Scientific Talos F200X G2 (S)TEM operating at 200 kV. Furthermore, STEM-EDS mapping was performed on this equipment utilizing the four in-column SDD Super-X detectors. EDS maps were recorded for approximately five minutes at a beam current of 0.12 nA and analyzed using Thermo Scientific Velox software.

Scanning Electron Microscopy. The nanocomposites before and after the addition of genomic DNA from the monkeypox virus samples were mounted on SEM stubs using double-sided carbon tape, air-dried, and then sputter-coated with an 80:20 platinum/palladium alloy to enhance conductivity. Surface topography was examined using a FEI Nova NanoSEM 450 field-emission scanning electron microscope (FE-SEM). High-resolution images were captured for each sample, and corresponding energy-dispersive X-ray spectroscopy (EDS) spectra were acquired to assess elemental composition.

X-ray photoelectron spectroscopy (XPS) analyses were performed using a Physical Electronics Versa Probe III system, equipped with a monochromatic Al Kα X-ray source (hv = 1,486.6 eV) and a concentric hemispherical analyzer. Charge neutralization was

facilitated by a dual-beam system employing low-energy electrons (<5 eV) and argon ions. The binding energy scale was calibrated using reference standards: gold (Au $4f_7/_2$ = 83.96 eV) and sputter-cleaned copper (Cu $2p_3/_2$ = 932.62 eV, Cu $3p_3/_2$ = 75.1 eV). All binding energies were referenced to the C 1s hydrocarbon peak (CH_x) set at 284.8 eV. Spectra were acquired at a 45° takeoff angle concerning the sample surface, providing a sampling depth of approximately 3–6 nm, from which 95% of the signal originates. Quantification was conducted using instrument-provided relative sensitivity factors (RSFs), incorporating corrections for photoionization cross-sections and the inelastic mean free path of electrons. For homogeneous samples, the standard deviation for major elemental concentrations (>5 at. %) was typically below 3%, while greater variability was observed for trace elements. The analysis area was approximately 200 μm in diameter.

UV–vis Spectroscopy. For absorbance measurements, 10 μL of target DNA samples (0.25 ng/mL) were mixed with 150 μL of AuNPs functionalized with ssDNA sequences 1a+1b and 2a+2b for Clade Ia, 3a+3b and 4a+4b for Clade II, and 5a+5b and 6a+6b for Clade Ib. The concentration of Mpox DNA was determined using aNanoDrop. Absorbance readings were recorded in 96-well plates using a Biotek Synergy Microplate Reader for both kinetic and spectral analysis. Each experiment was conducted at least three times, and the average spectra was reported. The normalized data were used to compare and standardize assay parameters independent of experimental conditions.

Preparation of the Multiplex Lateral Flow Assay Strips (in-house). The lateral flow assay (LFA) strip consisted of four sequentially assembled membranes with partial overlaps: a sample pad, a conjugate pad, a nitrocellulose (NC) membrane, and an absorbent pad. The sample pad was pretreated with 1× PBS containing 0.5% BSA, 0.15%

Tween-20, and 1% sucrose, then dried at room temperature for 1 hour. The conjugate pad was treated with 100 mM phosphate buffer (PB, pH 7.4) containing 0.1% Triton X-100, 0.1% BSA, 20% sucrose, and 5% HAMA blocker. On the NC membrane, the test lines were dispensed at 1 µL/cm using 0.5 mg/mL of antibodies: Anti-FITC antibody for T1, Anti-DIG antibody for T2, and Biotin–BSA for the control line. The spacing between the test and control lines was maintained at 3.5 mm, defining the reaction zone. The NC membrane was 25 mm long, with T1 positioned 10 mm from the sample application end, T2 located 3.5 mm downstream, and the control line 3.5 mm beyond T2. An additional 10 mm space was kept after the control line to avoid cross-signaling from backflow. Finally, the sample, conjugate, NC, and absorbent pads were assembled with a 2 mm overlap, cut into 4 mm-wide strips, and stored in a desiccator until use. The antibody dispensing on the NC membrane was performed using the Claremont dispenser.

Preparation of the ASOs for the development of the Lateral flow assay:

The antisense single-stranded DNA (ASO-ssDNA) was incubated with RecA protein (New England Biolabs) at a molar ratio of 3:1 (bases to protein) in the presence of 1 mM ATPγS (Sigma Aldrich), 10 U/mL pyruvate kinase, 3 mM phosphoenolpyruvate (PEP), and single-stranded binding protein (SSB; Abcam). All reagents were prepared in RecA reaction buffer consisting of 70 mM Tris–HCl, 10 mM $MgCl_2$, and 5 mM dithiothreitol (DTT), pH 7.6. The reaction mixture was incubated at 37°C for 10 minutes to allow RecA to form a stable nucleoprotein filament along the ssDNA. Subsequently, 10 µM of Mpox double-stranded DNA (dsDNA) was introduced to the reaction to facilitate strand invasion and hybridization, and the mixture was maintained at 37°C for an additional 15 minutes. This process enabled RecA-mediated unwinding of the dsDNA and promoted the exposure of

complementary single-stranded regions for efficient hybridization. Following this reaction, the mixture was immediately transferred to ice for 2 minutes to halt RecA activity and stabilize the newly formed DNA complexes, thereby preventing any nonspecific reannealing. To the cooled mixture, 2 μL of each 10 μM oligonucleotide was added to obtain a final 1× working concentration. These oligonucleotides were specifically designed to hybridize with target sequences within the Mpox genome, ensuring sequence-selective recognition during subsequent hybridization. A 1× SSC buffer was freshly prepared by diluting 5 mL of 20× SSC stock with 95 mL of deionized water. This buffer provided the necessary ionic strength and pH conditions to support stable nucleic acid duplex formation while minimizing nonspecific binding events. The hybridization reaction was then performed at 30°C for 10 minutes, a temperature optimized to maintain hybridization efficiency under moderately stringent conditions. After the hybridization step, 50 μL of the reaction mixture was mixed with 50 μL of 1× PBS containing gold nanoparticles (GNPs). The GNPs acted as optical reporters, enabling downstream visual signal generation through plasmonic enhancement during lateral flow immunoassay (LFIA) analysis. Finally, the prepared mixture was applied onto the LFIA strip, where the migration and capture of hybridized complexes were evaluated. The resulting colorimetric signal on the strip confirmed the specific detection of the target Mpox DNA.

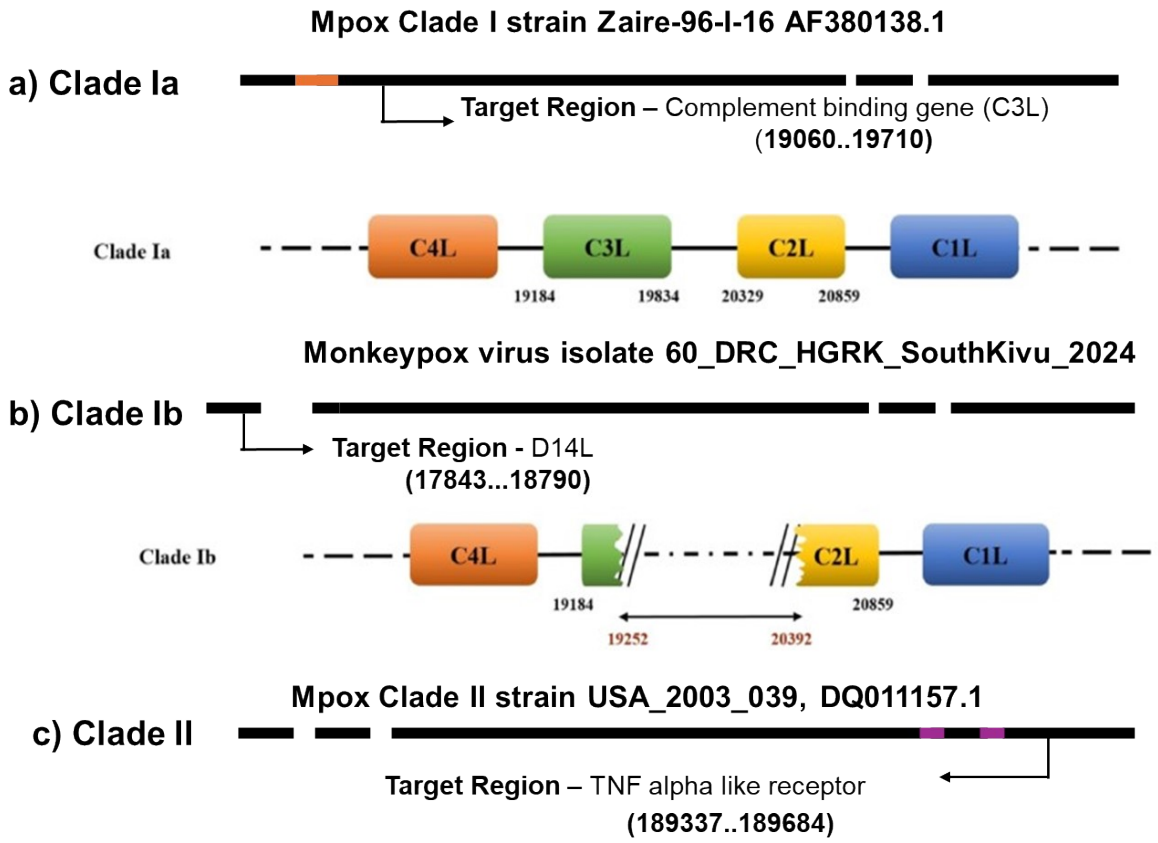

Figure S1. Genomics Sequence of Mpox Clade Ia strain Zaire-96-I-16 AF380138.1, Mpox Clade Ib strain-Southkivu, and Mpox Clade II strain USA_2003_039.

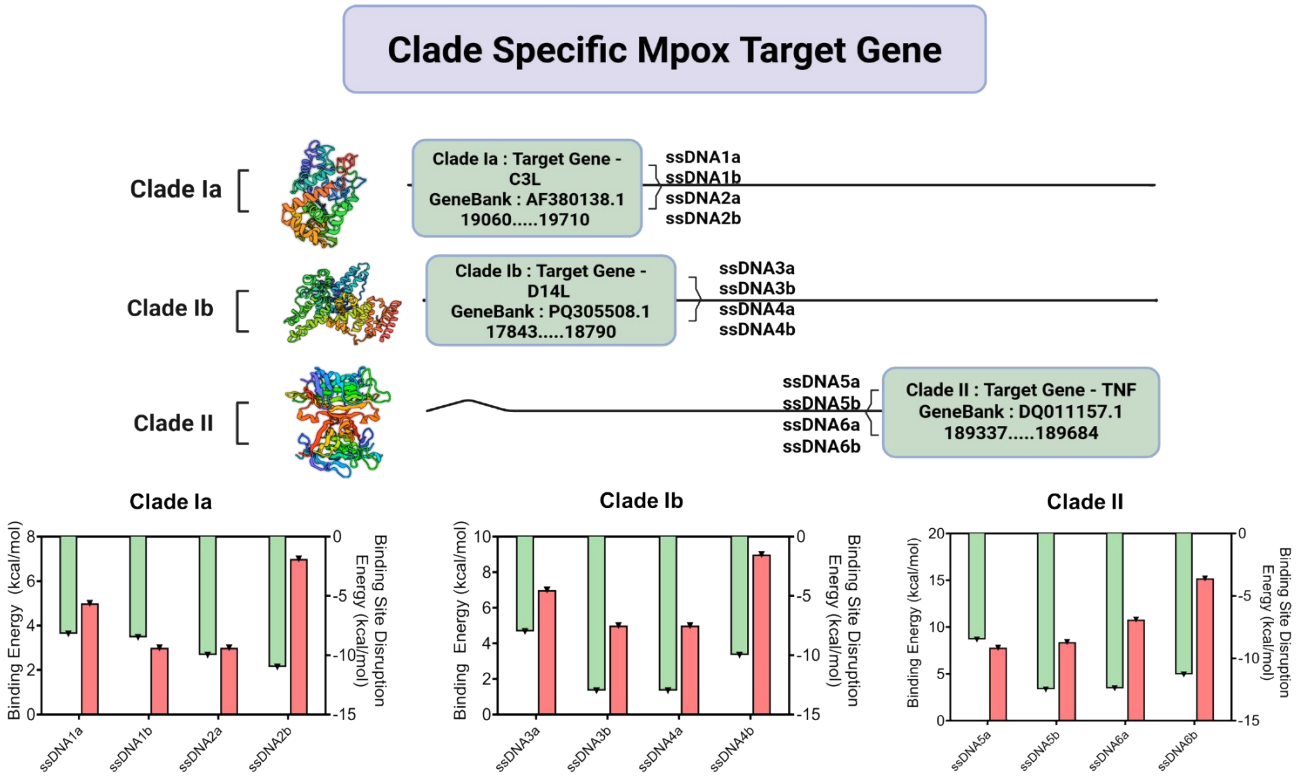

Figure S2. Proteins of the Clade Ia, Ib and II and the target binding energy, compared to the energy associated with the disruption of the binding site Clade Ia, Clade Ib, Clade II.

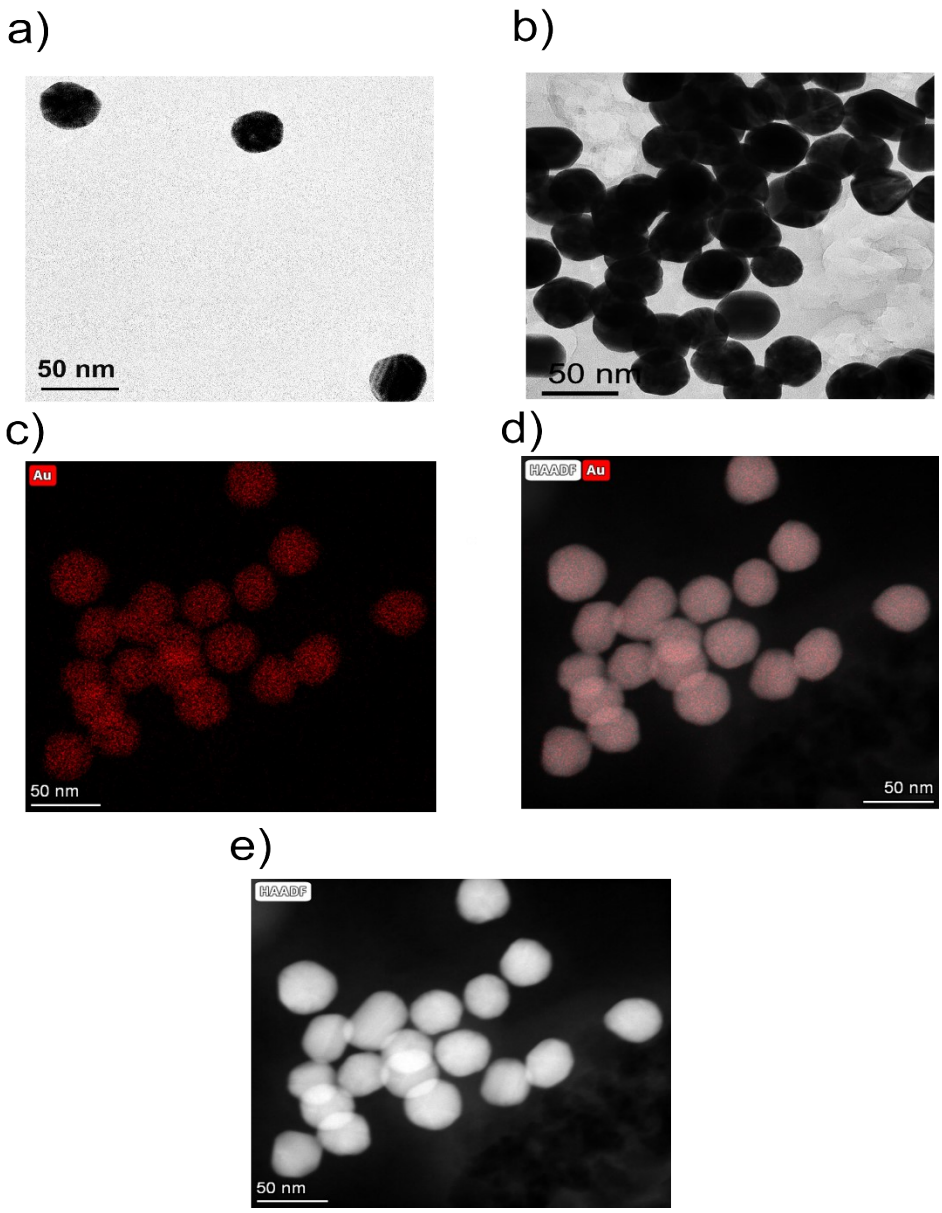

Figure S3. (a, b) TEM images show ssDNA-capped AuNPs before and after adding Mpox target DNA. Initially, the AuNPs were well-dispersed, but aggregation occurred upon target DNA addition across all Clades. (c-e) The figure presents high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) images.

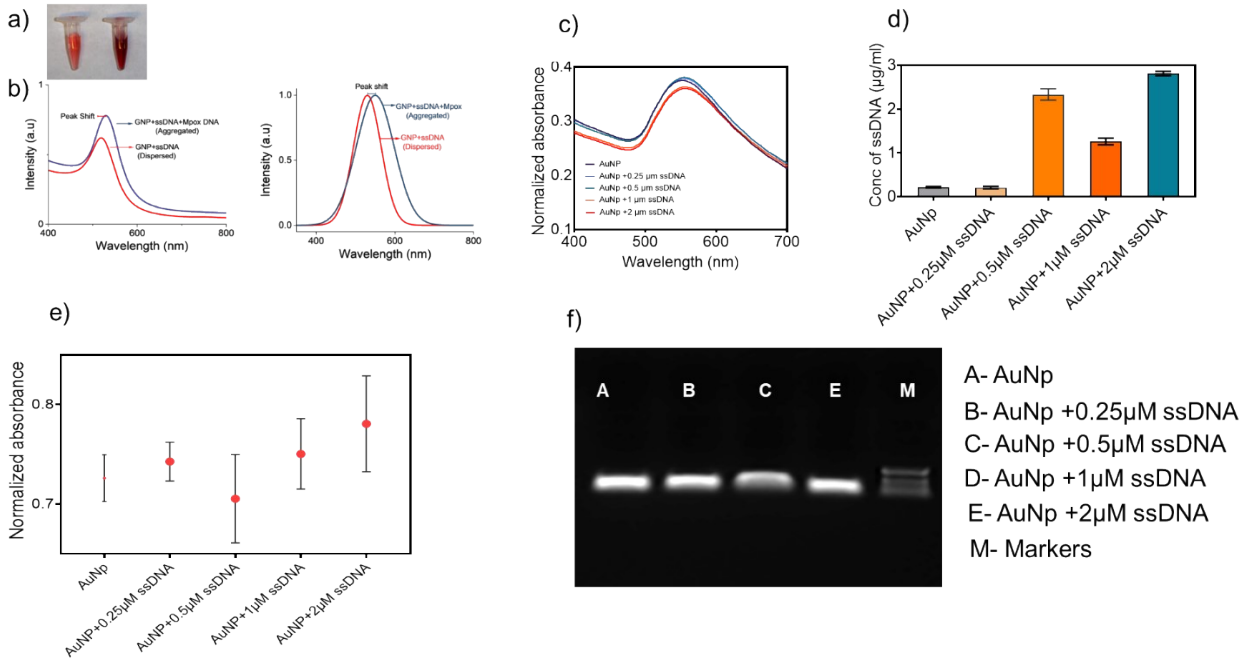

Figure S4. a) Photograph showing the color change of ssDNA-functionalized gold nanoparticles: bright red when dispersed (GNP+ ssDNA) without the Target Mpox DNA and dark red upon aggregation with Mpox DNA. b) **UV–Vis spectra of ssDNA-functionalized gold nanoparticles in the presence of Mpox DNA:** dispersed GNP+ ssDNA shows a characteristic SPR peak dispersed without Target Mpox DNA, while hybridization with Clade I or Clade II DNA induces aggregation, causing a red-shifted SPR band c) UV-visible absorbance spectra of as-synthesized gold nanoparticles and gold nanoparticles conjugated with ssDNA sequences at different concentrations. d) The stability of AuNPs was confirmed during the conjugation process using intensity measurements at 520 nm. e) UV-visible absorbance spectra with different concentrations of the ssDNA. f) The gel electrophoresis picture for the ssDNA and gold nanoparticle conjugation stability.

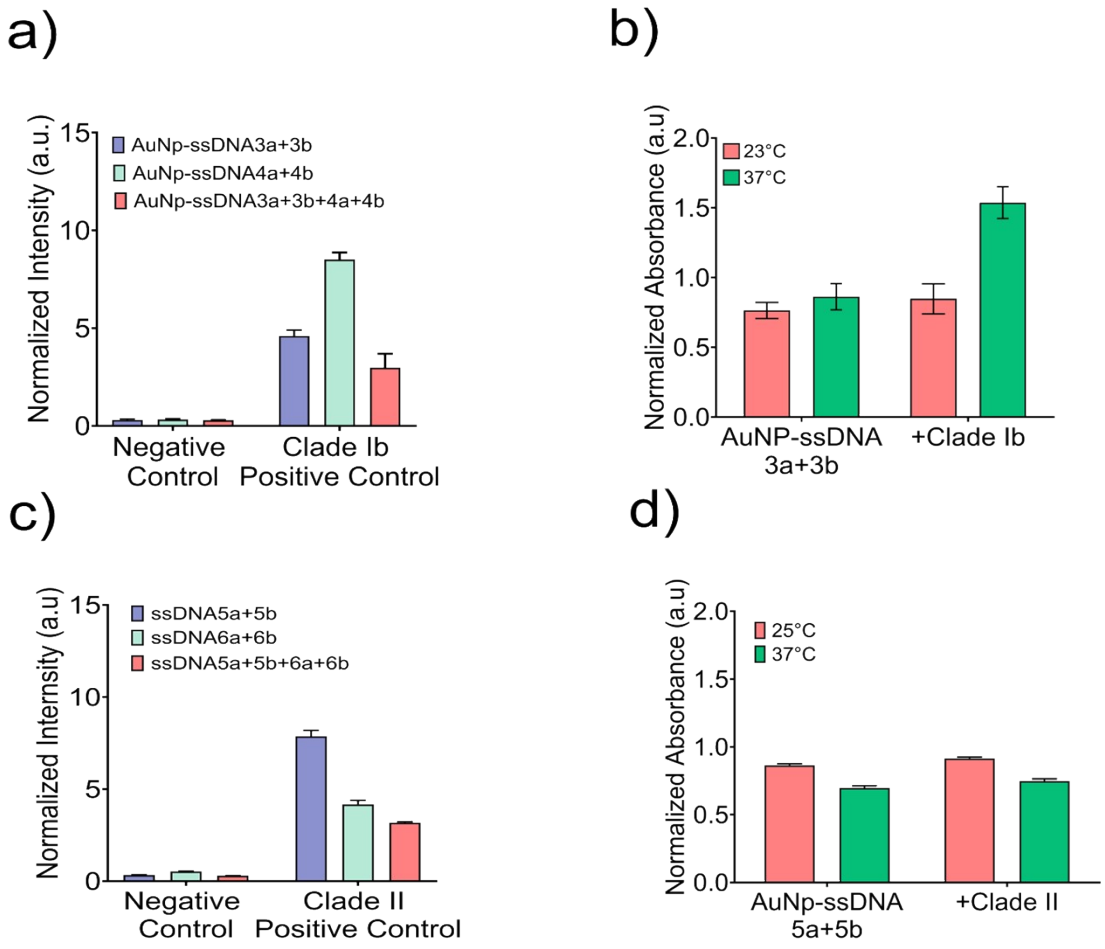

Figure S5. (**a, c**) Normalized intensity of ssDNA-AuNPs with Mpox DNA (0.25 ng mL^{-1}) compared to negative controls (mean ± SD, n = 3). (**b, d**) Normalized intensity of ssDNA-AuNPs (Thaxton et al., 2006) after incubation with Mpox DNA (0.25 ng mL^{-1}) at two temperatures.

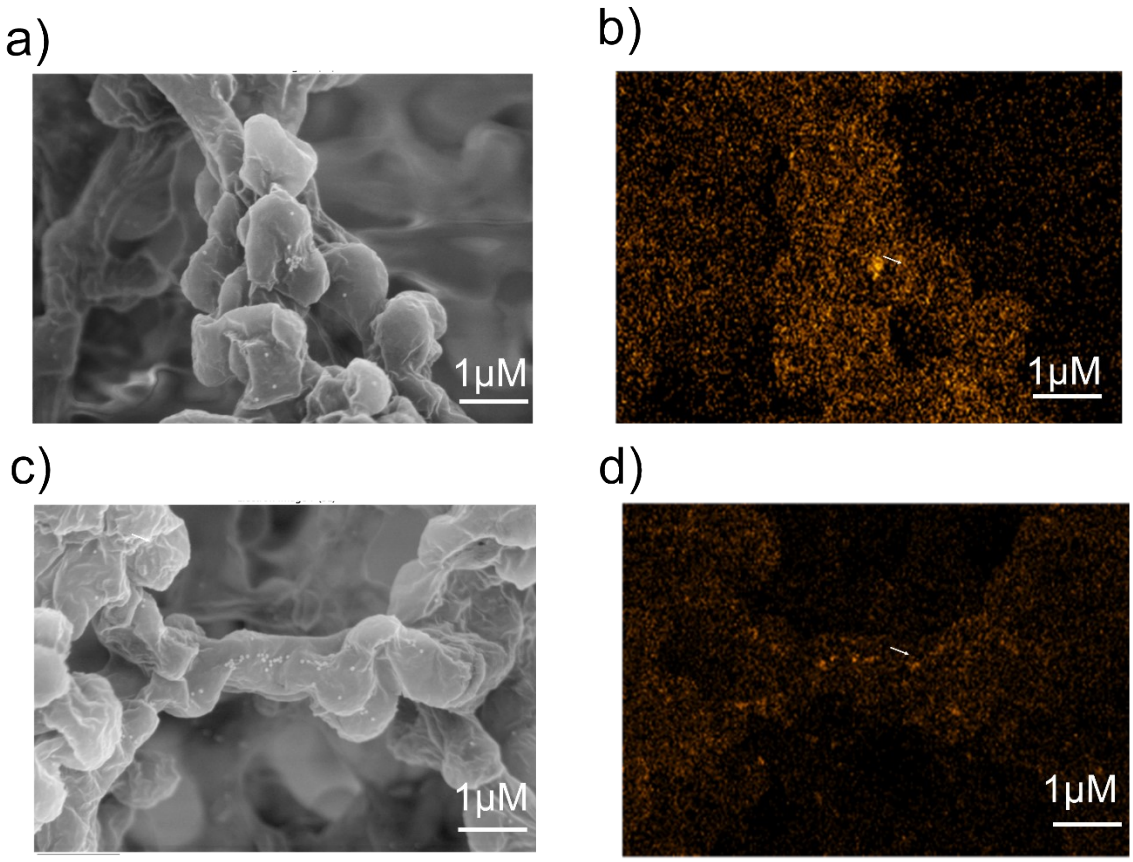

Figure S6. SEM images of the nano-assembly: (a) before and (d) after the introduction of Mpox target DNA at a rate of 0.25 ng mL^{-1}. The agglomeration of AuNPs is evident in the overlay EDS images **(a, c)** upon the addition of Mpox DNA. In contrast, the separate EDS maps for Au **(b, d)**

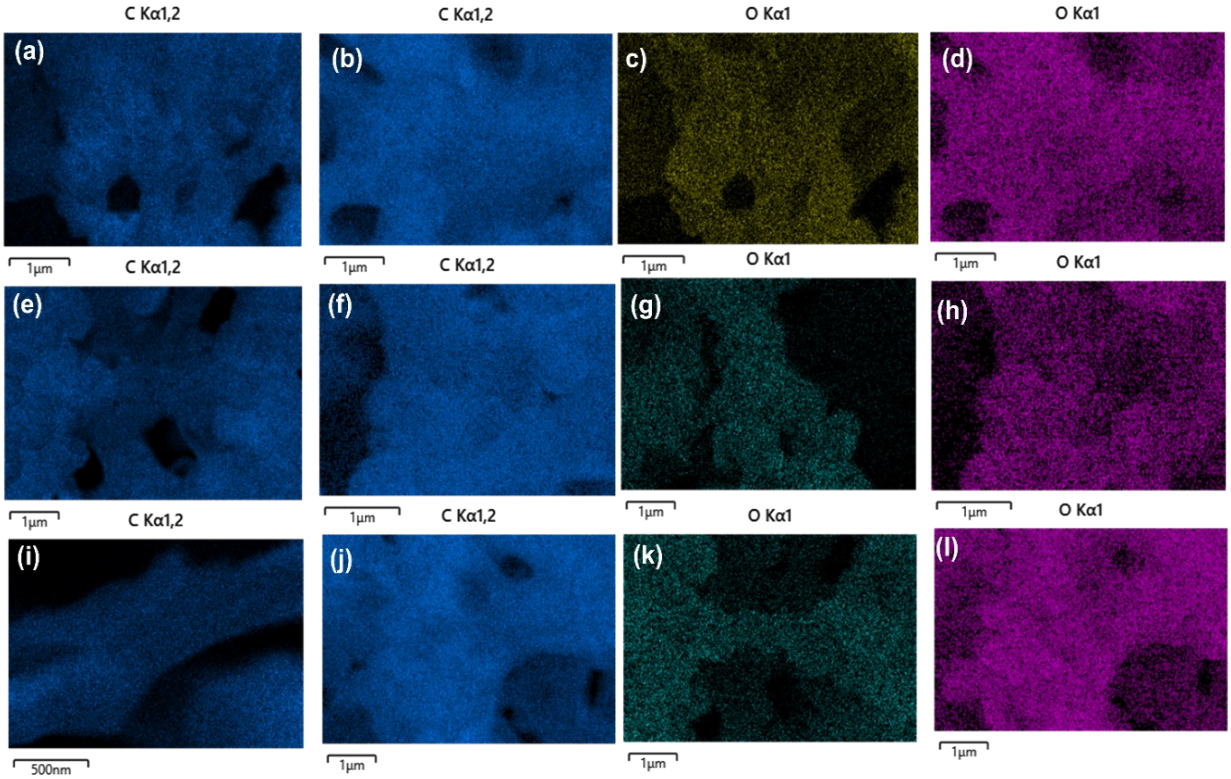

Figure S7. Scanning electron microscopic EDS images of the nano-assembly. EDS image of the C profile of the surface after the addition of Mpox DNA of Clade Ia, Ib, and II at 0.25 ng.µl^{-1} concentration (**a, e, i**) and before the addition **(b, f, j**). O profile of the surface after the addition of Mpox DNA of Clade Ia, Ib, and II at 0.05 ng/µl concentration (**c, j, k**) and before the addition (**d, h, i**).

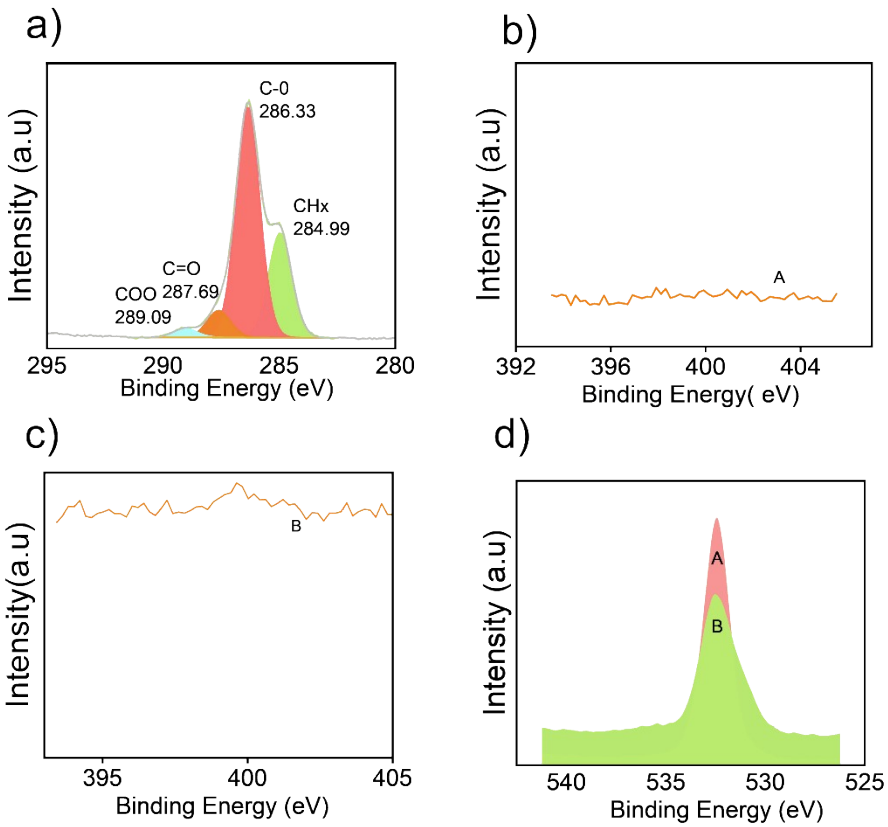

Figure S8. XPS data before and after introducing Mpox DNA to ssDNA-capped AuNPs. Panels (**a**) display carbon, (**b and c**) the nitrogen component, and (**d**) the oxygen component. (A, B) indicates before and after the incorporation of the target Mpox DNA into the nano assembly in each figure.

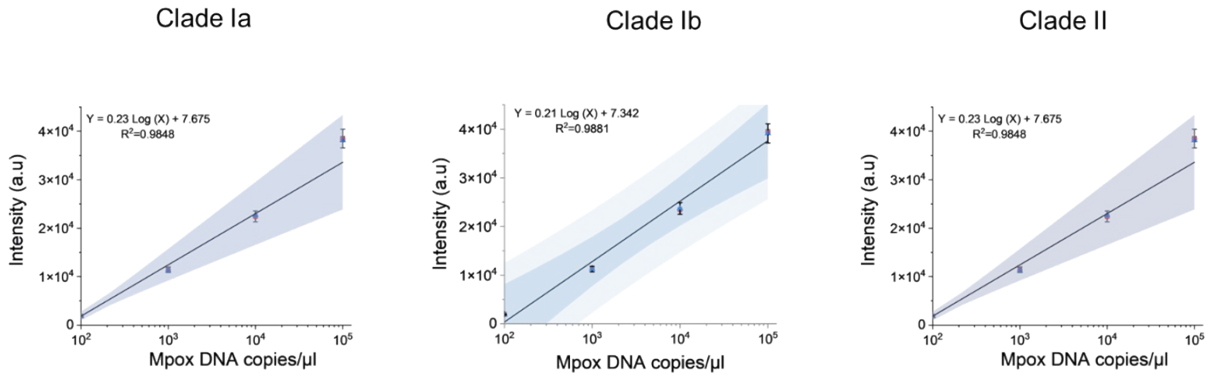

Figure. S9: Analytical limit of detection for gonorrhea using Clade Ia, Ib and II targeted ssDNA conjugated to gold nanoparticles. The assay was tested using the genomic DNA serially diluted 10-fold times with concentrations ranging from 10^5 copies/µL to 10 copies/mL. The limit of detection was calculated using the equation $3.3 * (Sy/S)$ where (Sy) is the standard deviation of the response of the curve and (S) is the slope of the calibration curve. The absorbance measurements were recorded from three (n=3) independent experiments.

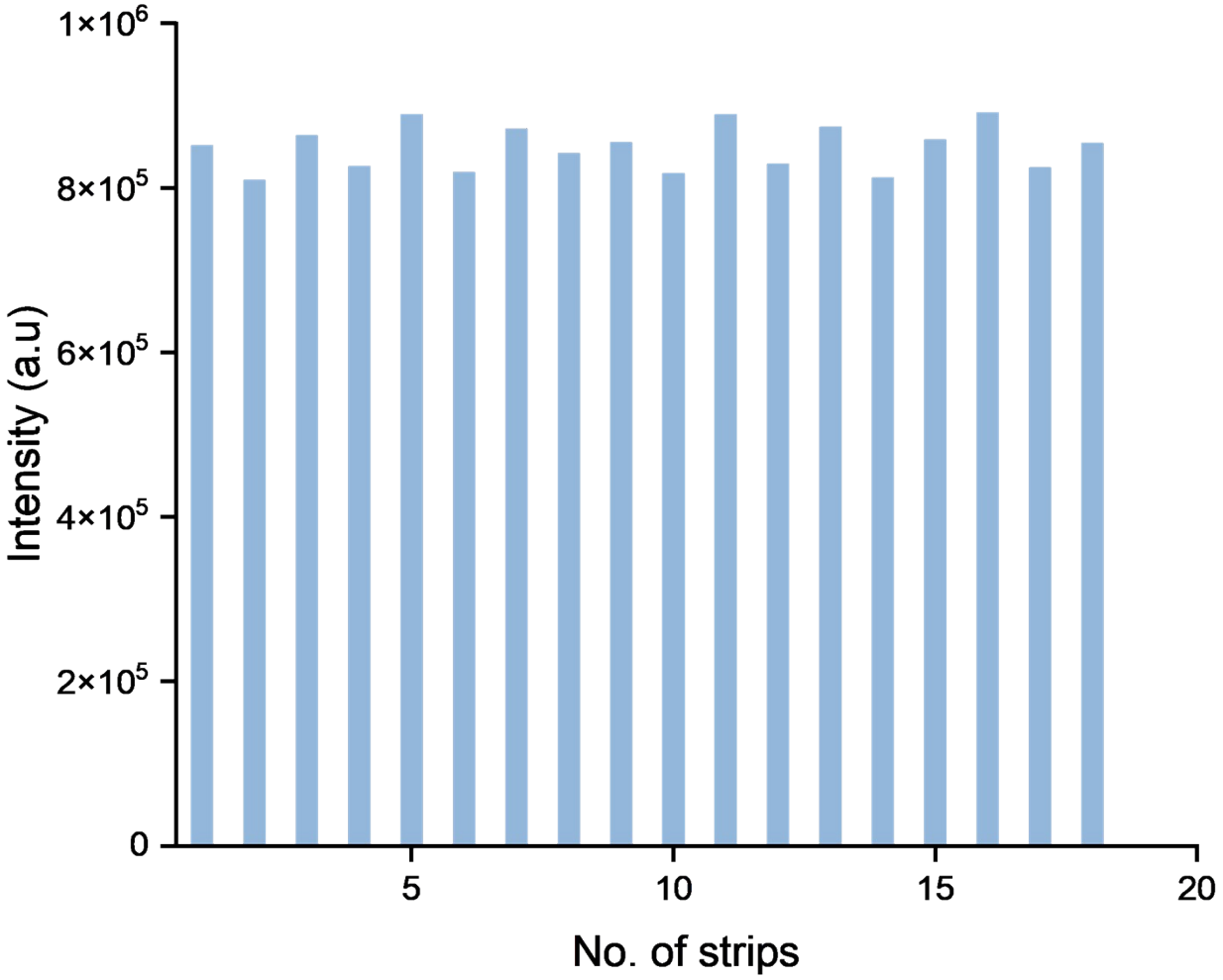

Figure S10. The intensity bar chart for the clinical swab samples for the Clade II

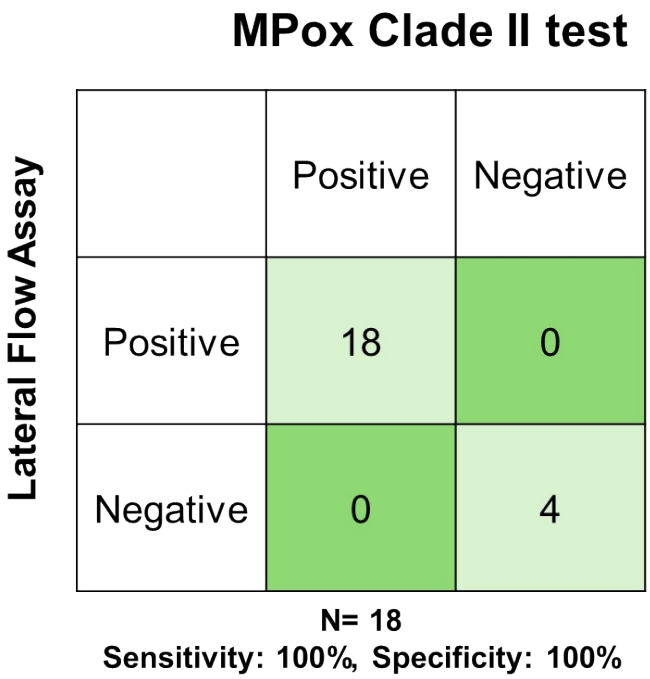

Figure S11. Confusion Matrix Chart for Mpox LFA Clinical Swab Samples (N=18).

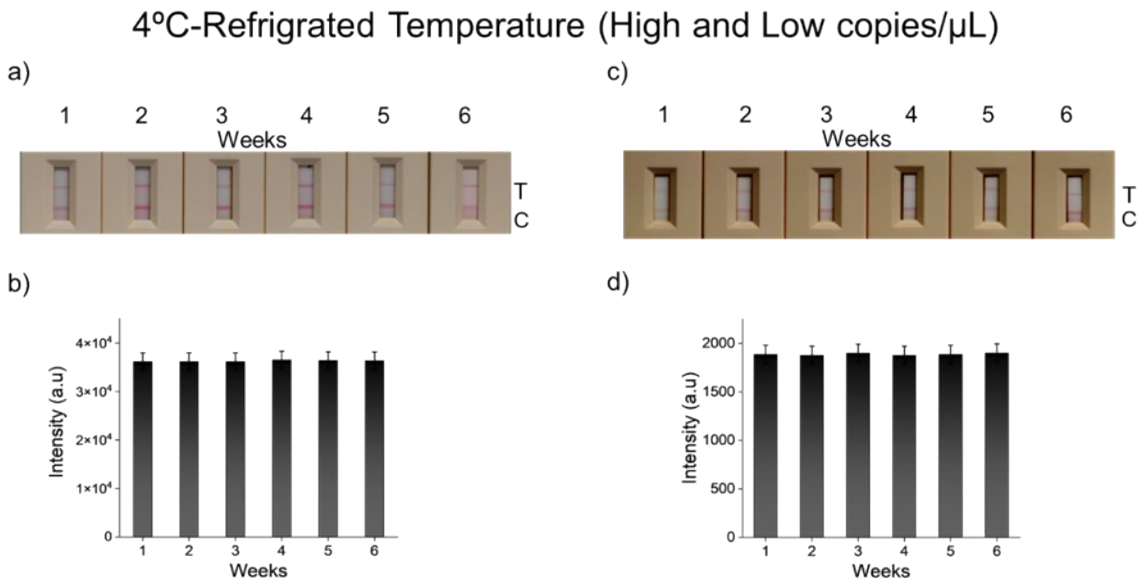

Figure.S12a. Stability test. **a-b)** Color images and their corresponding intensity bar chart for the 1, 2, 3, 4, 5 and 6 weeks at 4 °C storing strips. **c-d)** Color images and their corresponding intensity bar chart for the 1, 2, 3, 4, 5 and 6 weeks at 4 °C storing strips.

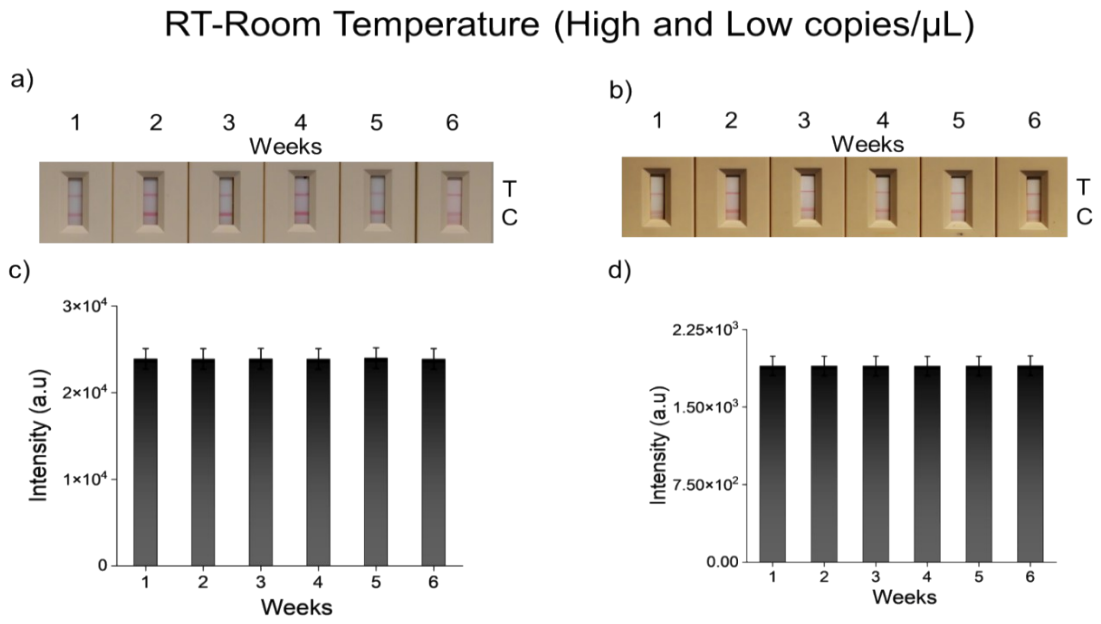

Figure. S12b. Stability test. **a-b)** Color images and their corresponding intensity bar chart for the 1, 2, 3, 4, 5 and 6 weeks at RT storing strips. **c-d)** Color images and their corresponding intensity bar chart for the 1, 2, 3, 4, 5 and 6 weeks at RT storing strips.

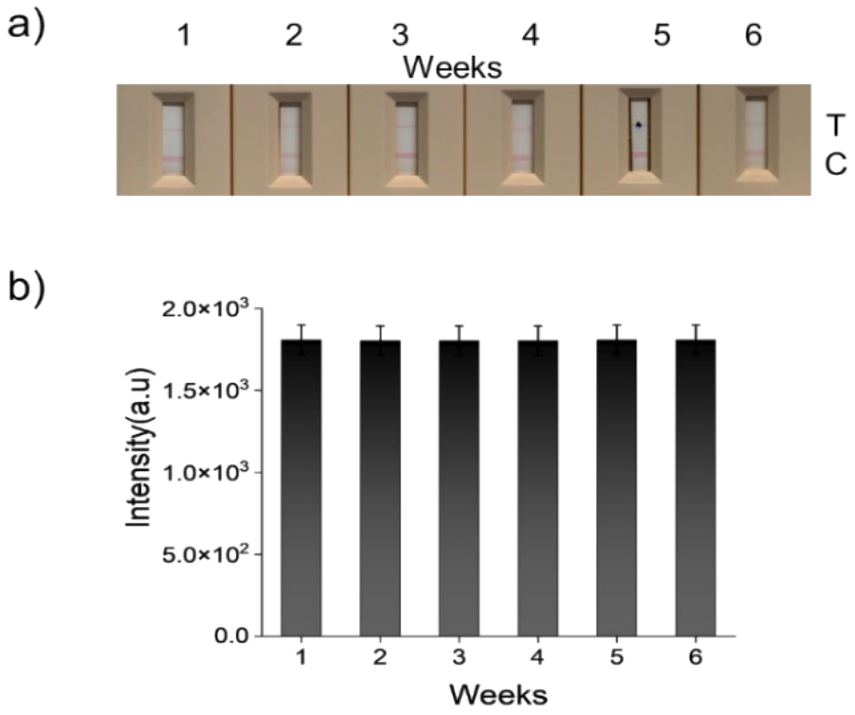

Figure. S12c. Gold conjugate stability test. **a-b)** Color images and their corresponding intensity bar chart for the 1, 2, 3, 4, 5 and 6 weeks at 4C storing strips.

Table S1. Details of designed ssDNA's with their target sequence and nucleotide gap positions.

Start Target Position	Ending Target Position	Nucleotide gap	Target sequence (5´-3´)	Complementary Oligos sequence (5´- 3´)
299	319	21	CUGCCAAGAUAGCUUCAAAG	CTTTGAAGCTATCTTGGCAG **Clade Ia ssDNA 1a**
340	360		UGCCAAGAUAGCUUCAAAGU	ACTTTGAAGCTATCTTGGCA **Clade Ia ssDNA 1b**
432	452	20	UUAACACUCUGCCAAGAUAG	CTATCTTGGCAGAGTGTTAA **Clade Ia ssDNA 2a**
472	492		UAACACUCUGCCAAGAUAGC	GCTATCTTGGCAGAGTGTTA **Clade Ia ssDNA 2b**
48	68	21	GCACUUCGAAAUGGAAAAGA	TCTTTTCCATTTCGAAGTGC **Clade Ib ssDNA 5a**
89	109		CUUCCAAACUUAAUCACUCC	GGAGTGATTAAGTTTGGAAG **Clade Ib ssDNA 5b**
71	91	24	CCAAACUUAAUCACUCCUAG	CTAGGAGTGATTAAGTTTGG **Clade Ib ssDNA 6a**
115	135		UCAGGCGCAUAUCCACCCAC	GTGGGTGGATATGCGCCTGA **Clade Ib ssDNA 6b**
562	582	23	GGUAACAGGUGGCCAAACUC	GAGTTTGGCCACCTGTTACC **Clade II ssDNA 3a**
605	625		GUAACAGGUGGCCAAACUCC	GGAGTTTGGCCACCTGTTAC **Clade II ssDNA 3b**
832	852	81	GAGAAGCAAAUGAUGACUUG	CAAGTCATCATTTGCTTCTC **Clade II ssDNA 4a**
933	953		UAUCUCCUCAAGGAAUACAC	GTGTATTCCTTGAGGAGATA **Clade II ssDNA 4b**

Table S2. The table indicates the components of the XPS analysis

Samples	Components						
	CHx	C-O-C	C=O	COO	Au	O	N
ssDNA+ AuNp before the addition of the Mpox DNA	19.2	40.4	5.1	1.7	0.7	29.8	-
ssDNA+ AuNp after the addition of the Mpox DNA	48.7	12.1	1.7	3.2	0.5	16.1	0.4

Table S3: The RT-PCR Probe and primers for Clade Ia, Ib, and II, and their PCR amplification conditions[5].

PCR Primers

Mpox Type	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
Clade Ia	TGTCTACCTGGATACAGAAAGCAA	GGCATCTGCCTTTAATACATTGAT
Clade Ib	TACACCGTCTCTTCCACAGG	GATACAGGTTTAATTCCACATAG
Clade II	CACACCGTCTCTTCCACAGA	GATACAGGTTTAATTCCACATCG

Probes

Mpox Type	Probe
Clade Ia	5-FAM CCCATATATGCTAAATGTACCGGTACCGGA-3_BHQ1
Clade Ib	5-FAM CCAACGTCGTAACCAGCAATACGAAC-3_BHQ1
Clade II	5-FAM AACCCGTCGTAACCAGCAATACATTT-3_BHQ1

PCR Protocol

Cycle Type	Stage	Cycle(s)	Temperature	Time
Enzyme Activation / Hot Start	1	1	95 °C	06.00
Amplification		40	95 °C 62 °C	00:15 00:30

Positive Control – Twist Synthetic Human Mpox Controls

- Clade Ia (Congo Basin) – 106056 – 1 x 10^5 copies/µL
- Clade Ib (South Kivu) – 654373 – 1 x 10^5 copies/µL
- Clade II (West Africa) – 106059 – 1 x 10^5 copies/µl

Journal of Virological Methods **2010, *169* (1), 223–227**

Table S4. Clinical swab samples list for the Mpox virus from Boca Biolistics, with the respective Ct values

Sample Code	Sample Type	Internal Sample Code	Matrix Type	Origin	PCR Status (Ct)	
					Clade I	Clade II
S000911565	Lesions	S1	Swab	Peru; Lima	--	26.86
S000911566	Lesions	S2	Swab	Peru; Lima	--	26.76
S000913082	Lesions	S3	Swab	Peru; Lima	--	26.48
S000913083	Lesions	S4	Swab	Peru; Lima	--	26.68
S000913094	Lesions	S5	Swab	Peru; Lima	--	26.61
S000913095	Lesions	S6	Swab	Peru; Lima	--	27.54
S000933134	Lesions	S7	Swab	Peru; Lima	--	26.48
S000933147	Lesions	S8	Swab	Peru; Lima	--	26.68
S000933154	Lesions	S9	Swab	Peru; Lima	--	26.18
S000933155	Lesions	S10	Swab	Peru; Lima	--	26.64
S000933190	Lesions	S11	Swab	Peru; Lima	--	26.86
S000933191	Lesions	S12	Swab	Peru; Lima	--	26.76
S000933194	Lesions	S13	Swab	Peru; Lima	--	--
S000933195	Lesions	S14	Swab	Peru; Lima	--	--
S000933226	Lesions	S15	Swab	Peru; Lima	--	--
S000933227	Lesions	S16	Swab	Peru; Lima	--	--
S000933230	Lesions	S17	Swab	Peru; Lima	--	26.61
S000933231	Lesions	S18	Swab	Peru; Lima	--	27.54

Table S5. This table shows the Competing diagnostic devices available on the market for detecting the Mpox virus variant.

Assay name and company	Clade detectable	Sensitivity	Specificity
Xfree monkeypox direct assay, USA	Detects Clade I and II	95	100
Monkeypox virus real-time PCR kit, Germany	Detects Clade I and II	95	100
LogixSmart Mpox (2-Gene) RUO test, Co-Diagnostics, USA	Mpox virus	95	100
DI Monkeypox virus Duplex PCR kit, France	Detects Clade I and II	95	100
SupremeDx Mpox Virus qPCR Detection Kit, Germany	Detects Clade I and II	95	100
GPS™ MPXV dtec-qPCR Assay, Spain	Detects Clade I and II	95	100
Novaplex™ MPOX/OPXV Assay, Korea	Mpox virus	95	100
Standard M10/Mpox, USA	Detects Clade I and II	95	100
Diaxxo PCR-MPXV, Germany	Mpox virus	-	-

Assay name and company	Clade I and II	Sensitivity	Specificity
Alinity M MPVX, Abbot USA	Detects Clade I and II	100	100
Bioperfectus Mpox test kit, Bioperfectus, china	Mpox virus	95	95
Viasure RT PCR Kit, Certec Biotech SL, Spain	Detects Clade I and II	95	100
Cue Mpox Test kit, Cue Health, USA	Detects Clade I and II	100	100
Quanti virus MPVX Test Kit, Diacarta Inc,USA	Detects Clade I and II	100	100
Rapi Fast Mpox detection kit, KH Medical kit	Detects Clade I and II	95	95
Cobas, Roche Diagnostics	Detects Clade I and II	95	100
Xpert Mpox(Cepheid)	OPXV*, Mpox clade II	100%	96.6%
Flashdetect Lycocart Monkeypox assay	Mpox only	-	-
Sanity 2.0, Monkeypox Virus Test Kit (ProDiag)	Detects Clade I and II	-	-
Cue Mpox molecular test (Cue Health)	Detects Clade I and II	100%	100%

Assay name and company	Clades detection	Status	Sensitivity	Specificity	Detection method
Pulselife monkeypox virus card	Mpox	Regulatory Achieved (US FDA EUA)	-	-	Cartridge based kit, with small instrument used for heating
Cue Mpox molecular test (Cue Health)	Mpox clades I, II	Regulatory Achieved (US FDA EUA)	100	100	Isothermal amplification based
Skin Tropic Virus Panel – Dragonfly (Proton Dx)	Mpox clades I, II	Research Use Only (RUO)	-	-	Isothermal amplification based
ZiP-MPx-P2 (ZiP Diagnostics)	Mpox	Research Use Only (RUO)	-	-	cartridge based kit
Our Kit- ssDNA based LFA kit	Clade I and II	-			Non amplification based LFA kit

Notes and References

1. I. Hammami and N. M. Alabdallah, *Journal of king Saud university-science*, 2021, **33**, 101560.
2. Y. Ding, C. Y. Chan and C. E. Lawrence, *Nucleic acids research*, 2004, **32**, W135-W141.
3. P. Moitra, M. Alafeef, K. Dighe, M. B. Frieman and D. Pan, *ACS nano*, 2020, **14**, 7617-7627.
4. J. Uhrovčík, *Talanta*, 2014, **119**, 178-180.
5. Y. Li, H. Zhao, K. Wilkins, C. Hughes and I. K. Damon, *Journal of virological methods*, 2010, **169**, 223-227.