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Supplementary Materials

Detection of coronavirus in environmental surveillance and risk monitoring for pandemic control

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Conflicts of interest

The authors declare they have no actual or potential competing financial interests.

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Table S1. Sampling, pretreatment and analysis methods of novel coronaviruses (SARS-CoV-1, SARS-CoV-2, MERS-CoV) in different environmental matrices.

Sample Type	Virus	Sampling site	Sampling method	Pretreatment method	Practical performances	Reference
Inanimate surface	MERS-CoV	MERS designated hospitals South Korea	1. Position: In MERS patients' wards 2. Time: after disinfection 3. Sampling: · Dacron swab, pre-moistened with viral transport medium	1. Clean-up: · Filtration (0.1-μm pore syringe filter) 2. RNA extraction	1. RT-PCR: · 42/68 positive 2. Cell culture (Vero E6): · 15/68 viability 3. EM: · Intact virions visible 4. Immunofluorescence assay(IFA): · Presence of MERS-CoV	1
Inanimate surface	MERS-CoV	MERS designated hospitals South Korea	1. Position: In MERS patients' wards 2. Sampling: · Dacron swabs, pre-moistened with viral transport medium	1. RNA extraction	1. RT-PCR: · 30/158 positive 2. Cell culture (Vero E6): · 6/158 viability	2
Inanimate surface	SARS-CoV-2	COVID-19 designated hospital Singapore	1. Position: COVID-19 patients' wards 2. Time: before the first cleaning cycle 3. Sampling: · Macrofoam swab, pre-moistened	1. RNA extraction	1. RT-qPCR: · 17/30 room positive (Total of 245 samples collected from 30 wards)	3
Inanimate surface	SARS-CoV-2	COVID-19 designated hospitals USA	1. Position: COVID-19 patients' wards and personal stuffs 2. Sampling: · 3 × 3 sterile gauze pads pre-wetted with PBS · wiping in an “S” pattern in one or two directions.	1. Desorption: · Manually shaking the conical for 1 min 2. RNA extraction	1. RT-PCR: (1) Personal items: 70.6% positive, 0.17-0.22 copies/μL (2) Toilet surface: 81.0% positive, 0.25 copies/μL (3) Room surface: 75.0% positive, 0.22 and 0.26 copies/μL (4) Floor surface: 100% positive, 0.45 copies/μL (5) Ventilation grate: 80% positive, 0.82 copies/μL 2. Cell culture (Vero E6): TEM confirm the presence of intact virions in culture.	4

Inanimate surface	SARS-CoV-2	COVID-19 designated hospital Singapore	1. Position: Inside and outside the wards 2. Time: Before and after daily disinfection 3. Sampling: Sterile pre-moistened swabs	1. RNA extraction	1. RT-qPCR: (1) After routine cleaning: all negative (2) Before routine cleaning: 17/28 positive, 30.64-38.24 Ct	5
Inanimate surface	SARS-CoV-2	COVID-19 dedicated hospital Wuhan, China	1. Position: · COVID-19 patients' wards and functional area 2. Sampling: · Pre-moistened swabs	Not mention	1. RT-qPCR: (1) Intensive care unit: 43/161 positive (2) General ward: 7/146 positive (More quantitative results showed in ref)	6
Inanimate surface	SARS-CoV-2	1. COVID-19 designated hospital and rehabilitation center 2. Apartment houses the Republic of Korea	1. Positon: (1) Hospitals for COVID-19 patients (after disinfection) (2) Rehabilitation center for COVID-19 patients (before disinfection) (3) Apartment houses (before disinfection) 2. Sampling: · Sterile flexible swabs pre-moistened with viral transport medium . Wiping within an area of 700 cm^2 (30 × 25 cm)	1. RNA extraction	1. RT-qPCR: · 2/80 positive (COVID-19 patient's room before disinfection)	7
Inanimate surface	SARS-CoV-2	COVID-19 designated hospital Italy	1. Position: · Emergency unit and the sub-intensive care ward 2. Sampling: · Flexible nasopharyngeal nylon flocked swabs pre-moistened with universal transport medium	Not mention	1. RT-qPCR: · 2/26 positive 2. Cell culture (Vero E6): · No viability confirmed	8
Inanimate surface	SARS-CoV-1	SARS designated hospitals Thailand and Taiwan	1. Position: · Wards and functional area 2. Sampling: · Sterile Dacron or cotton applicator pre-moistened with viral transport media · Wiping within circular area ~5 cm in diameter	1. RNA extraction	1. RT-qPCR: · 26/94 positive 2. Cell culture(Vero E6): · No viability confirmed	9
Inanimate surface	SARS-CoV-2	COVID-19 designated hospital Wuhan, China	1. Position: · Wards, functional room, and public area 2. Time: · After routine cleaning 3. Sampling: · Swabs pre-moistened with saline · Wiping within *c.* 5 cm^2	Not mention	1. RT-PCR: · 2/90 positive (from the inside of a patient's mask)	10
Aerosol	MERS-CoV	MERS designated hospitals South Korea	1. Position: MERS patients' wards 2. Sampling: MD8 airscan sampling device · Sterile gelatin filters (80 mm diameter/3μm pores) · Airflow rate of 50 L/min, 20 min	1. Desorption: · Dissolution of gelatin filter 2. Clean-up: · Filtration: 0.1-μm pore syringe filter 3. RNA extraction	1. RT-PCR: · 4/4 positive 2.Cell culture (Vero E6): · 4/4 viability 3. EM: · Intact virions visible 4. Immunofluorescence assay(IFA): · Presence of MERS-CoV	1

Aerosol	SARS-CoV-2	COVID-19 designated hospital Singapore	1. Position: COVID-19 patients' wards 2. Sampling: (1) NIOSH Sampler · 15 mL tube (>4μm particles), 1.5 mL tube (1-4μm), PTFE filter (<1μm particles, 37mm diameter, 3μm pores) · Airflow rate of 3.5 L/min, 4h (2) Filter cassette · PTFE filter, 37mm diameter, 0.3μm pore size · Airflow rate of 5 L/min, 4h	1. RNA extraction	1. RT-qPCR: (1) 4/10 positive (2) >4 μm: 927 and 2000 RNA copies/m^3 air (3) 1-4 μm: 916 and 1384 RNA copies/m^3 air (4) <1μm: None detected	3
Aerosol	SARS-CoV-2	COVID-19 designated hospitals USA	1. Position: · COVID-19 patients' wards · Hallway space 2. Sampling: (1) Sartorius Airport MD8 air sampler: · Gelatin filter (80 mm) · Airflow rate of 50 L/min, 15 min (2) Personal Button Sampler: · Gelatin filter (25 mm) · Airflow rate of 4 L/min	1. Desorption: · Dissolution of gelatin filter 2. RNA extraction	1. RT-PCR: (1) Wards: 63.2% positive, 2.42 copies/L air (2) Hallway: 58.3% positive, 2.51 copies/L air Personal air sampler: 100% positive, 5.37-67.16 copies/L air 2. Cell culture (Vero E6): · Immunofluorescent staining confirm the presence of viruses in culture.	4
Aerosol	SARS-CoV-2	COVID-19 designated hospital Singapore	1. Position: Inside and outside the wards 2. Time: Before and after daily disinfection 3. Sampling: (1) Filter cassettes: · PTFE filters (37mm,0.3μm) · Airflow rate of 5 L/min, 4 hours (2) Sartorius MD8 microbiological sampler: · Gelatin membrane filter · Airflow rate of 6 m^3/h, 15 minutes	1. RNA extraction	1. RT-qPCR: · All negative	5
Aerosol	SARS-CoV-2	COVID-19 dedicated hospital Wuhan, China	1. Position: COVID-19 patients' wards and functional area 2. Sampling: SASS 2300 Wetted Wall Cyclone Sampler · Airflow rate of 300 L/min, 30min	Not mention	1. RT-qPCR: (1) Intensive care unit: · Patient's room: 8/18 positive, 1.4 copies/L · Air outlet:5/14 positive, 3.8 copies/L · Doctor's office: 1/8 positive, 0.52 copies/L (2) General ward: · Patient's room: 2/16 positive, 0.68 copies/L	6
Aerosol	SARS-CoV-2	1. COVID-19 designated hospitals 2. Public places Wuhan, China	1. Position: · In hospitals (patient area and functional area) · In public area 2. Sampling: (1) Styrene filter cassette sampler(collection of total suspended particles) · Gelatin filter (25 mm diameter) . Airflow rate of 5 L/min (2) Miniature cascade impactor(collection of aerodynamic size-segregated aerosols)	1. Desorption: · Dissolution of gelatin filter (incubated at 37°C for 10 minutes by a block heater） 2. Inactivation: · Addition of 4:1 ratio of TRIzol LS Reagent 3. RNA extraction	1. ddPCR (1) Hospitals: · total suspended particles: 12/19 positive, 1-21 copies/m^3 · aerodynamic size-segregated aerosols: 3/3 positive, 20-42 copies/m^3 · total aerosol deposition sample: 2/2 positive, 31 and 113 copies/m^3 (2) Public places: · total suspended particles: 4/11 positive:3-11	11

			· Gelatin filter (25 mm and 37 mm diameter) · Airflow rate of 9 L/min (3) Filter packed in holder (collection of aerosol deposition sample) · Gelatin filer (80 mm diameter)		copies/m^3	
Aerosol	SARS-CoV-2	COVID-19 designated hospital Iran	1. Position: COVID-19 patients' wards 2. Sampling: Standard midget impinger · Tube containing 20 mL of DMEM · Airflow rate of 1.5 $L \cdot min^{-1}$, 1 h	1. Concentration: · ultracentrifugation 2. RNA extraction	1. RT-qPCR: · 0/10 positive	12
Aerosol	SARS-CoV-2	COVID-19 designated hospital Wuhan, China	1. Position: · Wards, functional room, and public area 2. Time: · After routine cleaning 3. Sampling: Impingement air sampler · 45 mL sampling buffer containing 150 μL positive potential of SLC-SiOH magnetic beads · Airflow rate of 80 L/min, 30 min	1. Concentration: (1) Immobilize and collect the magnetic beads from sampling buffer (2) Resuspend the beads in 200μL phosphate buffered saline pH 7.0 in a 2 mL sterile tube.	1. RT-PCR: · 0/135 positive	10
Aerosol	SARS-CoV-2	Clinic for evaluation of potential COVID-19 patients USA	1. Position: · In clinic 2. Sampling: Viable virus aerosol sampler (VIVAS) . 35 mm Petri dish with liquid collection media . Airflow rate of 6.5 L/min, 1 h	1. RNA extraction	1. RT-qPCR: · 1/2 positive, 0.87 genome equivalents/L 2. Cell culture (Vero E6): · No viability confirmed 3. Sanger sequencing	13
Aerosol (PM10)	SARS-CoV-2	Industrial area which is the epicenter of the Italian COVID-19 epidemic Bergamo, Italy	1. Position: · Ambient area 2. Sampling Low-volume gravimetric air sampler . Quartz fiber filters . Airflow rate of 38.3 L/min, 24 h	RNA extraction	1. RT-PCR: · 20/34 positive	14
Aerosol (PM2.5)	SARS-CoV-2	COVID-19 designated hospital Kuala Lumpur, Malaysia	1. Position: · Wards 2. Sampling · PM2.5 Sensor light scattering technique to measure PM2.5 Low Volume Sampler . Glass microfiber filters (0.6 μm - 0.8 μm particles retention)	1. Desorption: · Immersing filters in sterile RNase-free water and vortex 2. Clean-up: · Collecting supernatants after centrifugation 3. RNA extraction	1. RT-qPCR: · 2/4 positive, 74 ± 117.1 copies/μL, and 10 ± 7.44 copies/μL	15
Water	SARS-CoV-1	Sewage of two assigned hospitals receiving SARS patients. Beijing, China	1. Samples: · Sewage 2. Sampling: Grab samples · Before chlorine disinfection · After chlorine disinfection	1. Neutralize the residual chlorine · Adding 10 mL $Na_2S_2O_3$ (10% w/v) 2. Concentration: · Electropositive filter media particle	1. RT-PCR: (1) All positive in sewage before disinfection (2) Some positive in sewage after disinfection 2. Cell culture (Vero E6): · No viability confirmed · Survivability of SARS-CoV-1 was tested through laboratory experiments.	16

				3. RNA extraction		
Water	SARS-CoV-2	WWTPs and three receptor rivers, Milano Metropolitan Area, Italy	1. Samples: (1) Pre-treated wastewater (2) Treated wastewater (3) Receptor rivers 2. Sampling Grab samples . Separate stainless steel buckets and transported in dark glass bottles	1. Clean-up (1) Filtration of initial water samples through glass fiber filters (Whatman GF/F, 0.7 μm nominal pore size, 47mmdiameter) (2) Filtration of prefiltered water through nitrocellulose Millipore MCE filters (0.22 μm nominal pore size, 47 mm diameter) 2. RNA extraction	1. RT-qPCR: (1) raw wastewater: 4/8 positive (2) treated wastewater: 0/4 positive (3) receptor rivers: 4/6 positive 2. Cell culture (Vero E6): · No viability confirmed 3. Whole genome sequencing	17
Water	SARS-CoV-2	Suburban pumping station and WWTPs, Southeast Queensland, Australia	1. Sampling: Techniques (1) Conventional refrigerated autosampler (2) Submersible in-situ high frequency autosampler (3) Grab sampling technique	Concentration: Method 1: Electronegative membrane (0.45-μm-pore-size, 90-mm-diameter) Method 2: Ultracentrifugation (centrifugal filter with a cut-off of 10 kDa)	1. RT-qPCR: · 2/9 positive, 1.9-12 copies/100 mL water 2. Sequencing: · Sanger and Illumina sequencing platform 3. Infection prevalence estimation	18
Water	SARS-CoV-2	Water Reclamation Facility Bozeman, USA	1. Samples: · Pre-treated wastewater 2. Sampling: (1) Grab samples (2) 24-h composite samples	1. Clean-up · Sequential filtration through 20 μM, 5 μM (Sartorius Biolab Products) and 0.45 μM (Pall Corporation) membrane filters 2. Concentration · Ultrafiltration (100 kDa molecular weight cut-off) 3. RNA extraction	1. RT-qPCR: · Quantitative results showed in ref 2. Sanger sequencing	19
Water	SARS-CoV-2	WWTPs, Israel Different districts, Tel Aviv metropolis.	1. Samples: (1) Sewage from districts (2) Wastewater from WWTPs (3) Effluent water from WWTPs 2. Sampling: 24-h composite samples · Automatic samplers	1. Clean-up · Centrifugation 2. Concentration · PEG driven precipitation Ultrafiltration (molecular weight cutoff of 30 kDa) 3. RNA Extraction	1. RT-qPCR: · Quantitative results showed in ref	20
Water	SARS-CoV-2	WWTPs Valencia, Spain	1. Samples: (1) Pre-treated wastewater (2) Treated wastewater	1. Concentration: · aluminum driven precipitation	1. RT-qPCR: · Quantitative results showed in tables	21

				2. RNA Extraction		
Water	SARS-CoV-2	WWTPs, Milan and Rome, Italy	1. Samples: · Influent wastewater 2. Sampling: 24-h composite samples	1. Inactivation · 30 min treatment at 56 °C 2. Concentration · PEG driven precipitation 3. RNA Extraction	1. Semi-nested RT-PCR and RT-qPCR: · 6/12 positive	22
Water	SARS-CoV-2	WWTPs Netherlands	1. Samples: Pre-treated wastewater 2. Sampling: 24 h Composite sewage samples . Autosampler . Four sampling time points: 3 Weeks before and 1, 2.5, and 4 Weeks after the First COVID-19 Case Was Reported.	1. Clean-up Centrifugation 2. Concentration · Ultrafiltration (centrifugal ultrafilters with a cut-off of 100 kDa) 3. RNA Extraction	1. RT-qPCR: (1) samples collected before COVID-19 reported: 0/6 positive (2) samples collected at 1 week after COVID-19 reported: 4/7 positive (3) samples collected at 2.5 and 4 week after COVID-19 reported: 17/17 positive (Quantitative results showed in ref)	23
Water	SARS-CoV-2	WWTPs Paris	1. Samples: (1) Pre-treated wastewater (2) Treated wastewater	Concentration: · ultracentrifugation	1. RT-qPCR: (1) Pre-treated wastewater:23/23 positive (2) Treated wastewater: 6/8 positive	24
Water	SARS-CoV-2	WWTPs Murcia (low COVID-19 prevelance area), Spain	1. Samples: (1) influent wastewater (2) secondary treated effluent water (3) tertiary treated effluent water 2. Sampling: · Grab samples	1. Concentration · Aluminum driven precipitation 2. RNA Extraction	1. RT-qPCR: (1) Influent 35/42 positive, 5.4 ± 0.2 log10 genomic copies/L (2) Secondary treated effluent water: 2/18 positive, 5.4 log10 genomic copies/L (3) Tertiary treated effluent water: 0/12 positive	25
Water	SARS-CoV-2	WWTPs Barcelona, Spain	1. Samples: · Pre-treated wastewater 2. Sampling: · 24-h composite sample	1. Concentration · PEG 6000 driven precipitation 2. RNA extraction	1. RT-qPCR: · The archival samples pre-collected before the pandemic were also analyzed Quantitative results showed in ref	26
Water	SARS-CoV-2	(1) WWTPs (2) Manholes nearby the pandemic hospitals Istanbul, Turkey	1. Samples: · Pre-treated wastewater 2. Sampling: (1) 24-h composite pre-treated wastewater samples from WWTPs (2) Grab samples from manholes	Method 1: (1) Clean-up: centrifugation (2) Concentration: ultrafiltration (10kDa cutoff) (3) RNA extraction Method 2: (1) Clean-up: centrifugation (2) Concentration: PEG 8000 driven precipitation (3) RNA extraction	1. RT-qPCR: (1) Pre-treated wastewater from WWTPs: 5/7 positive, 2.89E3 and 1.80E4 copies/L (2) Wastewater from manholes: 2/2 positive, 4.49E4 and 9.33E4 copies/L	27
Water	SARS-CoV-2	WWTPs, Massachusetts, USA	1. Samples: · Pre-treated wastewater 2. Sampling: · 24-h composite samples	1. Inactivation · 60℃ for 90 min 2. Clean-up · Filtration (0.2 μm membrane)	1. RT-qPCR · Quantitative results showed in ref 2. Sanger sequencing	28

				3. Concentration · PEG 8000 driven precipitation		
Water	SARS-CoV-1	Sewage of two assigned hospitals receiving SARS patients Beijing, China	1. Samples: · Sewage 2. Sampling: Grab samples · Before chlorine disinfection · After chlorine disinfection	1. Neutralize the residual chlorine · Adding 10 mL $Na_2S_2O_3$ (10% w/v) 2. Concentration (1) Electropositive filter media particle (2) PEG driven precipitation 3. RNA extraction	1. Semi-nested RT-PCR: (1) All positive in sewage before disinfection (2) Some positive in sewage after disinfection 2. Cell culture (Vero E6): · No viability confirmed	29
Water	SARS-CoV-2	1. WWTPs 2. Influent pump stations 3. Interceptor lines Syracuse, and Onondaga County, NY	1. Samples: · Pre-treated wastewater 2. Sampling: · 24-h composite samples	1. Concentration: · Ultracentrifugation 2. RNA extraction	1. RT-qPCR: · Quantitative results showed in ref	30
Water	SARS-CoV-2	1. COVID-19 designated hospitals' wards 2. WWTPs of COVID-19 designated hospitals 3. Pipeline in Seafood market 4. Pipeline around seafood market Wuhan, China	1. Samples: (1) Pre-treated wastewater (2) Disinfected water (3) Pipewater	For PCR analysis 1. Clean-up: · Centrifugation 2. Concentration: · PEG 6000 driven precipitation 3. RNA extraction 4. For SERS analysis: no pretreatment	1. RT-qPCR and SERS: (1) Pre-treated wastewater: 4/8 positive (RT-qPCR), 6/8 positive (SERS) (2) Disinfected water: 0/3 positive (3) Pipewater: 1/6 positive	31
Water	SARS-CoV-2	WWTPs Federal State of North Rhine-Westphalia, Germany	1. Samples: (1) Pre-treated sewage (2) Treated sewage 2. Sampling: · Autosampler · 24-h composite samples	1. Clean-up: · Centrifugation 2. Concentration: · Ultrafiltration (10kDa cutoff) 3. RNA extraction	1. RT-qPCR: (1) Pre-treated wastewater: 1.8 copies/mL(aqueous) and 25 copies/ mL(solid) (2) Treated wastewater: 8.8 copies/mL (aqueous) and 13 copies/mL(solid) (More quantitative results showed in ref) 2. Cell culture (Caco-2 cells): · No viability confirmed 3. Sanger sequencing	32
Sludge	SARS-CoV-2	WWTPs Istanbul, Turkey.	1. Samples: (1) Primary sludge (2) Waste activated sludge 2. Sampling: Grab samples	1. Desorption: · Shaking to desorb virus in solid particles into aqueous phase. 2. Clean-up	1. RT-qPCR · 9/9 positive, 1.17E4 - 4.02E4 copies/L	33

			· Collecting before the sludge were dewatered (Sludge samples contain 98-99 % water)	· Centrifugation and filtration (0.45 µm and 0.2 µm pores) 3. Concentration · PEG 8000 driven precipitation 4. RNA Extraction		
Sludge	SARS-CoV-2	Wastewater treatment Facility New Haven, USA	1. Samples: · Primary sewage sludge 2. Sampling: Grab samples : . Collecting at the outlet of a gravity thickener (Solids content ranging from 2.6% to 5%)	RNA Extraction	1. RT-qPCR: · All positive, 1.7E3 - 4.6E5 copies/mL	34

NIOSH: the National Institute for Occupational Safety and Health, VIVAS: viable virus aerosol sampler, PTFE: polytetrafluoroethylene, WWTP: wastewater treatment plant, PEG: polyethylene glycol, PBS: phosphate buffer saline, DMEM: Dulbecco's modified eagle medium, EM: electron microscopy RT-PCR: reverse transcription-polymerase chain reaction, RT-qPCR: RT-quantitative real-time PCR, ddPCR: droplet digital PCR, SERS: surface enhanced Raman scattering.

Table S2. Techniques used for the detection of coronaviruses.

Method	Detection Principle	Key Feature	Sample	Sensitivity	throughput	Time to result	Reference
Nucleic acid-based analysis							
Direct hybridization							
Dot-blot, or southern blotting and northern blotting	Direct nucleic acids hybridization without amplification	Low sensitivity and demanding operation	Synthetic nucleic acids	1-10 pg/reaction	Several samples	Several hours to days	35
Dual-functional plasmonic biosensor		Easy, quick and high sensitivity, but need further optimization for clinical sample detection	Synthetic SRAS-CoV-2 RNA	0.22 pM/reaction	One sample	Several minutes to hours	36
Colorimetric assay based on gold nanoparticles (AuNPs)				0.18 ng/μL of RNA	One sample	10 min	37
Gene Microarray	Binding assays against hundreds of DNA	High throughput, accuracy and multitarget detection	Clinical samples (blood, sputum, plasma)	1-10 copies/reaction	Hundreds of samples	Several hours	38
PCR-based amplification before detection							
PCR-based	Nucleic acids amplification under thermal cycling	High sensitivity, requiring well-trained technician and lacking of quantitative and real-time results	SARS clinical samples (sputum, pharyngeal swab)	1-10 copies/reaction	Several samples	1-5 days	39
RT-qPCR	Nucleic acids amplification process in “real-time” under thermal cycling quantitatively	High sensitivity, offering real-time results, requiring expensive facilities and well-trained technician	SARS and SRAS-CoV-2 Clinical samples (sputum, pharyngeal swab)	1-10 copies/reaction	96 samples	3-4 hours	40
ddPCR	Nucleic acids amplification process in “real-time” under thermal cycling with absolute quantification	High sensitivity and immune to background, but dependent on thermal cycling	Mostly synthetic nucleic acids	1-10 copies/reaction	96 samples	Several hours	41
Traditional Isothermal amplification-based							
RCA	Nucleic acids amplification under constant temperature	High sensitivity, amplification at a single temperature and no need for expensive instruments, but need further optimization for clinical sample detection	Synthetic nucleic acids	10 copies/μL	Several samples	Several hours	42
NASBA							43
LAMP							44
RPA							45
CRISPR-based Isothermal amplification-based							
SHERLOCK	Collateral activity of CRISPR protein	High sensitivity and specificity, low expense, no need for thermal cycling and	Synthetic SARS-CoV-2 RNA	10 copies/μL	One sample	One to several hours	46
DETECTR				10 copies/μL			47

HOLMES		expensive equipment, but need further optimization for clinical sample detection	Synthetic nucleic acids	Attomolar			48
CRISPR-Chip	Specifically, binding and cleaving dsDNA of Cas9			1.7 fM			49
CRISDA	Cas9 mediated isothermal amplification			Attomolar			50
Next generation sequencing							
NGS (nanopore sequencing, Helicos sequencing and real-time single-molecule sequencing with polymerase)	de novo sequencing of a whole genome	NGS can provide a variety of fundamental information regarding viral nucleic acid sequences such as the origin, revolution, mutations, etc. but high expense of equipment and regents, requiring tedious sample preparation and lengthy turnaround time	Clinical samples (sputum, pharyngeal swab)	1-10 copies/reaction	NA	Several days	51
Protein-based analysis							
Antigen or Antibody Immunoassay							
RIA	Antigens binding to antibodies with high specificity and affinity	A highly sensitive method but the main drawback is the handling and disposal of hazardous radioactive substances	Recombinant proteins	10 pg/mL to 10 ng/mL	96 samples	Nearly 15 min	52
EIA (ELISA, FPIA, MEIA, CLIA)		Inapplicable to early-stage detection, but timeless, easy and quick operation	Clinical samples (blood, sputum or plasma)		96 samples		53
LFA					One sample		54
Mass spectrometry							
MALDI-TOF MS	Mass spectrometry technology combined with machine learning algorithms as an alternative fast tool for SARS-CoV-2 detection	A high-throughput and high-content analysis strategy aiming at the any biomolecules in a sample	nasopharyngeal swabs samples	NR	Hundreds of samples	NR	55
Virion detection							
EM	Observing virus particles directly	Demanding sample preparation and high cost, but meaningful in virology research	SARS and SRAS-CoV-2 infected cells or tissues	10^5 to10^6 virus particles per mL	One sample	Several hours	56
IEM				10^3 to10^4 virus particles per mL			56
Cyro-EM				NR			57
AFM				NR			58

COVID-19 FET sensor	Detecting the change of electrical response signal derived by the conjunction of antibody and target SARS-CoV-2 spike protein	A highly sensitive and requiring no sample pretreatment or labeling	Nasopharyngeal swabs	242 copies/mL	One sample	Minutes	59
QCM	The mass variation on the quartz crystal sensor will induce the change of frequency of oscillation	The label-free and real-time detection with sensitivity up to ng level	Oral swab samples	NR	One sample	Minutes	60
Flow cytometry technique	Identify and analyze virion based on fluorescence signal and particle size	Careful and technical preparation process including fixing and labeling of virus particles, which may bring barrier to the onsite detection	NR	NR	85-90 samples	180 min	61
Viability and infectivity evaluation							
Cell culture	Virus growth in cultured cells	Some viruses hard or impossible to cultivate，time-consuming, technically very demanding and required well-controlled laboratory environment, but meaningful in virology research	Isolated from SRAS-CoV-2 clinical samples such as respiratory and stool samples	NR	Several samples	Several days	1, 16, 62
Integrated Methods							
Microfluidics (a portable microfluidic immunoassay system)	Combining advanced biomarker detection methods with microfluidics (able to precisely manipulate and control the movements of tiny volume of fluids)	High sensitivity and specifically for easy-to-use, sensitive, rapid, multiple, and onsite detection of IgG/IgM/antigen of SARS-CoV-2 simultaneously	Clinical human serum and pharyngeal swabs	NR	One sample	15 min	63
Lab-on-a-chip (SPR-based biosensor)	The diagnosis of SARS using SCVme anchored onto a gold substrate	Integrated system without the need for any external equipment or human intervention	The fusion proteins	906 RU for anti-SCVme	One sample	10 min	64

PCR: polymerase chain reaction, RT-qPCR: reverse transcription quantitative real-time PCR, ddPCR: digital droplet PCR, RCA: Rolling-circle amplification, NASBA: nucleic acid sequence-based amplification, LAMP: loop-mediated isothermal amplification, RPA: recombinase Polymerase Amplification, SHERLOCK: specific high-sensitivity enzymatic reporter unlocking, DETECR: DNA Endonuclease-Targeted CRISPR Trans Reporter, HOLMES: One-Hour-Low cost Multipurpose highly Efficient System, CRISDA: CRISPR–Cas9-triggered nicking endonuclease-mediated Strand Displacement Amplification method, NGS: next-generation sequencing, RIA: radioimmunoassay, EIA: enzyme immunoassay, ELISA: enzyme linked immunosorbent assay, FPIA: fluorescence polarization immunoassay, MEIA: micropartical enzyme immunoassay, CLIA: chemiluminescent immunoassay, LFA: lateral flow assay, MALDI-TOF MS: matrix-assisted laser desorption/ionization-time of flight mass spectrometry, EM: Electron Microscopy, IEM: Immune electron microscopy, Cyro-EM: Cryo-electron microscopy, AFM: Atomic force microscopy, COVID-19 FET sensor, QCM: quartz crystal microbalance, SPR-based biosensor: surface plasmon resonance-based biosensor, SCVme: SARS coronaviral surface antigen, NR: not report.

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