Supplementary file 1

COVID-19: An overview on possible transmission ways, sampling matrices and diagnosis

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Table S1. Various methods for virus diagnosis along with some explanation about them

Test type	Sample Source	Detected analytes	Sensitivity	Advantages	Disadvantages	Ref
RT-PCR combined with a commercial kit						
TaqMan probe-based real-time PCR	Nasopharyngeal and/or oropharyngeal swab	Mainly targeted on viral genes like ORF, N, E, S, RdRp and Rnase P (human)	Most accurate sensitivity and specificity- the sensitivity of the test (66–83%). 0.001 parasite per reaction and it can get higher due to the target utilized (kinetoplasts) and the employing the TaqMan probes	The gold standard for Covid-19 testing is known for its time-consuming process and is best suited for large, centralized diagnostic labs. It has the ability to detect viruses from the beta-coronavirus group (specifically the E gene), as well as identify the SARS-CoV-2 virus. This method eliminates the need for post-	It is not possible to detect past infections using this method, and there is a chance of up to 67% false negative results due to the absence of SARS-CoV-2 in the oropharyngeal area. False-positive results are also possible due to swab contamination. This testing requires a laboratory setting, dedicated equipment, extensive human labor, and timing the detection of antibodies. The presence of	74-77

				amplification handling and makes automation and processing of large sample sizes easier. It also boasts an impressive dynamic range in template determination of up to six orders of magnitude.	alcohol near the testing device can interfere with sensors, leading to inconclusive results. This protocol is complex and expensive, with tests typically taking 4-6 hours to complete. However, logistical requirements for sending clinical specimens mean that execution time must not exceed 24 hours. In contrast, a standard RT-qPCR test takes an average of 90-120 minutes to test a set of samples.	
BioMerieux nucliSENS Easy MGA	Nasopharyngeal swab, nasal swab,mid-terminate nasal swab, oropharyngeal swab, bronchoalveolar lavage,lower respiratory tract aspirates, sputum	Nucleic acid	-	-	-	[78, 79]
ThermiFisher Scientific TaqPath Covid-19 combo kit	Nasopharyngeal,nasal swab, mid-terminate nasal swab, oropharyngeal swab, bronchoalveolar lavage	Orflab,N gene, and S gene	250 GCE/ml for the Geotek or 100 collector and 375 GCE/ml for the copane swab	-	-	[80]
ThermiFisher TaqMan 2019 .nCOV assay kit v1 (singleplex) combo kit	Nasopharyngeal,nasal swab, mid-terminate nasal swab, oropharyngeal swab	Orflab, N gene, and S gene	0.625 copies/µL	-	-	[81]
UTHSC/UCH SARS-CoV-2 RT-PCR assay	Nasal swab	Nucleic acid	100 PFUs	-	-	[7]

Dd-PCR Promega Wizard® Genomic kits	DNA from a variety of tissues and organisms, including peripheral blood, skin, hair, saliva, and microbes.	Genomic material	The accuracy rates for spleen aspirates range from 95% to 98%, while liver and bone marrow aspirates have accuracy rates of 76% to 91% and 52% to 89%, respectively. Lymph node aspirates have accuracy rates ranging from 52% to 69%.	Faster Results. Each BioFire Panel returns results in about an hour. Shorter time to optimal therapy. Improve Treatment Decisions. Avoid unnecessary antibiotics. Support antimicrobial stewardship efforts. Reduce unnecessary testing. Reduce healthcare costs	There is a chance of not detecting individuals who have eradicated the virus and recuperated from the ailment. The dispersion of the virus in the respiratory tract differs from one patient to another; hence, despite being infected, the virus may only be noticeable in sputum or nasopharyngeal swabs but not in both locations simultaneously. Additionally, it only indicates whether a person is currently infected with this particular coronavirus and cannot offer information on other diseases or symptoms.	82-84
RT-LAMP combined with a commercial kit						
RT-LAMP (Isothermal amplification) 1) SHERLOCK METHOD: uses Cas 13 a ribonuclease 2) DETECTOE METHOD: Uses Cas 12 a ribonuclease	Nasopharyngeal swabs, nasal swabs, and saliva, sputum, stool	SARS-CoV-2 genes, including OFF 1ab, S, E, and/or N gene,	The positive agreement of its specificity is exceptionally high, reaching 100% for samples with Ct <30 and 69-91% for those with Ct <40, when compared to RT-LAMP carried out with the isothermal fluorimeter.	Less time consumption for testing (may be completed in 30 min)	LAMP technology is newer when compared to RT-PCR, and due to the complexity of building these tests, it is more challenging. However, these tests can leave room for possible omissions of patients who have already recovered from the disease and cleared the virus, as the detection in the respiratory tract could vary between patients. It is plausible that even when an individual is infected, the virus may only be noticeable in either sputum or nasopharyngeal swabs, but not	85-89

					necessarily in both places at once. These tests can only indicate if a person is presently infected with the specific coronavirus and will not provide information regarding other diseases or indications of prior infection or immunity.	
RT-LAMP Primer and DNA fragment	Throat swab	Orf1ab, S and N gene	80 Copies viral RNA mL^{-1}	-	-	[90]
RT-LAMP primer	Nasal swab	Nuceocapsid gene	10^6 RNA copies	-	-	[91]
Viral RNA extracted using QIAamp Viral RNA Mini Kit	Swab/Bronchoalveolar Lavage fluid	Orf1ab and S genes	2×10^1 copies and 2×10^2copies / reaction with primer sets orf1ab and S gene	-	-	[92]
LAMP primers	Saliva	Orf1ab and n genes	$\sim10^2$ Viral genome/ reaction	-	-	[93, 94]
Immunoassay						
Lateral flow immunoassay	Whole human blood, blood plasma, serum, stool, urine, sweat, cerebrospinal fluid, or even tears	Three surface proteins of SARS-CoV 2, *e.g.,* spike, envelope, and membrane	Lowest detection limit utilizing the standard format, 3×10^{-11} mol/L	This test is rapid (about 15-20 min), easy to use. Various samples can be used, and the required amount of samples is small.	The low sensitivity of this method causes false negative results. There is a high probability that the operator will be infected.	[95-99]
Immunoenzymatic and immunofluorimetric assays	Blood	IgM and IgG	-	This test is rapid, cost-effective, and reasonably sensitive.	The delay in antibody increase is the reason behind the high incidence of false negative outcomes.	[97]
Protein microarray method	Serum	ORF1ab protein, surface glycoprotein, ORF3a protein, coat protein, membrane	-	This test is rapid, Low consumption in the sample, automatic, highly sensitive and can be performed in large-scales.	False negative results	[97]

		glycoprotein, ORF6 protein, ORF7a protein, ORF8 protein, nucleocapsid phosphoprotein, and ORF10 protein				
Antibody assay	serum/plasma/blood	Antibodies against virus	According to the team's findings, the specificity of the negative samples tested was 100%, indicating that all of them were correctly identified as negative. The overall sensitivity was 83.87%, which increased to 87.0% when tested 14 days after the onset of symptoms, 87.7% after 21 days, and 100% after 40 or more days.	IgM indicates the 3-5 days post onset IgG indicates the past infection and also gives the information for vaccination	Inadequate levels of antibodies or poor sensitivity are leading causes of false negatives in antibody testing. The former is mainly affected by assay format, antigens targeted, the quality of test antibodies, and the isotypes of antibodies that are being detected.	70, 100, 101
Enzyme-linked immunosorbent assay (ELISA)	Serum	IgG Antibodies	With an accuracy of 98.3%	ELISA is a straightforward and uncomplicated technique with high specificity and sensitivity levels. It works on the principle of antigen-antibody interaction and is highly efficient. Additionally, ELISA allows simultaneous testing without complex sample pre-treatment and is considered safe and	Preparing antibodies is a laborious and costly process that necessitates sophisticated techniques and expensive culture media. False positive/negative results are highly possible due to inadequate blocking of immobilized antigens. Antibody instability is another issue, and since antibodies are proteins, they necessitate refrigerated transport and storage.	102, 103

				environmentally friendly since it eliminates the need for radioactive substances or large amounts of organic solvents. Moreover, this cost-effective assay is relatively inexpensive, as reagents are available at lower costs.		
Colorimetric and spectroscopic methods						
Colorimetry	Blood/Serum/Plasma	Pattern of VOC concentration	With a single-stage process that eliminates the need for separate RNA extraction, the method can detect as few as 80 copies of viral RNA per 1 mL in the sample.	The test allows visual interpretation of amplified viral RNA results, eliminating the need for costly equipment. Furthermore, the test is non-intrusive and provides prompt results. The sensor response is unique to the targeted analyte, and it's feasible to avoid interactions with other VOCs.	Taking measurements can become challenging on certain surfaces that reflect light. The method cannot operate in UV and IR regions, nor is it compatible with colorless compounds. Additionally, a range of parameters is set rather than a specific wavelength.	[104]
Cavity-enhanced absorption spectroscopy (CEAS)	Exhaled breath	Nitric Oxide, Carbonyl Sulphide, and Ethane	-	The technique offers excellent spectral resolution and a favorable signal-to-noise ratio, while the equipment setup is uncomplicated, cost-efficient, and useful for conducting field measurements. The approach accommodates	The reflective capacity of the cavity mirrors dictates the range of spectral coverage, which is the only constraint. However, to achieve broad detection, conventional spectrographic instrumentation is typically utilized, which can limit the resolution as determined by the spectrometer system.	[34, 105]

				very long path lengths up to several hundred meters, which can be achieved with relatively compact absorption cells. A noteworthy advantage of incoherent broadband CEAS (IBBCEAS) is that it can operate with broadband sources like Xenon lamps, LDLS, and Synchrotron radiation, which cover a broad range from under 200 nm to above 10 mm, allowing detection of multiple species during a single measurement.		
Photoacoustic spectroscopy (PAS)	EB	Pattern of VOC concentration	-	A key advantage of the photoacoustic effect is that the level of sensitivity is not impacted by the optical path distance. This allows for a linear concentration response across a wide dynamic measurement range and high sensitivity with a short absorption path. Furthermore, this can be achieved even with small quantities of samples.	-	[89]
Laser spectroscopy	EB	Pattern of VOC concentration	-	In comparison to traditional light sources,	Obtaining appropriate standards for semi-quantitative analysis is	[106, 107]

				lasers offer numerous advantages, such as high power output, a monochromatic emission profile, stability, and quick tuning.	a challenging task. Additionally, there are significant interference effects to contend with, such as matrix interference and potential interference from particle size in the case of laser-induced breakdown spectroscopy in aerosols. Furthermore, the technique's detection limits are typically not as efficient as traditional solution-based methods.	
Laser-induced fluorescence (LIF)	EB	Pattern of VOC concentration	ppb/ppt	LIF is a useful technique for the analysis and detection of various molecular species, as it can provide valuable insights into their electronic structures and identities. Its wide detection range allows for the identification of a diverse range of molecular species. Additionally, LIF only requires a small sample for highly localized measurements, making it possible to measure spatial and temporal variations along tiny dimensions. Furthermore, its ability to detect weak signals even down to a single photon is	One of the primary challenges associated with LIF is distinguishing the fluorescence signal from the Rayleigh scattering of air molecules caused by the exciting radiation.	[108, 109]

				facilitated by its lack of significant background noise.		
Tunable diode laser absorption spectroscopy	EB	Pattern of VOC concentration	-	Laser absorption spectroscopy offers several advantages, including fast response times, real-time measurement capabilities, and simplicity, making it a cost-effective option for online measurement in industrial environments. Additionally, it has high sensitivity through selective detection of gas absorption lines, allowing for the measurement of concentrations at or below desired limits. It is also immune to interference from other gases, laser pulsation, and particles. The measurement results provide an average value of the path, which effectively captures the overall situation of the environment being tested, and can also measure gas temperature simultaneously, thereby providing a wealth of information beyond gas	The technique is based on detecting a minor fluctuation in a signal that is superimposed on a significant background.	[110]

				concentration measurement.		
PAS	EB	Pattern of VOC concentration	Compared to conventional optical-based spectroscopy, PAS exhibits greater sensitivity, capable of identifying absorption coefficients down to $10{-}7\ cm^{-1}$. Furthermore, it is less susceptible to the optical scattering effect of tissues.	The sensitivity of the measurement remains unaffected by the length of the optical path. Thus, a short absorption path length yields high sensitivity. The concentration response is highly linear and can measure small gas volumes over a broad dynamic range of low sample volumes. This direct measurement of absorption is one of the most effective techniques for detecting trace gases. PAS is less expensive than other gas analysis methods, and the microphones exhibit stable response, requiring infrequent calibration due to low drift.	The primary limitation of PAS is the background signal produced by the absorption of the incoming beam by the cell walls and window. Yet, the use of optically transparent windows and polished cell walls can mitigate this problem by reducing their absorbance.	[111]
Quartz-enhanced photo-acoustic spectroscopy (QEPAS)	EB	Pattern of VOC concentration	-	QEPAS is small in size, portable, anti-interference, and low power in consumption.	-	[49, 112]

Optical frequency comb spectroscopy (OFCS)	EB	Pattern of VOC concentration	-	OF-CEAS offers excellent spectral resolution and signal-to-noise ratio, resulting in a high level of sensitivity.	-	[49, 108]
Integrated cavity output spectroscopy (ICOS)	EB	Pattern of VOC concentration	-	It requires a relatively simple set-up. a single breath is needed to measure multiple points.	-	[49, 113, 114]
Raman spectroscopy	Serum	The Raman spectrum exhibits distinct peaks that correspond to particular carbohydrates present in the viral glycoprotein.	-	High sensitivity of this method in symptomatic and asymptomatic people	The existence of different and complex biochemical components makes it difficult to interpret the spectrum obtained from the sample. Various factors such as insufficient viral material in the sample and low viral load of the patient can cause false negative results.	[115]
Nanoparticle based optical sensors						
Spectrophotometry using thiolated modified ASO AuNPs	Serum	N-gene	0.18 ng μL^{-1}	-	-	[116-118]
Spectrophotometry using gold nanoparticles conjugated with Covid-19 antigen	Blood/serum/plasma	IgM/IgG Ab	With an accuracy of 88.66%	-	-	[119]

Spectrophotometry using Mouse anti-human IgG antibody labeled lanthanide-doped polystyrene NPs	Serum	Anti-SARS-CoV-2 IgG	Sensitivity (88.66%) and specificity (90.63%)	-	-	[119]
Spectrofluorimetry using Lanthanide Eu(III) fluorescent microsphere	Serum	IgM/IgG	With an accuracy of 98.72%	-	-	[120]
Spectrophotometry using anti-human IgM conjugated colloidal gold nanoparticles	Serum	IgM Ab	With an accuracy of 93.3% and 100%	-	-	[121]
Nanomaterial-based sensor Array Photonic crystals	EB	Viral nucleic acids (DNA and RNA), viral proteins, and antibodies, produced by the infected individual's immune system to fight the virus	-	Efficient screening of a large population within a short duration enables active searching of potential cases in the community, thereby facilitating early identification of the disease in asymptomatic contagious individuals.	-	[78]
Other methods						
Electronic nose	Exhaled breath	Patern of VOC concentration	-	This test is non-invasive and rapid.	The results of this test can be affected by the environment including temperature and humidity.	[122, 123]

Aeonose	Exhaled breath	VOCs	With an accuracy of 92%	The triage test is advantageous due to its quick, affordable, and non-invasive nature. Results are obtained promptly owing to real-time analysis, and the device is user-friendly, eliminating the need for specialized personnel. Additionally, it boasts a relatively low dropout rate of about 3%.	The performance of the sensor deteriorates when water vapor or high concentrations of certain components, such as alcohol, are present, which leads to reduced sensitivity. Additionally, the sensor is limited by its tendency to drift, inability to provide absolute calibration, and the relatively short lifespan of some of its sensors.	123-126
Nanowires (Laser ablation, chemical vapor, thermal evaporation and alternating current electrodeposition)	EBC	Covid-19 spike protein antibody	-	Compared to their thin film counterparts, they prove to be more efficient as bioelectrochemical transducers. This can be attributed to their sizable surface-to-volume ratio and unidirectional conduction channels, which are remarkably receptive to the slightest disturbances on their surface during binding events.	-	127, 128
Metal oxide semiconductors -based sensors	Saliva	ORF8 and E2 surface glycoproteins of SARS-CoV-2	Highly sensitive	This method is rapid, reusable, cheap and highly selective	Operating at temperatures exceeding 400 •C, there is a notable ineffectiveness in detecting gases present in low concentrations, as well as challenges encountered when attempting to determine grain	129-132

					size limit values. The latter issue has a notable impact on the processes of physisorption and chemisorption.	
Quartz crystal microbalance	Oral swab	Spike glycoprotein of SARS-COV-2	Less than 1 ng/cm^2	The method proves useful for directly measuring biologically active molecules, without requiring labeling or the use of extra chemicals. As a result, it has a relatively brief detection time, typically less than an hour.	-	[133]
Solid state biosensors	Nose swabs, throat swab, saliva sputum, and blood	S1 spike protein antigens of the virus	The method boasts high sensitivity levels, clocking in at less than one part per billion. Additionally, it has a high electrical conductivity, and the carrier mobility is theoretically around 100,000 cm^2/V.	The low cost, biocompatibility, substantial mechanical strength, and exceptional sensitivity are among their notable features.	False positives are a concern with this method, as it may result from low selectivity or intricate and costly fabrication procedures.	[134, 135]
Quasi-free-standing epitaxial-graphene based biosensors (QFS EG)	Mid-turbinate swabs and exhaled breath aerosol samples	S1 spike protein antigen of the virus	With an exceptional signal-to-noise ratio of 49.1 dB (on average), the ultra-high sensitivity of this method significantly outperforms others. It is capable of detecting the spike protein antigen in mid-turbinate swabs and aerosols from Covid-19 patients at concentrations as low as 1 ag/mL and 60 copies/mL, respectively, and does so in just 0.6 seconds. Interestingly, the detection limit is comparable to	This method ensures rapid detection due to an enhanced thickness uniformity, reduced phonon-carrier scattering, and higher mobility through hydrogen intercalation, making it a more efficient alternative to conventional EG. Additionally, it is available in a portable and user-friendly form, with straightforward device	The observed response of these sensors cannot be fully explained by a simple charge transfer mechanism from the antibody-antigen binding to the underlying QFS EG, especially within the atto-gram range.	[98, 136, 137]

			the molecular weight of a single spike protein.	fabrication, thus eliminating the need for signal amplification or the integration of a gate, unlike other graphene-based biosensors. The use of polyclonal antibodies in the heterostructure with EG can result in higher signal levels than monoclonal antibodies because polyclonal antibodies can recognize different epitopes on the same protein antigen. Furthermore, this approach is more sensitive in detecting the binding between antigen and antibodies, as revealed by the voltage change, than traditional methods.		
One-dimensional photonic crystals	Exhaled breath	Patern of VOC concentration	The sensor resolution and detection accuracy were capable of registering readings of 7 parts-per-million (ppm), 0.36 nanometers per square (nm^{-1}), and 2 nanometers.	Self-assembly has the benefit of creating structures with thousands of periodic layers, while photonic crystals offer the advantage of visibility even under bright sunlight. Additionally, these materials have a soft texture, which allows them to be deformed with minimal mechanical force,	-	138-140

				effectively reducing the layer thickness without altering the volume.		
Chest computed tomography (CT)	X-rays absorbed by different parts of the lung	-	a chest CT pooled sensitivity of 94.6% (95% CI: 91.9%, 96.4%)	CT scanning is a quick, painless, and non-intrusive procedure with a high level of accuracy. It excels at detecting small nodules in the lungs, making it particularly effective for early-stage detection of lung cancer, when odds of successful treatment are highest.	Expensive and time consuming-, require trained personnel to expose patients to X-rays.	139, 141-143
Field effect transistor detector	Nasopharyngeal swab	The genetic material of the SARS-CoV-2 virus	After 35 minutes of amplification, it has demonstrated the capability to identify positive and negative clinical samples and detect the presence of the spiked SARS-CoV-2 virus, ranging from 10 to 104 copies/μL.	The ability to rapidly detect small biomolecule amounts in biological fluids is essential for early disease discovery. This type of sensing technology boasts rapid electrical detection, allowing for a system-on-a-chip device integration that is portable and includes both the sensing component and the read-out system. Furthermore, it allows for multiplexing, meaning it can identify multiple biomolecules simultaneously.	It is expensive and special handling is required during installation.	144-147

Gas chromatography - mass spectrometry	Exhaled breath	Gases or small organic molecules like alcohols, fatty acids, aldehydes, esters, steroids, etc	-	High sensitivity and universal applicability	It is expensive, bulky and requires experienced personnel for operation. The probability of sample contamination is high in one of the stages of sample collection, storage and processing. Uncontrollable factors like environmental contamination can affect the results.	[49]
Clustered regularly interspaced short palindromic repeats (CRISPR) + Isotachophoresis (ITP)	Nasopharyngeal swabs, Oropharyngeal swabs, Saliva, Sputum, Urine, Stool	Nucleic acid (RNA) of virus	With a 93.8% detection rate of positive samples (30 out of 32) and a 100% identification rate of negative samples (32 out of 32), ITP-CRISPR showcased high sensitivity and specificity.	With the capability to detect as low as 10 virus gene copies in only 45 minutes, the process is both uncomplicated and dependable.	Off-target effects may disrupt gene function and lead to genomic instability, thereby impeding its potential usage and application in clinical procedures.	[148-151]