

■ Role of Breath Biopsy in COVID-19

Jyoti Kanwar Shekhawat ^a and Mithu Banerjee^{b,*}

Background: COVID-19 is a highly contagious respiratory disease that can be transmitted through human exhaled breath. It has caused immense loss and has challenged the healthcare sector. It has affected the economy of countries and thereby affected numerous sectors. Analysis of human breath samples is an attractive strategy for rapid diagnosis of COVID-19 by monitoring breath biomarkers.

Content: Breath collection is a noninvasive process. Various technologies are employed for detection of breath biomarkers like mass spectrometry, biosensors, artificial learning, and machine learning. These tools have low turnaround time, robustness, and provide onsite results. Also, MS-based approaches are promising tools with high speed, specificity, sensitivity, reproducibility, and broader coverage, as well as its coupling with various chromatographic separation techniques providing better clinical and biochemical understanding of COVID-19 using breath samples.

Summary: Herein, we have tried to review the MS-based approaches as well as other techniques used for the analysis of breath samples for COVID-19 diagnosis. We have also highlighted the different breath analyzers being developed for COVID-19 detection.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected every continent around the globe with a high rate of transmission and mortality among patients. Through human exhaled breath, coronavirus disease (COVID-19) is transmitted from person to person when an infected person exhales, coughs, or sneezes. The infection may lead to a cytokine storm causing acute respiratory distress syndrome (ARDS) and multi-organ failure culminating in death. (1). Presently, to detect SARS-CoV-2, infection specimens are collected from the upper (nasopharyngeal swab [NPS] and oropharyngeal swabs) or lower respiratory tract (induced sputum, endotracheal aspirate,

and bronchoalveolar lavage). The gold standard for diagnosing COVID-19 is RT-PCR (reverse transcription-polymerase chain reaction). Various methods being employed for COVID-19 diagnosis are summarized in Table 1. RT-PCR is a laborious, time-consuming method and, theoretically, it is dependent on a single molecule, a nucleic acid. Moreover, there is an exponential amplification of nucleic acid that makes PCR a powerful tool for the identification of specific nucleic acid. Although the PCR technique is sensitive and effective for the diagnosis of COVID-19, there are many limitations associated with it, including sampling quality, sample pretreatment, and the turnaround time (TAT). The false-negative rate for SARS-CoV-2 RT-PCR remains highly variable up to 67% within

^aDepartment of Biochemistry, All India Institute of Medical Sciences, Jodhpur-342005, Rajasthan, India; ^bDepartment of Biochemistry, All India Institute of Medical Sciences, AIIMS, Road, MI Phase-2, Basni, Jodhpur, Rajasthan 342005, India.

*Address correspondence to this author at: AIIMS, Road, MI Phase-2, Basni, Jodhpur, Rajasthan, India—342005. E-mail: mithu.banerjee.3@gmail.com. Received February 15, 2022; accepted April 21, 2022; published online June 20, 2022.

<https://doi.org/10.1093/jalm/jfac040>

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IMPACT STATEMENT

Diagnosis of COVID-19 patients using standard processes takes a long time. Analysis of breath using advanced technologies like mass spectrometry, biosensors, and gas chromatography will help in rapid diagnosis of these patients and their treatment. This will also help in decreasing the spread of COVID-19 infection.

the first 5 days of exposure (2). This has increased the urge to develop new diagnostic methods for COVID-19. Researchers have expanded their horizons to improve the diagnostic accuracy for COVID-19 detection by considering other clinical samples such as blood, urine, saliva, feces, and breath for screening of virus or virus-specific metabolites.

Human exhaled breath comprises a gaseous phase and a liquid phase. Breath contains water, volatile organic compounds (VOCs), and droplets that are composed of non-volatile metabolites, salts, proteins, and microorganisms such as viral and bacterial particles. Exhaled breath aerosols (EBAs) and exhaled breath condensate (EBC) are a potential source of SARS-CoV-2 as they can be suspended in contaminated air and cause infection by respiratory action (3). Inorganic and organic compounds detected in EBC include nitrite, nitrate, arachidonic acid metabolites, leukotrienes, prostanoids, cytokines, glutathione, proteins, and metabolites. (4). Breath analysis is a noninvasive technique, allowing the detection of markers present in it. It has been studied for the diagnosis of chronic airway diseases, such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease (COPD) (5). Leung et al. reported the presence of the SARS-CoV-2 virus in exhaled breath and cough of patients with respiratory illness. Additionally, they found significant decrease in the presence of SARS-CoV-2 in breath aerosol (6). Metabolomic fingerprinting of EBC samples provides information on more than one analyte

related to pulmonary diseases using mass spectrometry (MS) and LC-MS (7, 8). Fumagalli et al. performed proteomic analysis of pooled EBC samples using LC-MS from non-smokers and healthy subjects (n = 45), COPD without emphysema (n = 15) and pulmonary emphysema associated with α 1-antitrypsin deficiency patients (n = 23) and identified 44 unique proteins. Another study conducted using scent dogs to discriminate between COVID-19 positive and negative showed average diagnostic sensitivity and specificity of 82.63% and 96.35% respectively, in respiratory secretions containing samples (saliva and tracheobronchial samples) (9). Ryan et al. performed RT-PCR for S/E/N/ORF1ab genes of SARS-CoV-2 on EBC samples collected from 40 patients, out of which 16 were NPS positive, 15 were NPS negative but clinically positive for COVID-19, and 9 were NPS negative with other clinical diagnosis. They found 21/31 (NPS positive + NPS negative with clinical COVID-19) positive by RT-PCR for the E/S genes while 29/31 were positive for all 4 genes. EBC samples from 15 NPS negative but clinically positive cases showed 66.6%, 73.3%, and 93.3% positivity for E/S genes, N/ORF1ab genes, and E/S/N/ORF1ab genes, respectively (10). These studies set the stage for breath matrixes to be used in identification of COVID-19. Currently, various technologies are being utilized for the development of breath analyzers, which include gas chromatography (GC), different forms of MS (such as proton-transfer reaction [PTR]), and nanosensors, which we discuss in detail. [Table 2](#)

Table 1. Different methods for diagnosing COVID-19.

Diagnostic methods	Samples	TAT	Cost	Performance	References
RT-PCR	NPS and oropharyngeal swab, feces	3-4 h	High	Sensitivity of 97.2%, 62.3%, and 73.3% for sputum, saliva, NPS or oropharyngeal swabs, respectively	Böger et al. (55)
Loop-mediated isothermal amplification	Throat swabs	30-60 min	Medium	LoD ¹ : 118.6 copies of SARS-CoV-2 RNA per 25 μ L	Augustine et al. (56)
High-throughput automated sequencing	Oropharyngeal swab, blood, serum, plasma	1-2 days	High	N/A	Sah et al. (57)
Lateral flow immunoassay	Blood, serum, plasma	<15 min	Low	Sensitivity: 88.66% Specificity: 90.63%	Li et al. (58)
CRISPR-Cas12-based lateral flow assay	NPS or oropharyngeal swabs	\cong 30 min	Low	Sensitivity: 90% Specificity: 100% LoD: 10 copies/ μ L	Broughton et al. (59)
Enzyme-linked immunosorbent assay	Blood, serum, plasma	1-5 h	Low	Sensitivity: 97.1% Specificity: 97.5% Accuracy: 97.3%	Rongqing et al. (60)
Colloidal gold-Immunochromatographic assay	Plasma	10 min	Low	Sensitivity: 82.4% Specificity: 100%	Xiang et al. (61)
Computed tomography scan	Human body (lung)	<1 h	High	Sensitivity: \cong 95%-100%	Böger et al., Kovács et al. (55, 62)
Mass spectrometry	Breath, blood, serum, plasma, urine, NPS, and throat swab	\cong 5 min	High	Specificity: 85.7%-100% Accuracy: 93%	Grassin-Delyle et al., Lazari et al., Ibrahim et al. (25, 63, 64)
Biosensor	Respiratory and blood samples	\cong 2 h.	Low	Sensitivity: 86.43%-93.75% Specificity: 90.63%-100%	Choi et al. (65)

LoD, limit of detection; II.

summarizes the breath analyzers that have been utilized for the diagnosis of COVID-19 infections.

MASS SPECTROMETRY

MS is a powerful analytical technique for exploring the genomics, proteomics, metabolomics, and microbiomics of human diseases, due to its unique advantages such as sensitivity, specificity, and speed (11–13). Various analytical techniques

based upon MS are used to investigate COVID-19 and are summarized in Table 3 (14, 15). Different methodologies coupled with MS such as liquid chromatography (LC), GC, inductively coupled plasma (ICP), and matrix-assisted laser desorption/ionization (MALDI) are used for biomarker discovery, omics research, and qualitative and quantitative detection (16). Pondering over the respiratory properties of COVID-19, analysis of human EBA profiles may reflect the clinical and pathologic state (17). Breath sampling

Table 2. List of breath analyzers developed for COVID-19.

Technology	Test name	Company	Other relevant information available	References
MS	BreathTest-1000™	Astrotech BreathTech Corp	≤60 s TAT	Astrotech (18)
PTR-TOF 6000X2 MS	BreFence™ Go COVID-19 Breath Test System	Breathonix Pte Ltd. Ionicon	Can detect up to 1 part per billion	Breathonix (19)
GC-MS	VOX System	NextGen Biomed (merging with Scentech Medical)	Fast with on-the-spot results and has accuracy of >90%	Scentech Medical (20)
Field asymmetric ion mobility spectrometry (FAIMS)	Breath Biopsy®	Owlstone	—	Owlstone Medical (21)
High-resolution mass spectrometry (HRMS)	SICRIT Breath Analysis System	Plasmion	No sample preparation required, flexible coupling with GC or liquid chromatography	Owlstone Medical (22)
LCMS-8060X MS (LC-MS)	—	Shimadzu Corporation	Collects approximately 1 mL of EBC by subject's own manipulation and gives results with help of MS in 5 min	Shimadzu Corporation (23)
Artificial intelligence (AI) and MS	“Worlds Protect” (kiosk)	Worlds Inc.	Not approved by FDA and the test may cost less than \$0.25	Texas A&M system (24)
Nanosensors and cloud-based artificial intelligence	ASU Detect CV19	Canary Health Technologies and SmartShape Design	Under clinical trial in Delhi, India, will detect persons who have not developed symptoms yet in less than 3 min	Canary Global (66)
Nanosensors and deep sensing algorithms	DSA Analyzer	Deep Sensing Algorithms	Will cost approximately 2 euros	Helinski (67)
Biosensor	CoronaCheck™	Exhalation Technology (ETL)	Detects SARS-CoV-2 in EBC, collects EBC with Inflammasome check™ device, TAT is <5 min	Exhalation Technology (68)
Semiconductor sensors	Breathalyzer	Ohio State University	—	Ohio University (69)
Nanomaterial-based hybrid sensor array	Multiplexed	University of Science and Technology of China and Technion—Israel Institute of Technology	—	Shan et al. (51)
Nanotechnology biomarker Tagging (NBT)	Virus Hunter 6	Ancon Technologies Ltd	Received approval from the Medicines and Healthcare Products Regulatory Agency and has CE marking.	Ancon Technologies (70)

(continued)

Table 2. Continued				
Technology	Test name	Company	Other relevant information available	References
Silicon microscale PCR cavities to capture viral particles and high-speed real-time quantitative PCR (RT-qPCR)	CoviDx	Imec	Signed licensing agreement with miDiagnostics for commercialization of breath analyzer	Imec (54)
Breath-borne VOC biomarkers for COVID-19	—	Peking University	—	Chen et al. (29)
Fluorescent genetic tags	Breathalyzer	University of California Los Angeles	Fluorescent tags light upon virus binding	University of California Los Angeles (71)

technologies have emerged with great potential in conjunction with MS methods. The presence of diverse analytes in human breath samples makes them easily available for introduction as well as a collection with well-designed devices for online or offline analysis. EBC, VOCs, and EBA are commonly analyzed via MS-based approaches in breath samples. A variety of MS-based methods using breath as the specimen have been developed, which are successfully used for the diagnosis of, and research into, COVID-19 (18–24). Grassin-Delyle et al. used PTR quadrupole time-of-flight MS to metabolically profile COVID-19 ARDS patients and non-COVID-19 ARDS patients using breath samples and identified methylpent-2-enal, 2,4-octadiene, 1-chloroheptane, and nonanal as the most prominent VOCs. They were able to differentiate the 2 groups with 93% accuracy, 90% sensitivity, and 94% specificity with an AUC (area under curve) of 0.94 to 0.98 (25). Indeed, use of MS-based breath analysis could provide a better diagnosis and understanding of COVID-19. Advantages of using MS-based methods for the analysis of breath include noninvasiveness, in vivo samples, good analytical performance, and applicability for COVID-19 diagnosis. Predominantly, metabolites, salts, proteins, and microorganisms are present in the breath that may provide useful information regarding

COVID-19. MS-based approaches used for COVID-19 detection include: (a) GC-MS analysis of volatile metabolites, (b) LC-MS analysis of non-volatile metabolites and proteins, (c) MALDI-MS analysis of proteins and microorganisms, (d) ICP-MS analysis of trace elements, and (e) direct ionization-mass spectrometry (DI-MS) analysis of EBA using online sampling methods. As discussed, multidimensional use of MS-based technologies may provide feasible avenues and comprehensive information on EBAs with regard to COVID-19 diagnosis and research.

GC and GC-MS

GC is an analytical technique used to separate the chemical constituents of a sample mixture that are usually organic molecules or gases and determine their quantities present in samples. Inorganic volatiles such as NH₃, N₂, O₂, H₂O, CO₂, and trace VOCs are present in exhaled breath originating from endogenous (produced from the respiratory tract and internal organ systems and their microbiomes) and exogenous VOCs (produced from food, drugs, and environment) and their metabolites. Measurement of breath volatiles may provide insight into the biochemical processes occurring in the human body. Volatile substances produced by pathogenic viruses like COVID-19 may serve as biomarkers. GC-MS

Table 3. MS-based approaches for diagnosis and investigation of COVID-19.

MS methods	Samples	Analytes	Sensitivity and specificity	References
GC-MS	Breath	VOCs	Sensitivity: 68%, specificity: 85%, positive predictive value (PPV): 89%, negative predictive value (NPV): 60%	Ibrahim et al. (64)
	Blood serum	VOCs	Sensitivity: 94%, specificity: 83%, accuracy: 89%	Mougang et al. (26)
	Feces	Metabolites	COVID-19-altered faecal metabolites were correlated with clinical features, gut microbes, and serum metabolites	Lv et al. (72)
LC-MS	Urine	Proteins	Detects molecular alterations associated with COVID-19 pathophysiology	Li et al. (73)
	NPS	Proteins	LoD ^I : 9×10^{-13} g, association between summed MS peak intensities of SARS-CoV-2 proteins and viral load Ct ^{II} values	Bezstarosti et al. (74)
	Saliva	Proteins	Identification of peptides originated from SARS-CoV-2	Ihling et al. (75)
MALDI-MS ^{III}	NPS	Proteins	Sensitivity: 61.76%, specificity: 71.72%, accuracy: 67.66%	Rocca et al. (76)
	Plasma	Proteins	Sensitivity: 93%, specificity: 92%, accuracy: 92%	Lazari et al. (63)
	Residual nasal swab	Proteins	Two machine learning models were identified with: accuracy: 98.3%, positive percent agreement (PPA): 100%, negative percent agreement (NPA), and 96%, accuracy: 96.6%, PPA: 98.5%, and NPA: 94%, respectively	Tran et al. (77)
	Nasal swabs	SARS-CoV-2	Accuracy: 93.9%, false positives: 7%, false negatives: 5%	Nachtigall et al. (78)
	Serum	Serum peptidome	Sensitivity: 98%, specificity: 100%, accuracy: 99%	Yan et al. (79)
ICP-MS ^{IV}	Blood	Metals and metalloids	Whole blood iron, age, and sex were determined to be independent factors associated with the disease severity, while chromium, cadmium, and the comorbidity of cardiovascular disease were determined to be independent factors associated with the mortality	Zeng et al. (80)
	Urine	Trace elements	Urinary creatinine-adjusted copper of $\geq 25.57 \mu\text{g/g}$ and $\geq 99.32 \mu\text{g/g}$ were associated with significantly increased risk of severe illness and fatal outcome in COVID-19, respectively	Zeng et al. (81)
DI-MS	Breath	VOCs	Sensitivity: 90%, specificity: 94%, accuracy: 93%	Grassin-Delyle et al. (25)
	Nasal swabs	SARS-CoV-2	Diagnostic accuracy: 86.7% and 84% for DESI-MS ^V and LD-REIMS ^{IV} , respectively	Ford et al. (82)
	Lysed cell	Lipids	93.3% correlation to the PCR classification by paper spray-MS	Silva et al. (83)

Ct, threshold cycle; III MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; IV ICP-MS, inductive coupled plasma-mass spectrometry; DESI-MS, desorption electrospray ionization-mass spectrometry; LD-REIMS, laser desorption – rapid evaporative ionization mass spectrometry.

analysis of breath VOC and blood metabolites has been proposed for COVID-19 diagnosis and research (26–28).

A study performed on COVID-19 patients has shown high levels of butanoate compared to

healthy controls and lung cancer patients. High levels of isopropanol and butyraldehyde were detected in patients with non-COVID respiratory infections using BreathSpec GC-IMS (G.A.S., Dortmund, Germany) consisting of a gas

chromatograph and an ion mobility spectrometer (IMS) (29). Researchers from Loughborough University (United Kingdom) and collaborators at the IMSPEX Group (Abercynon, United Kingdom) conducted a study on 98 patients, out of which 31 were positive for COVID-19 by RT-PCR while others had asthma, COPD, and other respiratory diseases. Multivariate analysis showed aldehydes (ethanal, octanal), ketones (acetone, butanone), and methanol differentiated COVID-19 from other respiratory conditions. The differentiation ability of the device was 80% and 81.5% from Edinburgh and Dortmund, respectively, while sensitivity, specificity, and AUC were 82.4%, 75%, and 0.87 for Edinburgh, and 90%, 80%, 0.91 for Dortmund (30, 31). Scentech Medical has shown that their breath analyzer using GC has both sensitivity and specificity >90% in a preliminary study that included 784 subjects (20). Barberis et al. have conducted a bidirectional study using GC-MS on EBCs of COVID-19 patients, healthy controls, and chronic cardiopulmonary edema (CPE) patients to identify potential new biomarkers. They found 2 small molecules and some potential biomarkers (3). They performed partial least-squares discriminant analysis (PLS-DA), which clearly indicated the association of metabolic profile with infection. Univariate analysis showed differential expression of 26 metabolites in EBC samples. ROC and box-plot analysis showed 8 potential biomarkers capable of differentiating COVID-19 from healthy controls. Later these identified biomarkers were employed to discriminate between COVID-19 and CPE patients. No significant difference was observed for these 8 biomarkers. However, with the help of a complete chemical fingerprint of small molecules and machine learning, they found that fatty acids present in EBC can discriminate between COVID-19 patients, healthy controls, and CPE patients (3). Another study conducted on COVID-19 positive and non-COVID-19 paediatric patients has reported 84 VOCs from breath samples using GC-ToFMS (time of flight MS). Later

they used a second cohort of pediatric patients to further investigate 84 VOCs and found 6 significant VOCs from them, out of which 3 were aldehydes (heptanal, nonanal, and octanal) also detected in adult COVID-19 patients (32). However, it is still unclear whether the breath signatures reported in these studies are specific for COVID-19 detection or not. For instance, 2-butanone has been reported in lung cancer patients (33) indicating their non-specificity. Interestingly, two different study cohorts identified heptanal in breath samples of COVID-19 (31, 32). Further validating studies are needed to confirm the breath signature specificity for COVID-19 diagnosis.

Breath samples are collected in gas bags or gas bottles to couple them with analytical techniques such as GC-MS. Additionally, to improve biomarker discovery using breath as the specimen, SPME (solid-phase microextraction) and needle trap device techniques are coupled with GC-MS (34–36). SPME masks can be worn for a longer duration, allowing concentrating of EBA samples. Even a portable GC-MS device, named Hexin portable GC-MS 2000, weighing 19 kg with battery, has been developed that analyzes breath samples within 15 min of starting up and provides results within 4 min. It can monitor for around 2 h and has a battery standby time of 4 h. Moreover, SPME-in-mask (wearable facemask microextraction) has been developed for collections of breath and can be directly coupled with GC-MS for analysis (34). Such portable GC-MS devices can be programmed for unskilled users to monitor biomarkers. The SPME-based breath sampling will improve sample collection and can be employed in schools, hospitals, etc., to decrease transmission as well as swiftly isolate infected persons.

LC-MS

Non-volatile organic compounds and biological matrixes present in EBC can provide useful biochemical information on respiratory disease (37). LC-MS is frequently utilized for the analysis of

organic and biological compounds and digested proteins from EBC (38). There are various methods for collection of EBC from breath such as the RTube kit, TURBO-DECCS collection device, and EcoScreen device and portable condenser. Various key factors may significantly affect EBC sampling, for example, collection should be done in cold collectors below 0 °C so that bioparticles, metabolites, and water vapor are condensed in them (4, 39–44). LC-MS has been employed for COVID-19 diagnosis (23,28,37). Shimadzu Corporation has reported use of an LC-MS-based breath analyzer for COVID-19 diagnosis that can give results in 5 min (23). Although the collection of EBC samples is easier, the concentration of proteins is very low, thus, these samples need to be further concentrated before analyzing them with LC-MS. Lyophilization has been proposed as the best method for pre-concentrating EBC samples. Previously for analysis of breath samples with LC-MS, proteins were collected, lyophilized, matrix was removed, and in-solution/gel digestion was performed (45, 45, 46). Pooling of samples was done before EBC analysis to improve protein detection and proteome coverage. Bredberg et.al have used pooled samples of EBC for LC-MS analysis composed of 6 (3000 L exhaled air) and 10 (4400 L exhaled air) healthy donors. It was reported that various proteins like albumin, surfactant protein A, α 1-antitrypsin, serotransferrin, and immunoglobulins are shared between blood and bronchoalveolar lavage (47). A comparative study conducted by Lacombe et al. revealed that the pooling of samples can affect the protein composition. Bioinformatics-based analysis of 153 proteins showed that most of the proteins identified corresponded to proteins secreted in the respiratory tract (e.g., lung and bronchi) (45). EBC sampling with a face mask may be beneficial. However, further studies are needed to improve the EBC sampling to use it with LC-MS for biomarker discovery, which may provide useful biomedical knowledge.

DI-MS

DI-MS in combination with artificial intelligence-MS (AI-MS) can be used to analyze EBA samples without pre-collection and preparation of samples. In DI-MS, samples from the human mouth are directly introduced in the ionization region for direct MS analysis. Previously, direct MS analysis of breath samples has been described using PTR-MS, extractive electrospray ionization mass spectrometry (EESI-MS), secondary electrospray ionization mass spectrometry (SESI-MS), selected ion flow tube mass spectrometry (SIFT-MS), and other MS techniques (17, 48–50). The introduction of gaseous breath samples is continuous and a noninvasive process allowing direct analysis of small metabolites online, giving real-time data. This attribute makes DI-MS an appropriate tool for COVID-19 rapid diagnosis. Breath VOCs were detected using PTR-MS, where breath samples from COVID-19 patients were directly introduced to the MS heat transfer line. The data obtained were analyzed with a multivariate approach, using principal component analysis (PCA) and machine learning algorithms using different mathematical backgrounds including linear vector machine, orthogonal partial least-squares discriminant analysis (OPLS-DA), elastic net, and random forest. OPLS-DA and PCA showed breath fingerprints of COVID-19 were associated with specific signatures. They were able to differentiate between COVID-19 and non-COVID-19 patients with an accuracy of 93%, a sensitivity of 90%, a specificity of 94%, and AUROC (area under the receiver operating characteristics) 0.94 to 0.98 (25).

Plasmion is using SICRIT (soft ionization by chemical reaction in transfer) technology which is high-resolution MS for the detection of COVID-19 in breath samples. In this method, whole samples are directly analyzed by the analyzer. The VOCs are sucked in by the negative pressure of the ionization source. Ionization occurs in the form of a cold, ring-shaped plasma. The

Table 4. Advantages and disadvantages of the COVID-19 studies based on MS for breath specimens.

Advantages	Disadvantages	Reference
Identified use of surgical masks to reduce risk of contamination		Leung et al. (6)
Identification of SARS-CoV-2 from EBC samples of NPS negative but clinically positive for COVID-19 patients using RT-PCR	Low sample size, time of collection of swab samples and EBC samples were different	Ryan et al. (10)
Can differentiate between COVID-19 ARDS and non-COVID-19 ARDS patients on mechanical ventilation.	Low sample size, breath signatures identified are not specific to COVID-19, did not consider different severity groups of COVID-19	Grassin-Delyle et al. (25)
Performed 2 independent studies and found both were able to differentiate between COVID-19 and other respiratory diseases using aldehydes and ketones	Large-scale studies are needed to confirm preliminary study	Ruszkiewicz et al. (31)
Differentiated between COVID-19, healthy controls, and CPE patients with help of fatty acids detected in EBC samples	Identified 8 markers, initially unable to discriminate between COVID-19 and CPE patients although machine models later were able to discriminate	Barberis et al. (3)
Performed study on COVID-19 pediatric patients and non-COVID-19 children and were able to discriminate between them. Also identified breath markers were similar to those detected in adult COVID-19 patients	Large-scale studies including children and adults are needed to further explore the generalized signatures which are similar between them during infection	Berna et al. (32)

analytes fly through the plasma ring on their way into the MS, whereby the ionization or charge transfer takes place by reactive species and UV radiation (22). However, VOCs present in EBA are in very low concentration, some in parts per trillion or even lower, thereby limiting the efficiency of the DI-MS techniques. This can be overcome by using an SPME wearable mask followed by ambient ionization. The sampling of EBA is separated from MS analysis in space and time. Additionally, using a facemask will also protect humans from air pollutants and infections. Table 4 summarizes the advantages and disadvantages of the studies on breath using different MS techniques.

BIOSENSORS

Biosensors are self-contained, integrated analytical devices consisting of a bioreceptor, transducer, and signal detector. The interaction of the bioreceptor with the target analyte produces an

electronic signal that the transducer transmits to be further amplified by a detector circuit, then processed and displayed. An observational study conducted in Wuhan, China, using a multiplexed gold nanomaterial-based assay recruited 49 confirmed COVID-19 patients, 33 non-COVID lung infection controls, and 58 healthy controls. Data from the training and test sets has shown 94% and 76% accuracy in separating patients from healthy controls, as well as 90% and 95% accuracy between patients with COVID-19 and patients with other lung infections, respectively. Also, a sensitivity of 100% has been observed for both the training set and the test set, while the specificity was 90% in the training set and 61% in the test set (51). Researchers from the Wyss Institute have come up with a wearable COVID-19 testing mask. Masks are based on wearable freeze-dried cell-free technology, which can detect the presence of SARS-CoV-2 in the breath of an individual. It is an “on the go” test with a TAT of 90 min. The technology involves extracting and freeze-drying the

molecular machinery that cells use to decipher DNA and produce RNA and proteins. The machinery is activated upon the addition of water. Synthetic genetic circuits can be added to create biosensors that can produce a detectable signal in response to the presence of a target molecule. This consists of three consecutive biological reactions that are activated after releasing water from the reservoir inside. First, the SARS-CoV-2 membrane is cut to reveal the RNA. Second, amplification of the spike protein-coding gene occurs. The third and final reaction uses CRISPR-based specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) technology, i.e., CAS12a, to detect any spike gene fragments. Activated CAS12a performs trans-cleavage of a co-lyophilized 6-FAM-(TTATTATT)-biotin single stranded DNA probe into smaller pieces that are then reported via a lateral flow assay strip. The presence or absence of spike protein is dependent on the presence of SARS-CoV-2 in the exhaled breath of an individual. The positive or negative results are depicted in the form of lines in the readout portion, very similar to an at-home pregnancy test (52). World Protect kiosk is a rapid onsite mass screening booth for COVID-19 patients. At the kiosk, the person has to blow air into the inlet, which activates the chemo-sensors present on the device. Signals are transformed and analyzed using machine learning algorithms. The TAT for these breath-based analyzers is from 30 s to 90 min (24). Wintjens et al. used an electric nose (Aeonose) to discriminate between COVID-19 positive and negative patients based on VOCs to triage patients who have elected for surgery. The 219 patients included in the study were asked to breathe through Aeonose for 5 min. Aeonose contains a metal oxide whose conductivity is changed on reacting with VOCs. The conductivity data is analyzed with machine learning to recognize patterns associated with VOCs. The test showed sensitivity, specificity, PPV (positive predicted value), and NPV (negative predicted value) of 86%, 54%, 0.40, and 0.92,

respectively. The NPV of the test was increased to 0.96 after applying logistic regression (53).

TECHNOLOGIES TO ANALYZE VIRAL PARTICLES IN BREATH

RT-PCR and serological testing provide evidence of current or past infections. Imec has come up with a breath analyzer that can detect to what extent a person can transmit the coronavirus particle or aerosols in the air. To achieve this goal, they have utilized silicon chip technology. A sample collector collects the aerosols as well as viral particles from breath and then they are exposed to silicon cavities and subsequent PCR (e.g., CoviDx). TAT for this technology is less than 5 min (54). A breath analyzer from Owlstone also uses viral particles or RNA in droplets from breath for detection of COVID-19 infection. They use the ReCIVA[®] Breath Sampler for the collection of breath during tidal breathing. The sampler consists of biopsy cartridges composed of

adsorbent tubes for simultaneous VOC collection for multiple replications. Adsorption with the help of tubes allows collection of more viral particles for further analytical processes. These contain breath pattern recognition means, i.e. collection of specific breath fractions and targeted analysis of different regions of the airways used in conjunction with CASPER portable air supply (21).

CONCLUSION

Breath is a noninvasive specimen and it can be used for screening of COVID-19 infection in a suspected person. Breath analyzers should have the attributes of being rapid, simple, inexpensive, and easily accessible. Since these devices have a rapid TAT compared to PCR, many man-hours will be saved by employers because ordinarily after collection of oral/NPS swab

samples for PCR, one continues to remain in isolation till PCR results are out. The cost of these tests is also less than RT-PCR. The collection of breath samples will be easier with a disposable collection device like SPME face masks, with reduced risk of transmission, and even the discomfort experienced by patients during sample collection will be obviated. Also, an MS-based multidimensional analytical platform offers a new strategy for detection of metabolites, proteins, microorganisms, and trace elements at low concentrations in breath samples. These analytical techniques will contribute to the development of new methods for diagnosis of

COVID-19 with a shorter TAT and an improved understanding of the underlying physiological, biochemical, and bioinorganic processes, and impact of COVID-19 on health. Technically, MS-based tools for breath analysis are powerful methods for investigating COVID-19 with many advantages. Biosensors are also a feasible option for the analysis of breath samples, as they are handheld devices that provide rapid results. Deployment of these tools in rural areas will aid in preventing transmission of COVID-19 since results will be available quickly. As the world is opening up after a nearly 2-year long hiatus, chemical breath biopsies hold a lot of promise.

Nonstandard Abbreviations: ARDS, acute respiratory distress syndrome; NPS, nasopharyngeal swab; RT-PCR, reverse transcription-PCR; TAT, turnaround time; VOC, volatile organic compound; EBA, exhaled breath aerosol; EBC, exhaled breath condensate; MS, mass spectrometry; GC, gas chromatography; PTR, proton transfer reaction; DI-MS, direct ionization-MS; CPE, cardiopulmonary edema; SPME, solid-phase microextraction.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 4 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved.

Authors' Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the author disclosure form. The authors declare no conflict of interest. **Disclosures and/or potential conflicts of interest:** **Employment or Leadership:** None declared. **Consultant or Advisory Role:** None declared. **Stock Ownership:** None declared. **Honoraria:** None declared. **Research Funding:** This work was supported by University Grants Commission, New Delhi, Fellowship no. 582/(CSIR-UGC NET JUNE 2018). **Expert Testimony:** None declared. **Patents:** None declared.

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