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SARS-CoV-2 breath tests implementation for the rapid COVID-19 surveillance: a game changer? A review of existing data

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ABSTRACT

The Coronavirus Disease 2019 (COVID-19) pandemic has been spread across the globe for almost a year, causing economic, social, and psychological impacts with yet unknown dimensions. In emerging and reemerging pathogen surveillance and detection, polymerase chain reaction (PCR) is a classic laboratory technique that has been widely used for the amplification and identification of nucleic acids. Analysis of volatile organic compounds in breath has been long reviewed as a potential diagnostics tool for many diseases. The overall specificity for SARS-CoV-2 of these methods was calculated and reveled a low value for reliable detection. Breath tests are not a sufficiently evidence-based approach for rapid screening and to "secure" or creating "sanctuary" regions for touristic purposes. Therefore, policymakers must cautiously point out the importance of further evaluation and structured studies confronting gold-standards with new devices. This review aims to evaluate the possible potential of this novel diagnosis test within a public health perspective considering its implementation on a resource limited environment.

BACKGROUND

The Coronavirus Disease 2019 (COVID-19) pandemic has been spread across the globe for almost a year, causing economic, social, and psychological impact [1] with yet unknown dimensions [2]. The World Health Organization's Chief director, as early as March 2020, called for a simple but very urgent message to test, test, and test, pointing on the importance of large-scale testing and contact tracing as a significant effort to limit the impact of the pandemic. Nevertheless, this call is of most significant importance when it relies on diagnostic tests that can offer a rapid and conclusive detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

However, in countries with limited resources, testing all populations and especially vulnerability communities, or most-at-risk populations is a cumbersome venture; reinforcing the importance of accurate isolation, early management- if symptoms are present-, and precise geographic interventions when hotspots are localized, all of this cemented by the appropriate testing capacities [4,5].

Therefore, the development of effective and validated methods for SARS-CoV-2 detection has been one of the main objectives of the scientific community

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since the emergence and completed sequenced virus.[6] In emerging and reemerging pathogen surveillance and detection, PCR is a classic laboratory technique that has been widely used for the amplification and identification of nucleic acids [6]. Considering that this technique's basis is already well established, the quantitative reverse transcription-polymerase chain reaction (RT-qPCR) has been applied for the molecular detection of SARS-CoV-2 even from biological tissues. It is currently the most robust in terms of the effective diagnosis of the disease [7-9].

Even though the RT-PCR technique presents high specificity, sensitivity, and reproducibility rates and allows a high number of tests performed in relatively short time intervals [10], it is also limited by the high costs of the appliances and equipment necessary PCR. Without mentioning operational errors during sample processing and the unavailability of adequate storage systems for reagents [11] In search of new, fast, and low-price detection methods, a Point-of-Care (PoC) approach is considered. PoC diagnostic tests offer immediate results without requiring specialized technical personnel or a diagnostic laboratory infrastructure [12].

PoC test types include the so-called "rapid test" or serology test. They consist of simple immunochromatographic examinations that detect the presence of IgM and IgG antibodies in response to SARS-CoV-2 infection in serum, plasma, or blood samples. IgM antibodies begin to be detectable in blood one week after the infection begins. In contrast, IgG antibodies appear in the late stage of the infection, which generally occurs after the second week and persists over time [14].

Serologic tests are based on the detection of antibodies against a specific targeted protein. Antibodies to the nucleocapsid protein are the most sensitive target for serological diagnosis of infection with SARS-CoV-2 [15]. Antibodies against the spike protein of SARS-CoV-2, the target of neutralizing antibody, emerge later than those against the nucleocapsid protein [16]. Despite this findings, further studies are needed to understand antibody dynamics in persons infected with SARS-CoV-2 to determine the most sensitive and specific antibody assays and to use these antibody-based tests to determine seroprevalence in different populations.

When it comes to this type of test's limitations, detecting specific immunoglobulins to a particular antigen makes it challenging to ascertain when they appear in blood samples so that a false negative diagnosis could be given [17]. Likewise, due to the SARS-CoV-2 profile, virus-specific IgG/IgM tests should not be considered a confirmatory diagnosis but as a complementary technique to molecular genetic analysis such as RT-qPCR [17,18]. In an earlier study that surveyed a total of 12,897 participants between April and June of 2020 in 10 provinces of the Dominican Republic, emerging hotspots yielded a positivity for all participants of anti–SARS-CoV-2 IgM of only 3.8% and IgG of 5.4% [19].

Recently another PoC test that attempts to diagnose COVID-19 using antigen (Ag) detection has been tested. These assays are currently in use based on a nitrocellulose membrane technology and nanoparticles based on colloidal gold sensitized with monoclonal antibodies directed against highly conserved SARS-CoV-2 antigens [20]. Some authors conclude that the assay is used to diagnose the disease within a few days after symptom onset when the upper respiratory tract's virus load is at its peak [21,22]. Understanding advantages and limitations of using Ag tests in different populations across a prevalence range will allow the tests to be deployed simultaneously with others to improve the COVID-19 response.

Given the current availability of different types of COVID-19 tests, countries are still struggling to meet crucial testing demands for patient management and surveillance. Molecular tests and Rapid Tests have different but complimentary roles in the pandemic response.

SARS-CoV-2 breath tests

A search of the PubMed electronic database was undertaken using the search terms "novel coronavirus", "COVID-19", "nCoV", "Breath-Test", "Breath", "Rapid Test" and "SARS-CoV-2" in various permutations and combinations. The literature search was performed with articles which were accepted before April of the year 2021.

Analysis of volatile organic compounds (VOCs) present in breath has been long reviewed as a potential diagnostics tool for many diseases [23-26]. Chen et al. reported possible breath-borne VOC biomarkers for SARS-CoV-2. Infected patients show to possess statistically significantly higher levels of ethyl butyrate (29.13-95.67) (95% CI, N = 10) than healthy controls (16-24.3) (95% CI, N = 12). Also, statistically, significantly lower levels of isopropanol (RI: 920.7; Dt: 1.2224) than healthy controls are considered as an infection proxy [27]. These two VOCs suggest that this methodology represents a "game-changer" in rapid viral detection as proposed. Mechanics of breath analysis based on gas chromatography (GC) coupled with mass spectrometry (MS) or ion mobility spectrometry (IMS) [29], as well as other slightly unorthodox yet interesting approaches like scent detection of VOCs specific to SARS-CoV-2 with trained dogs. [30] In the first case, this study implies a particular limitation with the use of GC-IMS, such as environmental contamination, which ultimately results in an incapacity to resolve signals because of the charge transfer [31].

According to Ruszkiewicz et al, for GC-IMS to be a more feasible option, there would have to be a significant development in an ionization source. Since this device requires photo-ionization mechanisms and atmospheric pressure ionization charge transfer for it to work correctly, there is a lot more buildout needed when it comes to the source for the technique to function adequately [29].

Another recent method described by Shan et al. consists of a portable device that evaluates the variation in electrical conductivity, which occurs due to the interaction of SARS-CoV-2 VOCs with specific ligands. The device consists of two parts: an inorganic part composed of gold nanoparticles and an organic part. Organic ligands are found, thus creating a useful matrix that reacts to VOCs [32] When performing the test on an infected individual, the VOCs will diffuse or remain on the matrix's surface, reacting with the organic part and with the functional groups found on the inorganic compounds, altering the volume, be it inflation or contraction. This volume alteration results in an increase or decrease in the electrical conductivity detected by sensors [33] Devices would assess these VOCs' presence, facilitating to quickly detect SARS-CoV-2 and discriminate it from others that might produce similar symptoms. VOCs are mostly present in a breath when the individual is in the first weeks of their infection, which could allow the early identification of COVID-19, thus diminishing the possibility of subsequent infections and improving the chances of rapid recovery individual [33].

To test the effectiveness of the technique, 140 patients from Wuhan took part in testing experiments. Three groups were considered: individuals infected with SARS-CoV-2, individuals with no signs of infection, and individuals that possessed other lung infections. The results showed that the test is close to PCR's exact detection percentages, which correspond to 82-98%. Regarding sensitivity, the values ranged between 83 and 90%, which exceeded the average of current rapid detection tests for SARS-CoV-2 [23]. These results suggest the portable 2-dimensional gas chromatography (p2d-GC) device is indeed suited for SARS-CoV-2 detection, which is still a fundamental shortcoming in the specificity values. The overall specificity for SARS-CoV-2 of this method was a calculated 69% [23], which is a low value for reliable detection.

It is important to note that, since these results can only be attributed to a pre-diagnosed population in Wuhan, China, these sensitivity, and specificity values cannot be taken as absolute to determine the average that this test would consistently present accurately. Due to the findings of sensitivity and specificity, some elements of internal and external validity remain answered.

Another two independent observational prevalence studies at Edinburgh, UK, and Dortmund, Germany were developed to evaluate the feasibility of using breath-analysis to differentiate SARS-COV-2 infection from other respiratory diseases [29]. These studies aimed to trial point-of-care testing using self-contained gas chromatography-ion mobility spectrometry (GC-IMS) breath-analyzers in two hospitals and evaluate the breath biochemistry for possible markers of SARS-COV-2. The following VOCs were found to be potentially discriminating for SARS-COV-2: ethanal, octanal, acetone, butanone, methanol, heptanal, and an unknown compound named feature 144.

From a total of 90 participants, 25 in Edinburgh and 65 in Dortmund, the VOC-based diagnosis agreed with the RT-q-PCR diagnosis using a Principal Component Analysis (PCA) multi-variate analysis. The Dortmund PCA stratification model had 90% sensitivity and 80% specificity and an area under the receiver operator characteristic (AUROC) of 0.91

for distinguishing SARS-COV-2 patients from other patients. Meanwhile, the resultant PCA stratification model in Edinburgh had 82.4% sensitivity and 75% specificity with an AUROC of 0.87 for distinguishing SARS-COV-2 patients from other patients [29].

The compounds identified indicated that changes in breath biochemistry followed the same pattern in both studies with elevated ketone; aldehyde and feature 144 signals accompanied by a suppressed methanol signal were proved to be significant. These biomarkers are in concordance with a combination of extrapulmonary, metabolic, and gastrointestinal manifestations of COVID-19 within the body and airway inflammatory responses, such as ketosis [32], impaired gastrointestinal function [33], and inflammatory responses [35]

Suppose further investigations with several populations, using confirmatory analytical techniques such as gas chromatography-mass spectrometry (GC-MS/IMS), show to be reliable. In that case, these SARS-CoV-2 breath tests offers the possibility for rapid diagnosis of SARS-COV-2 in emergency rooms and primary care units that have the infrastructure and equipment for required for this analysis. The results of this studies are summarized in Table 1.

Location	Principle	Population studied	Selected groups	Sensitivity (values ranged)	Specificity	References
Wuhan	P2D-GC	140 patients	Three groups were considered: individuals infected with SARS-CoV-2, individuals vwith no signs of infection, and individuals that possessed other lung infections.	83-90 %	69 %	23
Edinburgh	GC-IMS	25 patients	Individuals infected with SARS-CoV-2	82.4 %	75%	27
Dortmund	GC-IMS	65 patients	Individuals infected with SARS-CoV-2	90 %	80%	27

CONCLUSIONS

When it comes to developing new diagnostic tests to meet public health demands in the face of the SARS-CoV-2 pandemic, technologies aimed at designing and generating fast and affordable tests. However, approving these tests for diagnostic use requires peer-reviewed studies that confirm their ability to offer a reliable result. Were these tests not subjected to rigorous research, the method in question would run the risk of yielding imprecise results that do not adequately reflect the epidemiological landscape, thus obstructing public health decision-making.

The number of studies available to date for the rapid detection method of SARS-CoV-2 in-breath is not sufficient to justify their immediate public use. It is necessary to carry out studies in different populations so that the specificity of the presented method increases compared to the standard RT-qPCR technique, which remains the preferred one for diagnosing SARS-CoV-2, despite its limitations.

Tests based on direct and indirect identification techniques of SARS-COV-2 are of high interest to the public health authorities. The SARS-CoV-2 breath test has characteristics that can facilitate its implementation since it makes implications of the viral presence in patients, and the costs would also be minimal. However, the rapid detection method of SARS-CoV-2 in breath has minimal references in peer review journals, representing a gap in its application knowledge.

Before having full confidence in this test and starting its commercialization, more studies with a robust scientific design were carried out to evaluate its precise detection capacity and the variability of the results in different geographical areas. Breath tests are not a sufficiently evidence-based approach for rapid screening and to "secure" or creating “sanctuary” regions for touristic purposes. Therefore, policymakers must cautiously take this, pointing to the importance of further evaluation and structured studies confronting gold-standards with new devices.

Contributions

AVD and RPR conceived the manuscript and thoroughly revised the literature. AVD, DMH, DH, EC, MP, MR and CM revised the existing literature on SARS-CoV-2 tests and provide insights on technical characteristics. AVD and RPR drafted the revised version of the manuscript; and critically revised the manuscript for intellectual content and approved the final version. RPR is the guarantor of the paper.

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