Abdimas Umtas: Jurnal Pengabdian Kepada Masyarakat
LPPM-Universitas Muhammadiyah Tasikmalaya
Volume: 6 Nomor: 1
E-ISSN: 2614 - 8544

Exhaled Breath Analysis for COVID-19 Investigation: Clinical instruments or Scientific Toys?

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Abstract

The COVID-19 pandemic was crucial worldwide. Ongoing COVID-19 disease investigation methods are primarily based on molecular and serological detection. These instruments are invasive and necessarily require the use of trained personnel. Non-invasive COVID-19 investigation methods could help diagnose and monitor the outbreak. Because the SARS-CoV-2 virus is non-living, it lacks its metabolism. Different infectious diseases can release volatile organic compounds (VOCs), resulting in specific VOCs. All of these are metabolic products primarily produced locally in the respiratory system and systemically via blood circulation. We overview the clinical applications in the COVID-19 investigation and summarize the methodological issue. Numerous VOCs in the exhaled breath have the prospects to distinguish patients from healthy people and people infected with COVID-19. It is hard to define COVID-19 using VOCs from exhaled breath. Due to a lack of standardization in data collecting and processing procedures, their use in clinical practice is hampered. There are studies validation and external validation to determine whether exhaled breath analysis adds value to the diagnostic and follow-up processes for COVID-19 infection. In conclusion, the use of VOCs in exhaled breath as a marker for COVID-19 infection has not been validated for clinical use.

Keywords: Virus, COVID-19, VOC, Exhaled Breath Analysis

INTRODUCTION

Last year was critical for the entire world. The unanticipated corona virus illness (COVID-19) pandemic had a profound effect on most of the nation's and people's lives (Ardillah et al., 2022). Severe acute respiratory syndrome is the etiology of COVID-19 disease. SARS-CoV-2 is a positive-sense single-stranded RNA virus (+ssRNA) with an envelope (Alsobaie, 2021). One of the foundations of pandemic control is rapid and reliable laboratory detection of active COVID-19 infection. Polymerase Chain Reaction (PCR) technology is the accepted practice for virus detection. PCR has a low rate of false positives and negatives (high specificity) as well as a high level of accuracy (Yüce et al., 2021). Nevertheless, sample shipping and laboratory facility overload result in a wait of several days before test results are available, adding to the healthcare system's burden (Loeffelholz & Tang, 2020). As a result, rapid antigen tests (Ag-RTDs) based on lateral flow assay or enzyme-linked immunesorbent assay (ELISA) technologies are often utilized as prescreening techniques (El Jaddaoui et al., 2021; Yüce et al., 2021). Rapid antigenic tests and

sensitive molecular tests both have practical limitations in terms of test protocols. This procedure is both upsetting and invasive for patients.

In recent years, a surge in scientific and clinical interest has seen in exhaled breath analysis. In the treatment of a range of respiratory ailments, including viral diseases, volatile organic compounds (VOCs) have been employed as diagnostic, prognostic, and treatment response biomarkers. Apart from individuals with COVID-19-specific clinical symptoms, screening for SARS-CoV-2 occurs in the elective, pre-operative, and asymptomatic population (Hojaij et al., 2020). Thus, healthcare systems demand tests that are speedy, affordable, and easy to use for identifying or ruling out infection at earlier stages, even before symptoms emerge, to lower the transmission and fatality rates. Diagnostic strategies that are low cost, fast, and easily available are crucial to contain the epidemic. In this review, we analyzed exhaled breath volatile chemicals as possible indicators for COVID-19 infection.

Current COVID-19 Diagnostic Methods

COVID-19 detection tools are currently classified into three groups. The first category comprises molecular assays for SARS-CoV-2 RNA, such as real-time reverse transcription-polymerase chain reaction (RT-PCR), isothermal amplification, and genome sequencing (Iyer et al., 2020). The second group comprises serological diagnostics that identify SARS-CoV-2 antibodies using chemiluminescence immunoassays or enzyme-linked immunosorbent assays (Yüce et al., 2021). Third, lateral flow immunoassays-based antigen detection kits. In the COVID-19 pandemic, these three detection tests complement one another.

Molecular Detection

RT-PCR is the current gold standard and preferred method of diagnosis. Swabs are used to take samples from the upper or lower respiratory tract. The genetic material taken from these samples is subsequently amplified using reverse transcription polymerase chain reaction (RT-PCR) to ascertain the SARS-CoV-2 genetic code. Concerns have also been expressed about the false negative rates of RT-PCR and sample preparation. Current sampling techniques place a high value on the sampler's ability and timing (Sawano et al., 2021). False-positive results arise as a result of swab contamination, whereas false-negative results occur in around 66–83 percent of patients due to the absence of SARS-CoV-2 in the oropharyngeal settings (C. Long et al., 2020; Pascarella et al., 2020). According to a comprehensive analysis, up to 29% of patients may have an initial false negative real-time polymerase chain reaction (RT-PCR) result (Arevalo-Rodriguez et al., 2020). Due to the relatively frequent occurrence of false-negative test findings, the creation of a new sampling equipment is required (Safiabadi Tali et al., 2021). As a consequence, if clinical suspicion is high, a single negative reverse transcription polymerase chain reaction (RT-PCR) test cannot rule out COVID19 and must be repeated (Caulley et al., 2021; Jamal et al., 2021). Furthermore, it is important to understand that accurate identification of any infectious disease involves collection of sufficient specimens at the anatomical site of infection and at a period when the pathogen is expected to be present.

Immunodiagnostic Method

Additionally, immunodiagnostic approaches are available for recognizing viral antigens and serology is available for detecting an immune response to a virus.

Serological based testing

COVID-19 serology tests look for antibodies that recognise SARS-CoV-2 antigens. When exposed to an antigen for the first time, IgM antibodies appear first, followed by IgG antibodies (West et al., 2021). After two weeks of infection, the majority of patients seroconvert or develop antibody positivity (Prévost et al., 2020; Tré-Hardy et al., 2020). Antibodies were initially thought to appear six days after symptoms and rapidly increase over the first two to four days (Lei et al., 2020; Marklund et al., 2020; West et al., 2021). Asymptomatic patients had lower levels of IgG than symptomatic patients (Q. X. Long et al., 2020). Serological data is instrumental in epidemiological studies for predicting attack and case fatality rates as well as evaluating control measures (Lee et al., 2020). Additionally, serological testing for prior SARS-CoV-2 infection may be quite beneficial in pediatric patients with the multisystem inflammatory syndrome (MIS-C) (Feldstein et al., 2020; West et al., 2021; Zhou et al., 2020).

False-negative antibody tests can occur as a result of insufficient antibody levels in the specimen or low sensitivity (Kontou et al., 2020; Liu & Rusling, 2021; Tollånes et al., 2021). False positive detection, on the other hand, may occur as a result of methods' insufficient specificity, which is usually caused by antibody cross-reactivity as well as contamination of samples or reagents (Liu & Rusling, 2021). A meta-analysis study that used RT-PCR or other NAT as the gold standard to evaluate IgM and IgG assays based on chemiluminescence immunoassays (CLIA), enzyme-linked immunosorbent assays (ELISA), and lateral flow immunoassays (LFIA) found specificity greater than 99% and sensitivity more significant than 93% (Kontou et al., 2020). Thus, a combination of strategies can be used to retest negative findings using different tests, as an example.

Antigen based testing

Antigen assays detect COVID-19 virus-expressed proteins in respiratory tract samples. A device for testing antigens that have been precoated with control (C) and test (T) lines. The test (T) region is coated with anti-SARS-CoV-2 monoclonal antibodies directed against the SARS-CoV-2 antigen. The quick assay for SARS-CoV-2 antigen detection (StandardTM Q COVID-19 Ag kit) demonstrated equivalent sensitivity (98.33 percent) to a real-time reverse transcription polymerase chain reaction (RT-PCR) (Chaimayo et al., 2020). The COVID-19 Ag test has the advantage of being a straightforward procedure with rapid results and a high NPV as a COVID-19 screening test, but it has the disadvantage of having a poor PPV in areas with low prevalence. Despite its limitations, the fast SARS-CoV-2 antigen test can assist all healthcare staff in more efficiently managing infected patients, especially in remote and outbreak locations. If the examined material contains a significant amount of antigen, a detectable line appears on the cassette test. Due to the fact that this type of test is only beneficial when the SARS-CoV-2 antigen concentration is high, it should be used only during the acute or early stages of infection.

VOC as A Promising Development of the COVID-19 Diagnostic Tool

VOC analysis of breath samples has been shown to be effective in identifying a wide variety of ailments and infections (Chambers et al., 2012). Volatile organic compound (VOC) diagnostics have the potential to be the next generation of pathogen identification and infectious disease management tools (Wang et al., 2018). VOCs are metabolic compounds with a low molecular weight that have a high vapor pressure and a low boiling point, allowing for evaporation at room temperature (Hong-Geller & Adikari, 2018). Since ancient times, when physicians diagnosed ailments using their senses, VOC patterns have been utilised as biomarkers for disease (Hong-Geller & Adikari, 2018). Furthermore, artificial intelligence (AI) has been utilized to examine the species specificity of VOCs using breath biochemistry, potentially providing a pathogen's species-level biological fingerprint (Mardian et al., 2021).

Different infectious diseases can release volatile organic compounds, which can induce specific VOCs. These are metabolic products produced mostly by cell metabolism and excreted by the breath, saliva, sweat, urine, feces, skin emanations, and blood (Amann et al., 2014; Jendrny et al., 2021). Due to the fact that VOC patterns reflect an organism's many metabolic states, they could be employed for medical diagnostics via scent recognition and disease outbreak control (Angle et al., 2016; Jendrny et al., 2021). An increasing number of studies show that VOC analysis effectively detects a variety of non-infectious diseases, including inflammatory disease, metabolic disorders, lung cancer, and even vascular dementia (Buljubasic & Buchbauer, 2015; Dent et al., 2013; Mazzatenta et al., 2015; Schnabel et al., 2015). VOC detection has clinical value in three aspects of infectious disease diagnostics, including determining the absence and presence of specific pathogen antigens. A study showed the use of breath VOCs in identifying TB infection compared with positive sputum cultures had a sensitivity of 82.6% and a specificity of 100% specificity (Phillips et al., 2007). This is also consistent with studies in which a comparison of VOC profiles between ventilator-acquired pneumonia (VAP) (+) and VAP (-) revealed a subset of 12 VOCs with a sensitivity and specificity of approximately 75.8 percent and 73.0 percent, respectively, that correctly discriminated between those two patient groups. Additionally, determine the pathogen's antibiotic resistance vs sensitivity to inform treatment regimens. An identification study of antibiotic-resistant *E. coli* causing urinary tract infections analyzed VOCs to

differentiate between resistant and susceptible bacteria based on the abundance of six VOCs with an overall accuracy of 85.7% (Hewett et al., 2020). The development of a VOCs test for virus identification would benefit not just pandemic-level threats, but will also aid in separating bacteria from viral infections, which will aid in combating antibiotic resistance and guiding treatment courses (Hewett et al., 2020). These capabilities based on VOCs will enable the creation of non-invasive, quick, and ideally extremely sensitive diagnostic procedures and instruments, ultimately resulting in improved patient outcomes.

Substances in exhaled breath gas are generally classified as: 1.) inorganic substances such as nitric oxide, oxygen, and carbon dioxide, 2.) organic substances such as ethylene glycol, 3.) Exhaled breath condensate, which contains cytokines, hydrogen peroxide, isoprostanes, and leukotrienes; 4.) Volatile organic compounds (VOCs), which include ethane, pentane, aldehydes, and isoprene (Dent et al., 2013). Exhaled breath samples have been used in the vast majority of studies on VOC biomarkers since they are the easiest to obtain (Boots et al., 2012; Hong-Geller & Adikari, 2018; Wickham et al., 2019). Volatile organic compounds (VOCs) are released by both exogenous and endogenous sources. In the human body, endogenous VOCs are created by biological processes such as oxidative stress and inflammation, as well as by invading pathogens (Boots et al., 2012; Schnabel et al., 2015; Yuan & Hu, 2021). On the other hand, exogenous volatiles are substances inhaled from the external environment, such as while consuming food or smoking cigarettes (Hong-Geller & Adikari, 2018; Stavropoulos et al., 2021). Exhaled breath analysis revealed that infection alters the microbial flora of the lungs, which in turn alters the exhaled metabolites produced by the respiratory tract and internal organ systems, as well as their microbiomes (Gould et al., 2020). VOCs are excreted into the blood after they are produced, and then they diffuse into the respiratory system, where they are exhaled (Schnabel et al., 2015). Oxidative stress and inflammation alter the content of VOCs emitted by the damaged organ and hence the exhaled breath. Additionally, microbes may produce unique molecules, resulting in a variation in the VOC profile of exhaled breath (Belizário et al., 2021; Janfaza et al., 2019). Bioinformation produced from volatile organic compounds (VOCs) in human exhaled breath can aid in the early detection and selection of appropriate medical therapies for a number of disorders (Dent et al., 2013). VOC assessment via exhaled breath analysis is one proposed strategy for identifying viral infections non-invasively. VOCs can be obtained both through cellular in vivo cell metabolism and pathological processes (Mancebo, M. C., Eisen, J. L., Sibrava, N. J., Dyck, I. R., & Rasmussen, 2014). Different pathogenic species produce various VOC profiles because of their unique metabolisms. While pathogens are capable of producing a diverse array of VOCs, very few metabolites are generated exclusively by a single bacterial species (Hong-Geller & Adikari, 2018). In the following, we summarize some of the identification of specific VOCs that characterize being infected with certain pathogens and can potentially be used to differentiate infected from uninfected patients, particularly in polymicrobial cases (Table 1). In addition, while infection causes common VOCs, unique VOC fingerprints have been discovered in response to specific pathogens.

Table 1. Volatomics of Respiratory Infections

Virus	Method	Sample	Design	Analytical method	Significant VOCs identified
HRV (Mancebo, M. C., Eisen, J. L., Sibrava, N. J., Dyck, I. R., & Rasmussen, 2014)	In vitro	TBE cells	HRV-infected cells were compared to uninfected	SPME GC-MS	Aliphatic alcohols Aliphatic compound (E-7-tetradecenol) Hydrocarbon, (2,3,4-trimethyl-hexane) Dimethyl sulfide Acetic acid Phenol Hydrocarbon, (2,3,4-trimethyl-2-pentene)

Virus	Method	Sample	Design	Analytical method	Significant VOCs identified
					Aliphatic alcohol (2-propyl-1-heptanol and 2-butyl-1-octanol) 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-ol 3-phenyl-2-propenal
HPAI (Aksenov et al., 2014)	In vitro	LC1RB cells	LC1RB compared LC1R B infected with H9N2 (avian), H6N2 (avian), or H1N1 (human) influenza	SPME GC-MS	2-methoxy-ethanol Thiirane propanoic acid 2-methyl butanoic acid 5-methyl-hexan-3-one heptan-3-one octan-2-one 1-phenylbut-1-ene 4-ethylbenzaldehyde Decanal
RSV (Purcaro et al., 2018)	In Vitro	Hep-2 cells	HEp-2 compared HEp-2 infected with RSV	GC-TOF MS	2-methyl-pentane methyl sulfone 2,4-dimethyl-heptane 4-methyloctane alkylated hydrocarbon

HRV -- human rhinovirus; TBE -- tracheobronchial epithelial; SPME GC-MS -- solid-phase microextraction fibers gas chromatography/mass spectrometry; HPAI -- Highly pathogenic avian influenza; LC1RB -- lymphoblastoid C1R B; RSV -- Respiratory syncytial virus; Hep-2 -- Human laryngeal cancer cell line; GC-TOF MS -- Gas chromatography time of flight mass spectrometry

VOC as Promising Markers in COVID-19

Most viral respiratory infections are transmitted via direct contact with other people or infected surfaces. The high rate of SARS-CoV-2 transmission shows that virus-containing inhaled droplets contribute significantly to the infection's rapid spread (Arianti et al., 2022; Maddali et al., 2021). SARS-CoV-2 can be identified in the air and in things that have the potential to affect the surrounding air (e.g., ventilation fans and hospital floors), mostly because the virus remains alive in the air for up to 3 hours (Patients et al., 2020). COVID-19 is spread via inhalation of minute aerosol particles composed of evaporated respiratory droplets that are small enough to remain airborne for hours (5 mm) (Asadi et al., 2020). As a result, these droplets are released during exhalation, coughing, and sneezing and can be sampled and examined. They are also collected during tidal breathing through the cooling and condensation of exhaled breath.

Viral respiratory infections have been shown to change the respiratory and gastrointestinal microbiota (Hanada et al., 2018). All of these microbiome changes are likely to manifest themselves in metabolite changes detectable in the breath (Hanada et al., 2018). The liquid phase, on either side, includes both exhaled breath condensate (EBC) and aerosols (EBA), which both contain a diverse range of non-volatile molecules such as entrapped semi-volatile and non-volatile compounds, such as proteins, metabolites, smaller polar compounds, chemokines, hydrogen peroxide, ammonia, adenosine, leukotrienes, isoprostanes, nitrogen oxides, peptides, fatty acids, cytokines, bacteria, and viruses (Brusselmans et al., 2018). As a result, the discovery of metabolomic, proteomic, and genomic fingerprints of exhaled breath for the early detection of

respiratory disorders has garnered considerable interest in recent years. Due to the fact that EBC analysis is a more contemporary, non-invasive approach that enables the detection of biomarkers originating mostly in the lower respiratory tract (Gould et al., 2020; Konstantinidi et al., 2015).

A review of the current state of knowledge regarding the mechanisms behind viral activation of cellular metabolism (Sanchez & Lagunoff, 2015). Infection biochemistry is a complex, multistep process that results in the synthesis of VOCs. To begin, SARS-CoV-2 infects cells of the lower respiratory tract by stimulating endocytosis via its Spike(S)-glycoprotein (Jackson et al., 2022). This fusion is associated with a drop in the endosome's pH, which results in particular modifications in protein synthesis and VOC production. Due to the fact that cellular infection influences a wide number of signal transduction and protein expression pathways simultaneously, a significant downstream effect on VOC generation can be expected (Aksenov et al., 2014). This viral entrance is distinct from that of influenza or rhinovirus, which utilize sialic acids and Toll-like receptor 3, respectively, implying the formation of SARS-CoV-2-specific VOCs (Woodby et al., 2021). The viral genome is then released into the cytoplasm, where it is translated into two polyproteins and structural proteins (Li et al., 2020). These components create a replication-transcription complex, which initiates the replication of the viral genome and results in the formation of accessory and structural proteins. This requires the use of several tiny molecules as cofactors, reactants, and (side) products that are capable of crossing the cell membrane and being detected in exhaled breath. The endoplasmic reticulum and Golgi apparatus assemble freshly generated genomic RNA, nucleocapsid proteins, and envelope glycoproteins into viral particle buds that fuse with the plasma membrane and release the virus into the airways (Malone et al., 2022).

Second, dendritic cells take up SARS-CoV-2 and display its antigens, activating the humoral and cellular responses and resulting in the formation of virus-specific B- and T-cells (Apostolidis et al., 2021). B-cells, in particular, will almost certainly produce unique and distinct VOCs following infection as a result of the individual virus-cell interactions, as cell lines with varying HLA gene expression profiles demonstrate distinctive VOCs as an immunologic fingerprint in response to antigen presentation (Aksenov et al., 2012, 2014; Apostolidis et al., 2021)

Third, while the immune response is critical for controlling and resolving the SARS-CoV-2 infection, it may result in a cytokine storm (Aksenov et al., 2014; Costela-Ruiz et al., 2020). Viral RNAs act as molecular patterns associated with pathogens and are recognized by endosomal pattern recognition receptors (Jensen & Thomsen, 2012). These molecules activate downstream cascades, resulting in the activation of NF-κB and the generation of IFN-α and pro-inflammatory cytokines, so triggering a cytokine storm in the body (Tang et al., 2020). This results in lung injury and may be associated with the critical condition of COVID-19 patients, as it results in the destruction of the cellular structure due to oxidative stress and the release of several additional volatile organic compounds (VOCs) that can vary in concentration depending on the severity of the damage (Q. X. Long et al., 2020; Tang et al., 2020). Thus, VOCs may be used to assess COVID-19 pulmonary infection and maybe predict illness outcomes, emphasizing their utility as diagnostic and prospective prognostic biomarkers.

Like all diagnostic tests, these VOCs should not be confused with exogenously produced VOCs associated with drugs, diet, or the environment, which can enter into equilibrium with the body (Pleil et al., 2013). Diet, humidity, and background pollution all have an effect on false-positives (Mardian et al., 2021; Ruszkiewicz et al., 2020; Shan et al., 2020). Thus, VOCs may be used to assess COVID-19 pulmonary infection and maybe predict illness outcome, emphasizing their utility as diagnostic and prospective prognostic biomarkers. Correlating the discovered VOCs to the biological pathways associated with viral infection may aid in the understanding of COVID-19. The basic concept for assessing EBA stemmed from an investigation into how canines could track humans long after they had vanished and VOCs had likely vanished as well (Buljubasic & Buchbauer, 2015). A proof-of-concept study employing sweat samples from COVID-19-positive patients revealed encouraging results, with diagnostic success rates ranging from 84% to 100% (Jendrny et al., 2021). EBA can be sampled by trapping the aerosols in a filter, although, as with

VOCs, caution is required to account for exogenous exposures (Wickham et al., 2019). Also, a study published recently demonstrated that VOC indicators can be successfully used to differentiate COVID-19 from influenza-A (Traxler et al., 2019). The breath analysis's screening and diagnostic capability for COVID-19 were also proved in a study that indicated a machine model was successful in identifying these various groups using different arrays of 12 endogenous breath-borne VOC species to identify specific VOC species in COVID-19. Propanol concentrations were found to be significantly higher in the exhaled breath of COVID-19 patients (Chen et al., 2021). Therefore, potential VOC biomarkers can be used. Here we describe the potential of breath VOC biomarkers and machine learning that can be used for high-performance COVID-19 screening.

Exhaled Breath Analyzer Implementation in COVID-19

Exhaled breath analysis has been found to be a more recent, non-invasive technique for detecting biomarkers originating primarily in the lower respiratory tract (Giovannini et al., 2021). It is collected during tidal breathing when the expelled breath is cooled and condensed. Additionally, exhaled breath contains exhaled droplets that contain semi-volatile and non-volatile components such as proteins, metabolites, smaller polar molecules, cellular fractions, fatty acids, cytokines, germs, and viruses. These droplets or aerosols occur in both the lower and upper airways as a result of surfactant disruption and turbulence (Smolinska et al., 2017). Recently, COVID-19 ARDS (acute respiratory distress syndrome) patients were successfully distinguished from non-COVID-19 ARDS patients using breath VOC profiles (four VOC species) acquired using proton transfer reaction time-off light mass spectrometry.

A promising development in the diagnostic field is based on the detection of COVID-19 using volatile organic compounds (VOCs). The following are several studies that demonstrate the exhaled breath analyzer's diagnostic performance in detecting COVID-19 (Table 2).

Table 2. Current implementations exhaled breath analyzer for COVID-19 Investigation

Reference	**Ibrahim *et al.*** (Ibrahim et al., 2021)	**Rodriguez-Aguilar *et al.*** (Rodríguez-Aguilar et al., 2021)	**Shaen *et al.*** (Shan et al., 2020)
Title	Diagnosis COVID-19 by Exhaled breath analysis using gas chromatography-mass spectrometry	Comparative analysis of chemical breath-prints through olfactory technology for the discrimination between SARS-CoV-2 infected patients and controls	Multiplexed Nanomaterial-Based Sensor Array for Detection of COVID-19 in Exhaled Breath
Country	UK	Mexico	China
Characteristic of included study			
Study design	Prospective, real-world observational study	Analytical cross-sectional	Cross sectional study
Start date	April 29. 2020	NA	March 9, 2020
End date	July 10, 2020	NA	March 27, 2020
Population description	All participants underwent-testing for SARS-CoV-2 using PRC of nasopharyngeal swab	Subjects with a targeted sampling of positive and negative subjects with RT-qPCR test from the Research Center for Health Sciences and Biomedicine of the	Participants were registered at a variety of locations in Wuhan and Hefei.

Reference	**Ibrahim *et al.*** (Ibrahim et al., 2021)	**Rodriguez-Aguilar *et al.*** (Rodríguez-Aguilar et al., 2021)	**Shaen *et al.*** (Shan et al., 2020)
		Autonomous University of San Luis Potosi (UASLP)	
Inclusion criteria	Patients with a suspected COVID-19 infection were addressed and informed consent was obtained. All individuals were tested for SARS-CoV-2 infection using a nasopharyngeal swab PCR.	• 18-70 years old, both sexes • symptomatic (patients presenting, headache, sore throat, body aches, general discomfort, loss of taste and smell, among other typical symptoms) • asymptomatic SARS-CoV-2 specific gene RT-qPCR ct below 38 to be considered as positive.	• CT, nasal, and pharyngeal swab specimens, RT-PCR, and anti-body assays were used to confirm the COVID-19 patients. • Each participant signed an informed consent form.
Exclusion criteria	NA	• pregnant patients • patients with confirmed pulmonary infection othet than COVID-19 (influenza, tuberculosis or other infectious disease) • subject who in the course of sampling acquired an infectious pathology • subjects who withdrew informed consent	NA
Controls	NA	Negative test for SARS-CoV-2 by RT-qPCR	Divided into 58 healthy controls and 33 non COVID-19 lung infection

Reference	**Ibrahim *et al.*** (Ibrahim et al., 2021)	**Rodriguez-Aguilar *et al.*** (Rodríguez-Aguilar et al., 2021)	**Shaen *et al.*** (Shan et al., 2020)
Method of recruitment of the participants	Patients admitted to the hospital with suspected COVID-19	Voluntary	Voluntary for controls, COVID-19 patients treated in hospital
Characteristic of Exhaled breath collector	Exhaled breath analysis using gas chromatography-mass spectrometry with features (benzaldehyde, 1-propanol, 3,6-methylundecane, camphene, beta-cubebene,iodobenzene and an unidentified compound)	Using the Cyranose 320 (sensigent) equipped with 32 chemoreceptors. Each has unique VOC adsorption qualities that result in varied degrees of reaction due to their polymeric makeup (polyvinyl butyral, polyvinyl acetate, polystyrene, and polyethylene oxide) and the conductive nanoparticles they are formed of (black carbon and carbon nanotubes).	The small, handheld analyzer is equipped with eight distinct sensors. Nanomaterial-Based Hybrid Sensor Array.
Total number of participants	81	84	140
Mean age	56.5	38 +/- 14	No data
Result characteristic			
RT-PCR result	At the time of admission, 52 (64%) of the individuals had a positive PCR test.	Positive: 41 Negative: 42	Positive: 49
Diagnostic performance			
Specificity	85.0%	97.6%	90.0%
Sensitivity	68.0%	100.0%	100.0%
PPV	89.0%	No data	88.0%
NPV	60.0%	No data	100.0%

UK – United Kingdom; NA - not applicable ; RT-PCR -- Real-Time Polymerase Chain Reaction ; SARS-CoV-2 **--** Severe acute respiratory syndrome coronavirus 2; PPV -- positive predictive values ; NPV -- negative predictive values.

The study of trace volatile organic compounds (VOCs) in exhaled breath may one day provide more convenient, less expensive, quicker, and non-invasive screening approaches for COVID-19 disease diagnosis. The critical requirement for non-invasive clinical breath tests demonstrates the path forward for future research and provides critical guidance toward the objective of creating non-invasive diagnostic tools for COVID-19 disease. Non-invasive collection methods, including exhaled breath, have recently become popular since these samples are easy to obtain and have

the potential for huge population-based surveillance. In Indonesia, breath VOC tests are clinically available. However, the performance is still in the validation process.

The diagnosis of COVID-19 through breath samples still requires much consideration. The following are some limitations and challenges, including drug sampling and a lack of reproducibility between studies. Concerning sampling, given this viral infection, the high rate of transmission of SARS-CoV-2 suggests that exhaled droplets containing the virus play an essential role in the rapid spread of infection (Patients et al., 2020). Exhaled breath samples were collected in accordance with strict biosafety criteria, and the collected samples were sealed and transported to a Level-2+ biosafety laboratory. Additionally, it is well established that subjects breathe spontaneously at varying rates, and that hypo- or hyperactivity during sampling alters the composition of exhaled air. When expired or end-tidal gas is used, the concentrations being measured will fluctuate. The end-tidal phase results in a substantial increase in exhaled breath concentrations, which corresponds to the maximum concentration of expired carbon dioxide (end-tidal carbon dioxide concentration) (Ghorbani et al., 2020). The effect of medication used to treat respiratory diseases, such as inhalation agents, corticosteroids, antibiotics, and anesthetics, as well as the effect of concurrent medications such as antihypertensive or anti-diabetic therapy, and the effect of co-existing disorders, on exhaled VOCs is unknown. As a result, a well-defined standard process and criteria are required. Additionally, many clinical research enrolled only a few hundred people (Ibrahim et al., 2021; Rodríguez-Aguilar et al., 2021; Shan et al., 2020). Therefore, clinical trials with larger samples are needed, and the developed technology is needed to provide a new concept for screening non-invasive rapid point of care tests for COVID-19 in various scenarios through breath analysis.

CONCLUSION

Current techniques for diagnosing COVID-19 using exhaled volatile organic compounds (VOCs) are intriguing but far from clinically effective. Additionally, the lack of standardization in data gathering and analysis procedures impedes their use in clinical practice. External validation is required to determine whether exhaled breath analysis adds value to the diagnostic procedure and follow-up of COVID-19. To summarize, the use of exhaled breath analyzers in the COVID-19 study is currently experimental and has not been validated for clinical usage

Author Contributions: Conceptualization, IM, NR; writing—original draft preparation, IM, NR, MM; writing—review and editing, IM, NR, MM; supervision, MM; project administration, MM, YAAR, Y.Y; funding acquisition, MM, YY. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: none.

Conflicts of Interest: The authors declare no conflict of interest.

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