Electronic Supplementary Information

CRISPR/Cas12a-enhanced DNA nanomachine for multiple respiratory pathogens detection

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Experiment Section

Reagents and Materials

Streptavidin-coated magnetic microspheres (MBs) were Dynabeads™ M-280 Streptavidin (10 mg/μL) purchased from Thermo Fisher Scientific Inc. (USA). 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-aceticacid-10-maleimidoethylacetamide (MMA-DOTA) was purchased from Macrocyclics, Inc. (USA). Terbium (III) chloride hexahydrate ($TbCl_3 \cdot 6H_2O$), holmium (III) chloride hexahydrate ($HoCl_3 \cdot 6H_2O$), and thulium (III) chloride hexahydrate ($TmCl_3 \cdot 6H_2O$) were purchased from Aladdin Reagent Inc. (Shanghai, China). Tris (2-carboxyethyl) phosphine (TCEP) was obtained from Adamas Reagent, Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris-HCl) stock solution, ethylenediaminetetraacetic acid (EDTA), and DEPC-treated water were purchased from Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). Sodium chloride (NaCl), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), ammonium acetate (NH_4Ac), acetic acid (HAc), and nitric acid (HNO_3) were bought from Chengdu Kelong Chemical Reagent Company (China). 3K Amicon Ultra-0.5 NMWL spin filters were purchased from Merck Millipore (Germany). Ultrapure water (UPW, 18.25 MΩ cm^{-1}) was produced using a ULUPURE (Chengdu, China) water purification system.

All the oligonucleotides listed in Table S1 were synthesized and HPLC-purified by Shanghai Sangon Biotech Co. Ltd. (Shanghai, China). The clinical serum samples were collected by Chengdu Seventh People's Hospital, and the throat swab samples were taken from our research group.

Buffers and Solutions

a. NH_4Ac buffer: 0.5 M NH_4Ac, pH 5.8.

b. Wash buffer: 10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7.4.

c. Tris-HCl buffer: 20 mM Tris-HCl, 150 mM NaCl, pH 7.4.

d. Binding and Washing (B&W) buffer: 5 mM Tris-HCl, 1 M NaCl, 0.5 mM EDTA, pH 7.4.

e. 2×B&W buffer：10 mM Tris-HCl, 2 M NaCl, 1 mM EDTA, pH 7.4.

f. NEBuffer r2.1: 10 mM Tris-HCl, 50 mM NaCl, 10 mM $MgCl_2$, 100 μg/mL Recombinant Albumin, pH 7.9.

NEBuffer r2.1 is diluted with DEPC-treated water, and the rest of the solution is prepared using ultrapure water.

Apparatus

In this experiment, the data of lanthanide metal elements ^{159}Tb, ^{165}Ho, and ^{169}Tm were collected by using a NexION 350 commercial inductively coupled plasma mass spectrometer (PerkinElmer Inc., USA), and the detailed instrument operating parameters are shown in Table S2. CRISPR/Cas12a inactivation experiments were performed with a K90 PCR amplification instrument (Hangzhou Lattice Scientific Instrument Co., Ltd.), and the rest of the experiments were performed in a thermo-shaker shaker (Hangzhou Aosheng Instrument Co., Ltd.). Electrospray ionization mass spectrometry (ESI-MS) of macrocyclic compounds and their chelates was recorded using a TSQ Quantum Ultra ultra liquid chromatography-mass spectrometer (Thermo Fisher Scientific, USA). The Zeta potential

of MBs and nanomachines was measured with the Zetasizer Nano ZS nanoparticle size and zeta potential analyzer (Malvern Psystemalytical, UK). The high-resolution field emission scanning electron microscope with dual probe spectroscopy and EBSD analysis system (manufactured by Thermo Fisher and Oxford Instruments) was used for EDS analysis of the samples.

Synthesis of Lanthanides Labeled DNA Substrate Strands

^{159}Tb, ^{165}Ho, and ^{169}Tm were selected to label three different DNA substrate strands, respectively. The specific experimental process is shown in Figure S1. First, 1,4,7,10 tetraazacyclododecane-1,4,7-triacetic acid-10-maleimide ethylacetamide (MMA-DOTA) forms a chelate complex with a lanthanide (III) ion. The lanthanides ($TbCl_3 \cdot 6H_2O$, $HoCl_3 \cdot 6H_2O$, and $TmCl_3 \cdot 6H_2O$) and MMA-DOTA were dissolved in NH_4AC buffer (pH 5.8). 20 μL of 5 mM MMA-DOTA solution and 20 μL of 10 mM Tb^{3+} solution were diluted with NH_4Ac buffer and incubated at 37 ℃ for 1 h to obtain DOTA-Tb chelate (DOTA-Ho chelate and DOTA-Tm chelate were also prepared according to the above procedure). Meanwhile, three different DNA substrate strands modified with disulfide bonds were dissolved in NH_4Ac buffer solution at a concentration of 100 μM. The three DNA substrate strands were mixed with 10 mM TCEP at a 1:1 volume ratio, reacted at 37 ℃ for 30 min, and purified with a 3K ultrafiltration tube to remove the remaining TCEP. The ultrafiltration procedure was as follows: centrifugation of the three DNA substrate strands at 4 ℃ for 15 min followed by centrifugation at 4 ℃ for 2 min to transfer the reduced strands of the three DNA substrate strands to a new PCR tube. Then, 150 μL of the prepared DOTA-Tb, DOTA-Ho, and DOTA-Tm chelates were added to the PCR tubes, and the DNA orbital strands labeled with ^{159}Tb, ^{165}Ho, and ^{169}Tm were obtained by shaking vigorously at 37 ℃ for 2 h. Finally, ultrafiltration was continued with washing buffer 5 times to remove excess Tb^{3+}, Ho^{3+}, and Tm^{3+} metal ions. The final product was diluted to 1220 μL for the sixth time with Tris-HCl buffer for later use.

Preparation of DNA Nanomachines

The DNA substrate strands labeled with lanthanide elements (^{159}Tb, ^{165}Ho, and ^{169}Tm) are connected to Streptavidin-modified magnetic beads (SA-MBs) to prepare DNA nanomachines. 100 μL of SA-MBs were dispersed in B&W buffer solution and washed 3 times. 500 μL of metal-labeled DNA track strand with biotin and 500 μL of 2×B&W buffer solution were added for 2 h at 25 ℃ with vigorous shaking. The prepared DNA nanomachine was washed 4 times with Tris-HCl buffer to remove excess orbital strands. Finally, it was diluted to 750 μL for later use. The above operations prepared three different DNA nanomachines.

Analysis of ICP-MS

10 μL of 100 nM DNAzyme, 10 μL of 20 nM Cas12a/crRNA (1:3 ratio), and 20 μL of different concentrations of target were mixed in PCR tubes for 60 min at 37 ℃. DNAzyme, Cas12a/crRNA, and different concentrations of targets were diluted by NEBuffer r2.1. The above experimental procedures were performed on a clean bench. After

the cleavage of Cas12a was completed, the Cas12a activity was lost by a PCR instrument at 85 ℃ for 5 min. 30 μL of each inactivated solution was mixed in a new PCR tube, and 45 μL of Mg^{2+} and 45 μL of the prepared nanomachine were added to it, and the reaction was violently shaken at 25 ℃ for 40 min to release Tb^{3+}, Ho^{3+}, and Tm^{3+} metal ions on the MBs. Finally, the supernatant was separated by magnetic suction, dispersed in 1 mL of 1% HNO_3, and then sent to ICP-MS for mass spectrometry analysis.

Actual Sample Preparation

The study was approved by the Ethical Committee of West China Hospital, Sichuan University (reference no. 193, 2020). The serum sample was centrifuged, and the resulting supernatant was then taken and diluted tenfold. The throat swab sample was extracted with a kit.

PAGE of Three DNA Substrate Strands Labeled with Lanthanides

The 12% nondenaturing polyacrylamide gel electrophoresis (PAGE) was employed to characterize DNA substrate strands labeled with lanthanides, this experiment was performed in standard 1× TBE buffer at room temperature for 1.5 h at 80 V constant voltage. Eventually, the gel was imaged by FluorChem M.

MALDI-TOF-MS of Three DNA Substrate Strands Labeled with Lanthanides

MMA-DOTA-modified DNA substrate strands are characterized by MALDI-TOF-MS. A mixture of 3-hydroxypicolinic acid (3-HPA) and DHCA (volume ratio 3:2) was used as the matrix solution in the determination procedure. The sample is mixed with an equal volume of the matrix solution, and then 4 μL of the mixture is dropped on the target plate and air-dried naturally at room temperature. The sample to be tested is finally sent to MALDI-TOF-MS for testing. The instrument conditions are: profiles: 100, shots: 2, tuning mode: linear, power: 165-175, mass range: 11500-16000.

Table S1 The DNA and RNA sequences used in this work.

Name	Sequences (5' to 3')
SARS-CoV-2 cDNA	CTGCGTTCTCCATTCTGGTT
H1N1 DNA	TACACCCAGTCACAATAGGA
MP DNA	AACATAATGGAGGTTAACCG
SARS-CoV-2-crRNA	UAAUUUCUACUAAGUGUAGAUAACCAGAAUGGAGAACGCAG
H1N1-crRNA	UAAUUUCUACUAAGUGUAGAUUCCUAUUGUGACUGGGUGUA
MP-crRNA	UAAUUUCUACUAAGUGUAGAUCGGUUAACCUCCAUUAUGUU
SARS-CoV-2-DNAzyme	ATCTCTTCTCCGAGCCGGTCGAAATAGTGT
H1N1-DNAzyme	CCGTATTCTCCGAGCCGGTCGAAATCTGAC
MP-DNAzyme	TATCCTTCTCCGAGCCGGTCGAAATGCATA
SARS-CoV-2-substrate	**Biotin**-T_{15}ACACTAT/rA/GGAAGAGATT$_5$-**SH**
H1N1-substrate	**Biotin**-T_{15}GTCAGAT/rA/GGAATACGGT$_5$-**SH**
MP-substrate	**Biotin**-T_{15}TATGCAT/rA/GGAAGGATAT$_5$-**SH**
influenza B	CGGGCCTCACTCTGCTCTTTACGTC
HIV	ACTGCTAGAGATTTTCCACAT
HAV	GGACTTGATACCTCACCGCC
RSV	AAAAATGGGGCAAATA

The same colors (underlines) represent the complementary sequence. The bold part represents the location and type of DNA modification.

Table S2 The working condition of the ICP-MS instrument.

Parameter	**Setting**
Vacuum Pressure (Torr)	3.50×10^{-7}
ICP RF Power (W)	1300
Nebulizer Gas Flow (L/min)	0.94
Auxiliary Gas Flow (L/min)	1.2
Plasma Gas Flow (L/min)	18
Dwell Time (ms)	50
Dead Time (ns)	35
Sweeps per reading	120
Isotope monitored	^{159}Tb、^{165}Ho、^{169}Tm

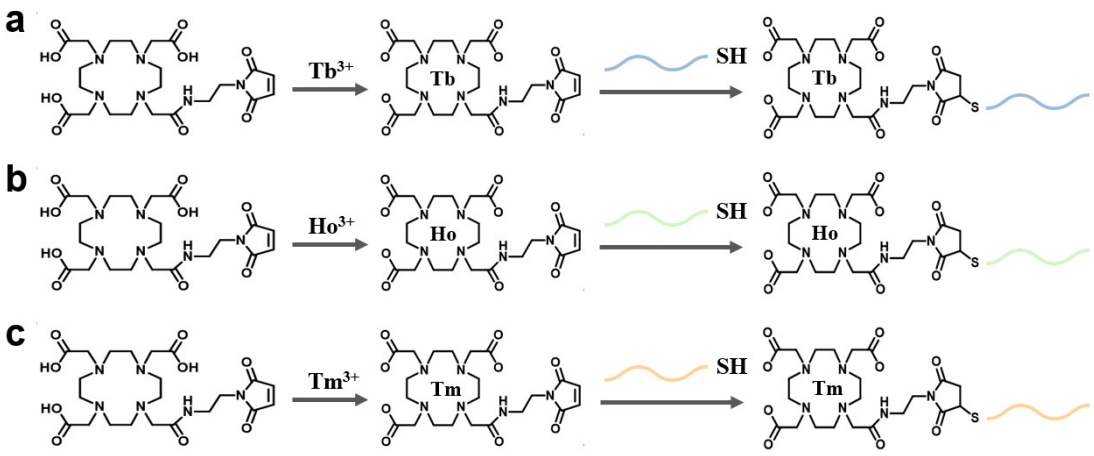


Fig. S1 Labeling process of DNA substrates with (a) DOTA-Tb, (b) DOTA-Ho, and (c) DOTA-Tm.

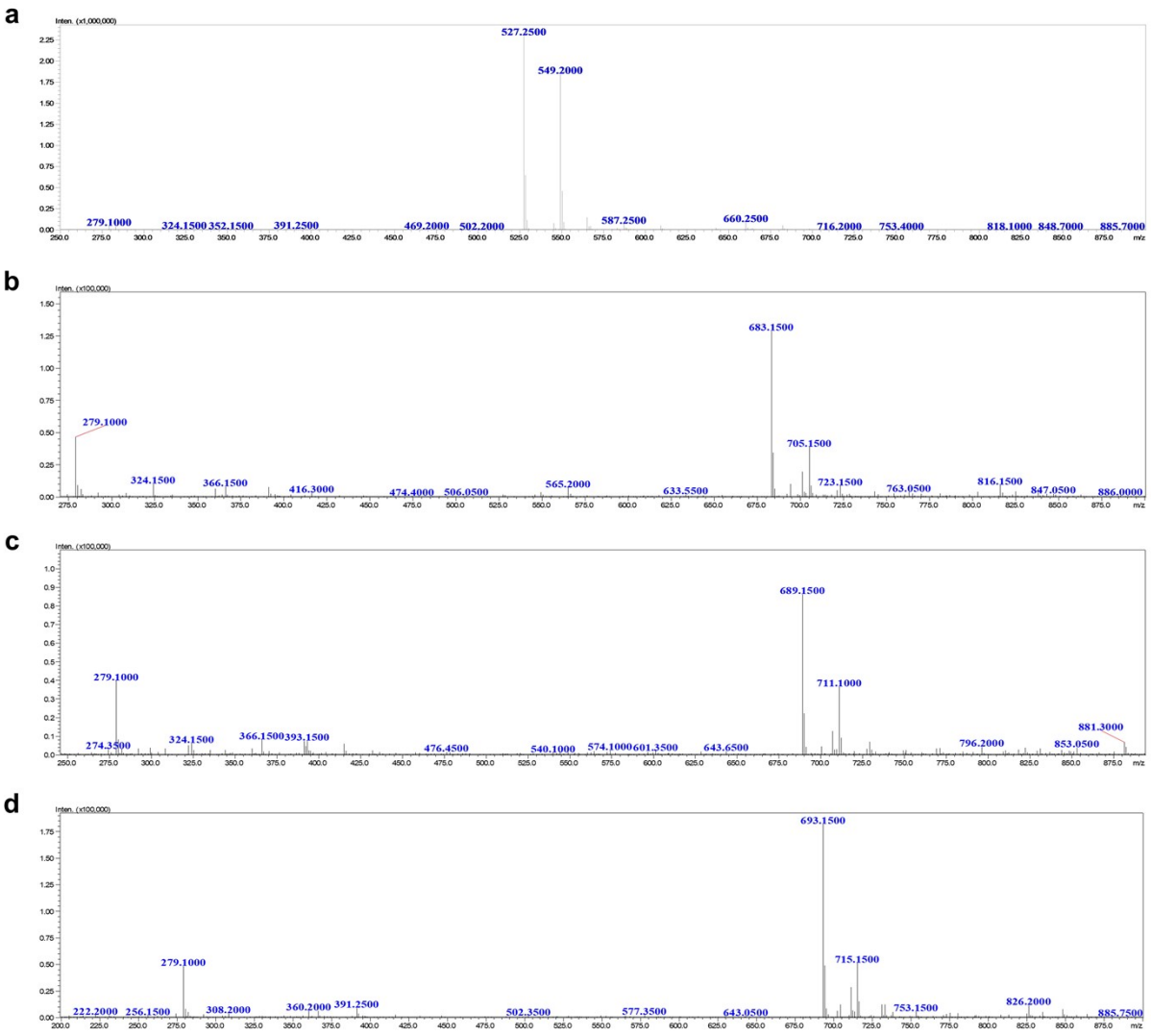


Fig. S2 ESI-MS of MMA-DOTA, DOTA-Tb, DOTA-Ho, and DOTA-Tm. (a) MMA-DOTA, ESI-MS, m/z, found: 527.2 ($[M+H]^+$), 549.2 ($[M+Na]^+$); calc.: 527.5 ($[M+H]^+$). (b) DOTA-Tb, ESI-MS, m/z, found: 683.1 ($[M+H]^+$), 705.2 ($[M+Na]^+$); calc.: 683.4 ($[M+H]^+$). (c) DOTA-Ho, ESI-MS, m/z, found: 689.1 ($[M+H]^+$), 711.1 ($[M+Na]^+$); calc.: 689.4 ($[M+H]^+$). (d) DOTA-Tm, ESI-MS, m/z, found: 693.1 ($[M+H]^+$), 715.2 ($[M+Na]^+$); calc.: 693.4 ($[M+H]^+$).

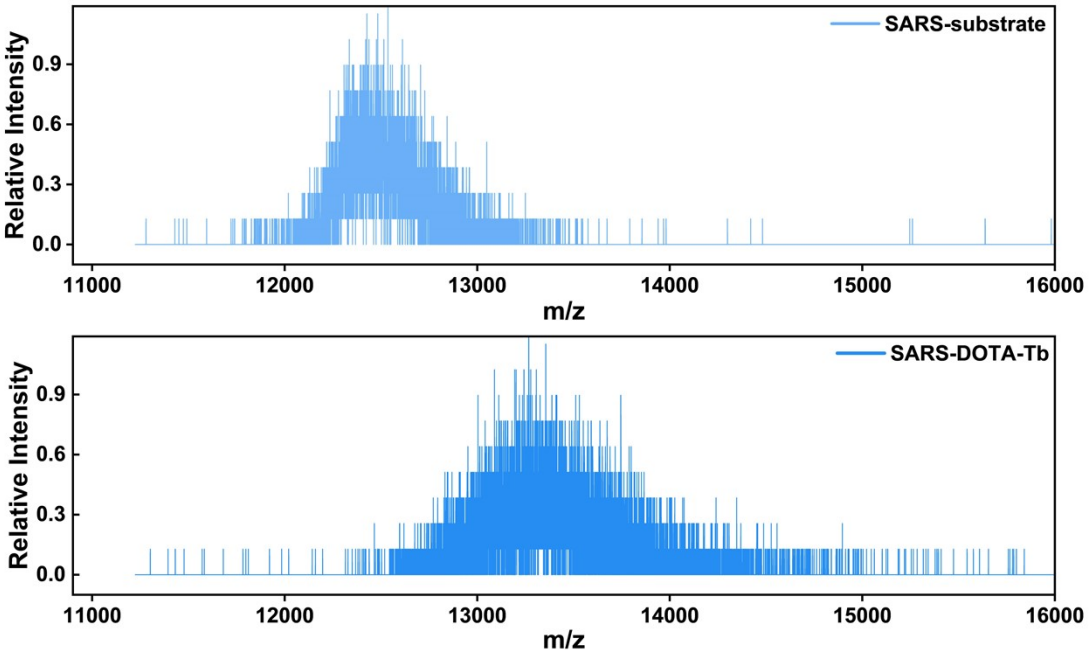


Fig. S3 MALDI-TOF-MS of SARS-substrate DNA and SARS-DOTA-Tb.

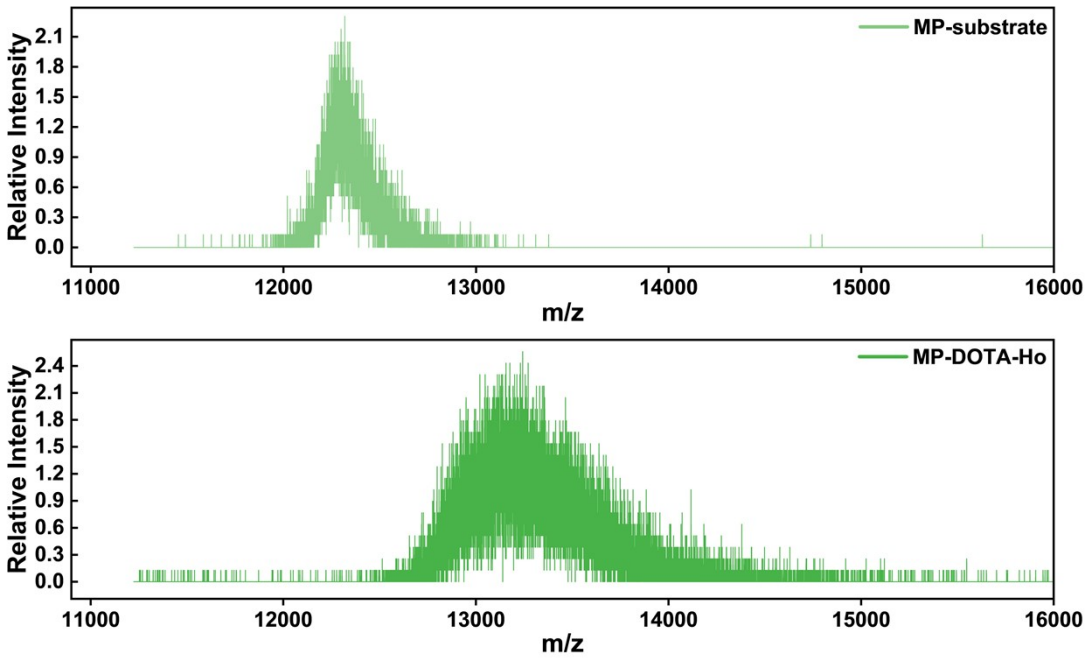


Fig. S4 MALDI-TOF-MS of MP-substrate DNA and MP-DOTA-Ho.

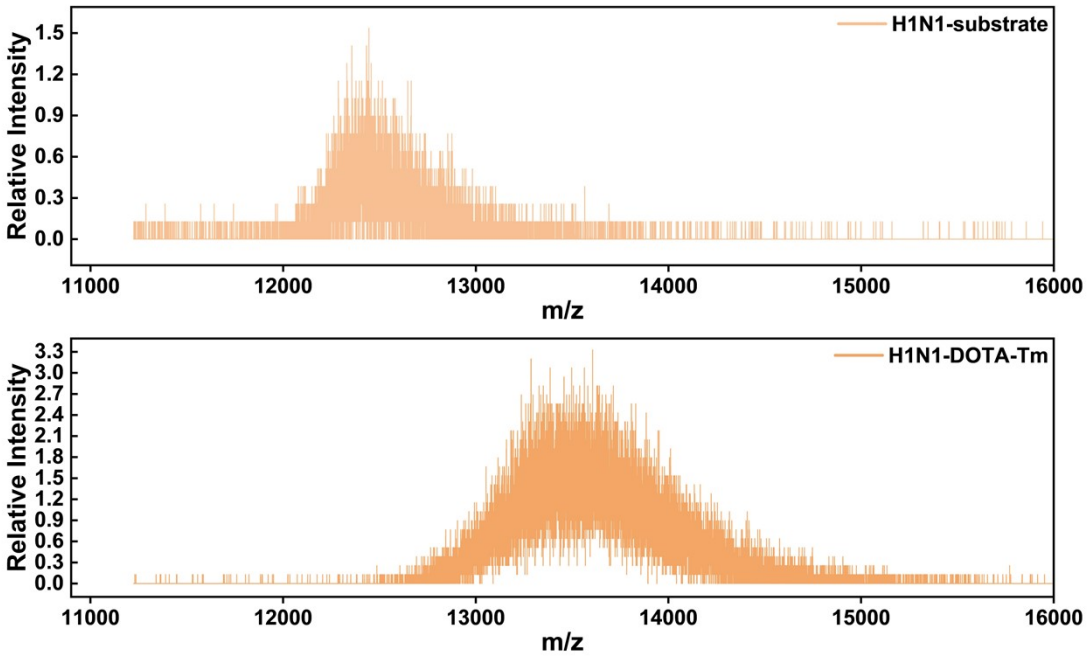
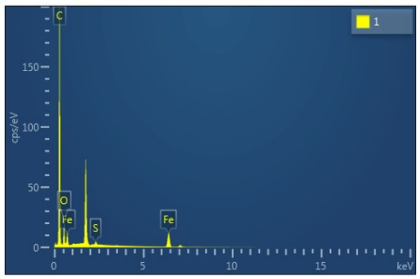


Fig. S5 MALDI-TOF-MS of H1N1-substrate DNA and H1N1-DOTA-Tm.

a

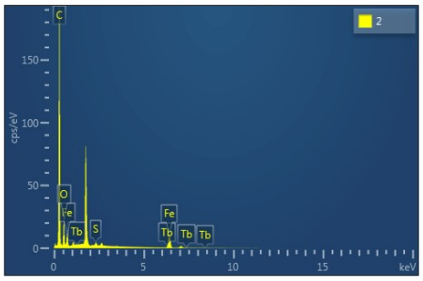


b

Element	Wt %
C	68.76
O	11.41
S	0.58
Fe	19.24
Tb/Ho/Tm	0
Total	100

Fig. S6 EDS spectrum of Bare MBs. (a) EDS spectrum. (b) Weight content distribution of different elements.

a

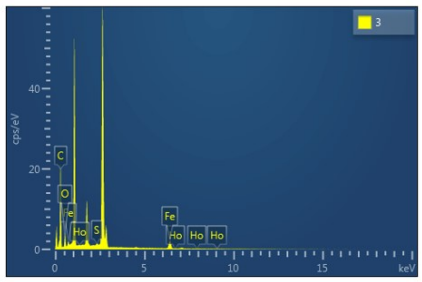


b

Element	Wt %
C	67.70
O	12.83
S	0.54
Fe	18.42
Tb	0.51
Total	100

Fig. S7 EDS spectrum of MBs with DNA-Tb probes attached (MBs-DNA-Tb). (a) EDS spectrum. (b) Weight content distribution of different elements.

a

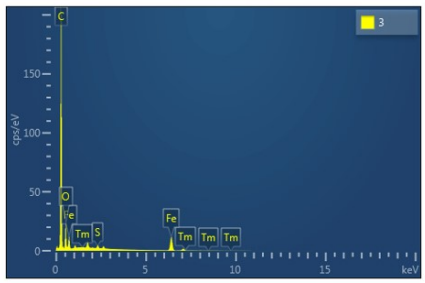


b

Element	Wt %
C	69.53
O	23.78
S	0.48
Fe	6.10
Ho	0.12
Total	100

Fig. S8 EDS spectrum of MBs with DNA-Ho probes attached (MBs-DNA-Ho). (a) EDS spectrum. (b) Weight content distribution of different elements.

a



b

Element	Wt %
C	68.65
O	12.45
S	0.55
Fe	18.24
Tm	0.11
Total	100

Fig. S9 EDS spectrum of MBs with DNA-Tm probes attached (MBs-DNA-Tm). (a) EDS spectrum. (b) Weight content distribution of different elements.

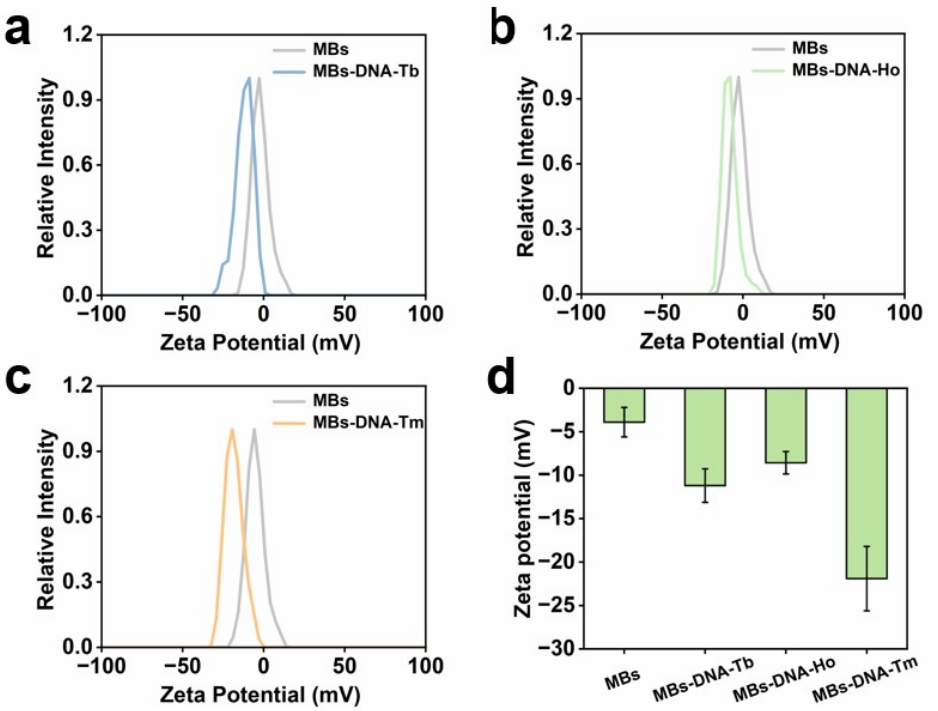


Fig. S10 Zeta potentials of MBs and MBs-DNA-Tb, MBs-DNA-Ho, MBs-DNA-Tm. (a) MBs and MBs-DNA-Tb. (b) MBs and MBs-DNA-Ho. (c) MBs and MBs-DNA-Tm. (d) The results of three Zeta potential experiments.

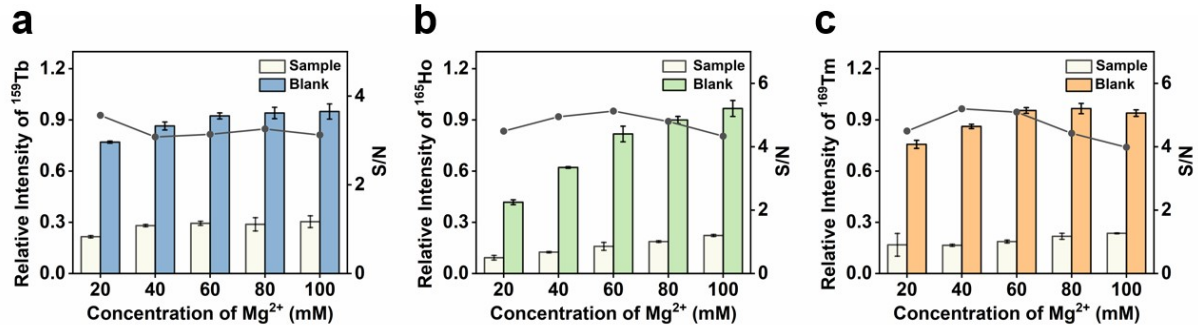


Fig. S11 Effect of the concentration of Mg^{2+} on the ICP-MS relative intensity and S/N value of (a) SARS-CoV-2 cDNA, (b) MP DNA and (c) H1N1 DNA.

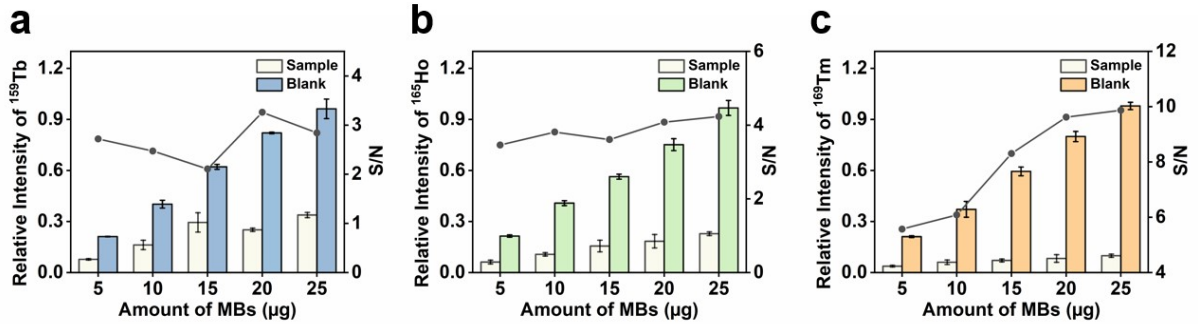


Fig. S12 Effect of the amount of MBs on the ICP-MS relative intensity and S/N value of (a) SARS-CoV-2 cDNA, (b) MP DNA and (c) H1N1 DNA.

Table S3 Analytical performance of the multiple DNA based on the proposed method.

Target DNA	Linear equation	Linear range	LOD	R^2
SARS-CoV-2 cDNA	Y=-13684 lg$X$+32342	10-200 pM	1.4 pM	0.98
MP DNA	Y=-7111 lg$X$+17288	10-200 pM	1.9 pM	0.98
H1N1 DNA	Y=-19370 lg$X$+39321	10-100 pM	1.5 pM	0.99

Table S4 Summary of the assays for detecting multiple respiratory pathogens.

Analysis strategy	Amplified method	Target analysts	Respiratory pathogens	LODs	Ref
ICA		Antigen	FLUA	2.4 pg/mL	1
			SARS-CoV-2	6.2 pg/mL	
SERS-based LFA strips		Antigen	SARS-CoV-2, RSV	8 pg/mL	2
			H1N1	85 copies/mL	
POCT	RT-LAMP	RNA	SARS-CoV-2	35 copies/μL	3
			IAV	100 $TCID_{50}$/mL	
			IBV	1000 $CEID_{50}$/mL	
			RSV	1000 $TCID_{50}$/mL	
Integrated microfluidic chip	PCR	Plasmid	HPIV-1, FLUA, HRV, HADV, FLUB, SP, HCMV, HMPV, MP, CP, LP, RSV-A	10 copies/μL	4
ePAD		DNA	SARS-CoV-2	0.12 pM	5
			H1N1	0.35 pM	
			RSV	0.36 pM	
POCT		Antibody (Ig G)/Antigen	SARS-CoV-2	17/18 ng/mL	6
			Influenza A	30/16 ng/mL	
			Influenza B	280/635 ng/mL	
			Adenovirus	110/33 ng/mL	
			RSV	12/41 ng/mL	

FLUA/IAV: influenza A virus; IBV: influenza B virus; RSV: respiratory syncytial virus; HPIV-1: human parainfluenza virus 1; HRV: human rhinovirus; HADV: human adenovirus; FLUB: influenza B virus; SP: streptococcus pneumoniae; HCMV: human cytomegalovirus; HMPV: human metapneumovirus; MP: Mycoplasma pneumoniae; CP: chlamydia pneumoniae; LP: legionella pneumophila; RSV-A: respiratory syncytial virus-A; Ig G: immunoglobulin type G.

ICA: immunochromatographic assay; SERS: surface-enhanced Raman scattering; LFA: lateral flow immunoassay; RT-LAMP: reverse transcription loop-mediated isothermal amplification; POCT: point-of-care testing; PCR: polymerase chain reaction; ePAD: electrochemical paper-based analytical device.

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