Detection of SARS-CoV-2 infection by exhaled breath spectral analysis: Introducing a ready-to-use point-of-care mass screening method

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Summary

Background The current SARS-CoV-2 pandemic created an urgent need for rapid, infection screening applied to large numbers of asymptomatic individuals. To date, nasal/throat swab polymerase chain reaction (PCR) is considered the "gold standard". However, this is inconducive to mass, point-of-care (POC) testing due to person discomfort during sampling and a prolonged result turnaround. Breath testing for disease specific organic compounds potentially offers a practical, rapid, non-invasive, POC solution. The study compares the Breath of Health, Ltd. (BOH) breath analysis system to PCR's ability to screen asymptomatic individuals for SARS-CoV-2 infection. The BOH system is mobile and combines Fourier-transform infrared (FTIR) spectroscopy with artificial intelligence (AI) to generate results within 2 min and 15 s. In contrast to prior SARS-CoV-2 breath analysis research, this study focuses on diagnosing SARS-CoV-2 via disease specific spectrometric profiles rather than through identifying the disease specific molecules.

Methods Asymptomatic emergency room patients with suspected SARS-CoV-2 exposure in two leading Israeli hospitals were selected between February through April 2021. All were tested via nasal/throat-swab PCR and BOH breath analysis. In total, 297 patients were sampled (mean age 57·08 ± SD 18·86, 156 males, 139 females, 2 unknowns). Of these, 96 were PCR-positive (44 males, 50 females, 2 unknowns), 201 were PCR-negative (112 males, 89 females). One hundred samples were used for AI identification of SARS-CoV-2 distinguishing spectroscopic wave-number patterns and diagnostic algorithm creation. Algorithm validation was tested in 100 proof-of-concept samples (34 PCR-positive, 66 PCR-negative) by comparing PCR with AI algorithm-based breath-test results determined by a blinded medical expert. One hundred additional samples (12 true PCR-positive, 85 true PCR-negative, 3 confounder false PCR-positive [not included in the 297 total samples]) were evaluated by two blinded medical experts for further algorithm validation and inter-expert correlation.

Findings The BOH system identified three distinguishing wave numbers for SARS-CoV-2 infection. In the first phase, the single expert identified the first 100 samples correctly, yielding a 1:1 FTIR/AI:PCR correlation. The two-expert second-phase also yielded 1:1 FTIR/AI:PCR correlation for 97 non-confounders and null correlation for the 3 confounders. Inter-expert correlation was 1:1 for all results. In total, the FTIR/AI algorithm demonstrated 100% sensitivity and specificity for SARS-CoV-2 detection when compared with PCR.

Interpretation The SARS-CoV-2 method of breath analysis via FTIR with AI-based algorithm demonstrated high PCR correlation in screening for asymptomatic individuals. This is the first practical, rapid, POC breath analysis solution with such high PCR correlation in asymptomatic individuals. Further validation is required with a larger sample size.

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Abbreviations: AI, artificial intelligence; BOH, Breath of Health Ltd; FTIR, Fourier-transform infrared spectroscopy; GC-IMS, gas chromatography ion mobility spectrometry; GC−MS, gas chromatography mass spectrometry; PCR, polymerase chain reaction; POC, point-of-care; SD, standard deviation; VOC, volatile organic compounds

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Research in context

Evidence before this study

The current SARS-CoV-2 pandemic created an urgent need for a rapid, mobile, usable, highly sensitive, and specific point-of-care SARS-CoV-2 screening method. The current "gold standard" nasal/throat swab PCR test has a prolonged result turnaround time and is uncomfortable to the person being tested. Exhaled air testing technologies offer great potential for providing this solution along with a non-invasive sampling method. We searched the PubMed database for combinations of the words and MeSH Terms "exhaled breath analysis", "breath analysis", "COVID-19", "SARS-CoV-2" and "diagnosis" in November 2021. We detected 15 research studies focused on SARS-CoV-2 detection via breath analysis. Some of these studies presented high values of sensitivity and specificity. However, none of them have reached a level of practical usability. Some require high level of expertise to run the tests, some require a prolonged testing interval, some were primarily initial proof-of-concept studies, and some had relatively poor sensitivity and specificity when compared with PCR. Most studies focused on symptomatic patients and did not demonstrate the ability to screen asymptomatic individuals. To date, despite its attractiveness and great promise, no breath testing technology has managed to present a usable, mobile, rapid, highly sensitive, and specific solution for rapid mass SARS-CoV-2 screening.

Added value of this study

This study presents a ready-to-use breath analysis method for SARS-CoV-2 screening that utilizes Fourier-transform infrared (FTIR) spectroscopy combined with an artificial intelligence (AI) generated algorithm applied to FTIR result interpretation. The entire process is performed by a mobile point-of-care machine within less than 3 min. The study focuses on using a disease specific spectrometric profile for SARS-CoV-2 diagnosis rather than the identification of disease specific molecules. This is accomplished by generating an AI-based algorithm for SARS-CoV-2 diagnosis via FTIR breath analysis. Subsequently, the study presents two phases of validation data obtained through expert determination of SARS-CoV-2 status based on AI algorithm generated criteria. In both phases the AI algorithm results are compared/correlated with PCR results. The results show a 1:1 correlation with the "gold standard" PCR. This device is the first to offer a practical solution for rapid, non-invasive SARS-CoV-2 screening in settings such as airports and sports arenas. In the current SARS-CoV-2 pandemic, the urgent international need for such technology is of paramount global importance.

Implications of all the available evidence

Multiple prior breath-analysis studies focused their efforts on SARS-CoV-2 detection in patients who were symptomatic. Their various employed technologies, predominantly focused on PCR or volatile organic compound (VOC) detection, and were limited in their ability to provide adequately rapid result turnover. They focused on symptomatic individuals, who are anticipated to have higher viral loads and greater VOC production, increasing the likelihood of VOC detection. The primary international need for quelling viral contagion demands rapid mass screening of asymptomatic populations early in their infection course at points of mass human congregation. This proof-of-concept study has promising results that provide the first evidence of a usable breath analysis method that makes this goal achievable. The results require further validation due to the study's proof-of-concept design and small sample size. Additional study is also warranted to demonstrate its usability in a variety of settings and geographic locales.

Introduction

The current SARS-CoV-2 pandemic sparked an explosion of human innovation and technological development in every aspect of pandemic spread; viral genome discovery, understanding of disease progression, treatment strategies and diagnostic testing. Many governments and regulatory bodies continue to focus on the latter as they strive to balance the public health need for viral contagion containment with the human need for social interaction and economic stability.[1-7] The deployment of mass screening in large public venues such as airports and sports events behooves the urgent need for point-of-care (POC) diagnostic screening that is minimally invasive, cost-effective, rapid, mobile, and highly sensitive and specific.[5-8] The currently employed testing methods of nasal/throat swabs for PCR or antigen testing fall short in meeting these criteria. The swabbing itself is often uncomfortable, if not painful. The PCR testing is relatively slow, mostly producing results after more than 1 hour.[9] The antigen tests have shown varying degrees of sensitivity, ranging from 36 to 82%, when used for screening asymptomatic persons.[10-14] Therefore, there is urgent need for such rapid POC SARS-CoV-2 testing, to allow humans to return to public congregation, ease the public laboratory workload, and relieve the economic burden caused by lengthy or unreliable testing methods.

The last decade has seen an uprising trend in disease diagnosis through exhaled air analysis. This spans from malignancy detection,[15,16] through analysis of metabolic states,[17,18] to host and invader diagnostics.[19-21] The term "breath-print" has been coined to express the multiplicity of techniques that have been applied to analyze the various contents of exhaled air, including particulate matter, aerosol, and gaseous content.[22]

Exhaled breath testing could provide an appealing minimally invasive solution for SARS-CoV-2 testing. Exhaled air testing, as opposed to blood drawing, nasal/

throat swabbing, or other testing methodologies, is a non-invasive method of objective human evaluation. To date, several technologies have been developed for exhaled breath analysis. To wit, SARS-CoV-2 viral particles are exhaled and detectable via exhaled PCR testing.[23-26] However, as with other PCR technology, this is neither rapid nor conducive to POC testing. Gas chromatography mass spectrometry (GC−MS) allows for the detection of hundreds of volatile organic compounds (VOC's) at the 10 part-per-trillion by volume (pptv) level and is considered the gold-standard testing method for exhaled VOC's.[27,28] However, it involves manual procedures of sampling and preparation, cannot perform rapid trace-gas measurements, requires expensive equipment, precise calibration, and administration by trained personnel. Overall, this precludes its application to single-breath analysis at the point of care.[28]

Mid-infrared (MIR) laser spectroscopy allows for the development of compact point-of-care optical instruments, enabling even single breath diagnostics.[28] Herein, we present the novel application of a breath test utilizing Fourier-transform infrared (FTIR) spectroscopy technology combined with artificial intelligence (AI) to detect SARS-CoV-2 infection via organic compound detection in exhaled breath. The spectrometer of FTIR is easily available, mobile, simple to operate, and has demonstrated a high degree of sensitivity for organic compound detection.[27] In this study, we aim to compare this methodology's sensitivity and specificity for SARS-CoV-2 detection to the current in-situ gold-standard in SARS-CoV-2 testing, the reverse transcriptase polymerase chain reaction (PCR) performed in hospital laboratories. If successful, this methodology can offer a sensitive and specific, rapid, non-invasive, point-of-care solution for mass SARS-CoV-2 testing.

Methods

Study design
This is a proof-of-concept study designed in two phases. The first phase involves AI algorithm development to identify exhaled breath FTIR wave frequency absorbance profiles that distinguish SARS-CoV-2 positive and negative states. This is achieved by comparing exhaled breath FTIR wave frequency absorbance profiles of SARS-CoV-2 PCR positive and negative patients. The second phase involves preliminary algorithm validation. Patients are diagnosed as SARS-CoV-2 positive or negative through applying algorithm rules to exhaled breath FTIR test results. Algorithm validation is achieved by correlating the algorithm-based diagnosis with same-patient PCR results. Conceptually, PCR status correlation with a particular wave frequency absorbance pattern associates the PCR status with the presence or absence of a specific molecule or molecule

combination. To date, SARS-CoV-2 breath analysis studies focused on diagnosing the disease state through identifying the presence or absence of such disease specific molecules.[25,26,29-35] The current study aims to achieve disease state diagnosis using disease specific wave frequency absorbance patterns without the need for identifying the underlying molecules.

Measuring device − pertinent details
The Breath of Health Ltd. (BOH) Merkava 1.1 device consists of an FTIR analytical system connected to an artificial intelligence (AI) Intel® computer portal (catalog number NUC IV790174816). An auxiliary patented system developed by BOH allows for the detection of non-volatile (e.g., C-reactive protein, interleukin-6), semi-volatile (e.g., steric acid) and volatile organic compounds (e.g., aldehydes and ketones) via a thermodynamic process in the gas cell mounted on the FTIR. The entire system dimensions are W 70 cm x L 90 cm x H 115 cm, roughly the size of a bank automated teller machine (ATM) or small video arcade game.

The analytical system consists of a Thermo Fisher Nicolet™ iS™10 FTIR spectroscope (catalog number IQLAADGAAGFAHDMAPC) with a Specac heated gas cell accessory (catalog number GS24652) with an optical length of 5 m capable of detecting 200 ppb, and a Thermo Fisher MCT detector (catalog number Thermo Scientific™ TN714−008,400) capable of reducing the signal-to-noise ratio related to our threshold detection. The gas cell accessory used for FTIR reading is warmed continuously to ~60 °C and keeps a constant flow of nitrogen (Oxygen and Argon Works, Ltd., Caesarea, Israel) into the cell (to which the FTIR is blinded). The Tedlar® bag containing the sampled exhaled air is cooled in a freezer at −20 °C for 90 s and is then emptied by vacuum into the measuring cell accessory. The FTIR spectroscope measuring chamber is heated constantly to 60 °C, pressurized to 2 atmospheres, and illuminated by an infra-red (IR) source. The entire process of measuring and obtaining results takes 45 s. Cleaning the machine between samples takes 45 s. Total cycle time takes less than 2 min. Total person wait time from sample collection till obtaining results is 2 min and 15 s (i.e., sample cooling and measuring times).

Background − How the BOH system generates its data: Each exhaled air sample is inspected by the BOH system using 32 screenings under the lowest FTIR frequency resolution of 4 cm^{-1}. This generates a vector of 7882 data points for each sample. The vector's X-axis represents light wavelength frequency, presented as wave numbers (cm^{-1}). The Y-axis represents the signal intensities, presented as the spectrometer signal absorbances' second derivative value. This data represents readings of various organic compounds. Peaks or nadirs in this second derivative value signify the

PCR RESULT	Total Number of Patients (n)	Age In Years (Mean±SD)	GENDER	Total Number of Patients (n)	Age In Years (Mean±SD) Median	AI Learning/Algorithm Development Number of Patients: (n)	Age In Years (Mean±SD) Median	Algorithm Validation: Single Expert Testing Number of Patients: (n)	Age In Years (Mean±SD) Median	Further Algorithm Validation: Two Expert Testing Number of Patients: (n)	Age In Years (Mean±SD) Median
			TOTAL	297*	18 - 95 (57•08 ± 18•86) Median=59	100	18 - 95 (60•62 ± 19•60) Median=62	100	22 - 91 (57•30 ± 17•55) Median=60	97*	19 - 91 (53•14 ± 18•63) Median=55
PCR Positive	96	19 - 90 (55•45 ± 17•28) Median=55.5	Male	44	22 - 90 (54•82 ± 17•46) Median=55.5	26	22 - 90 (55•50 ± 18•13) Median=57.5	14	23 - 82 (57•28 ± 14•38) Median=57	4	26 - 74 (49•00 ± 17•71) Median=48
			Female	50	19 - 90 (55•72 ± 17•37) Median=56	24	19 - 90 (56•08 ± 17•92) Median=57.5	20	24 - 89 (55•55 ± 16•85) Median=56.5	6	29 - 80 (52•33 ± 16•83) Median=52
			Unknown	2	unknown	NA	NA	NA	NA	2	unknown
			Male	112	18 – 95 (57•61 ± 19•25) Median=62	29	18 - 95 (63•86 ± 18•47) Median=66	34	22 - 91 (56•67 ± 17•40) Median=61.5	49	19 - 85 (54•57 ± 20•05) Median=60
PCR Negative	201	18 - 95 (57•85 ± 19•51) Median=61	Female	89	19 – 93 (58•14 ± 19•82) Median=61	21	21 - 93 (67•66 ± 21•54) Median=70	32	22 – 87 (59•06 ± 19•15) Median=62.5	36	19 – 91 (51•77 ± 16•74) Median=51.5
			Unknown	NA	NA	NA	NA	NA	NA	NA	NA

Table 1: Patient Demographics — Age and Gender Distribution by PCR-Result. Table 1 presents participating patient demographics by age and gender divided into subgroups by study phase (AI Learning/Algorithm Development, Algorithm Validation Single Expert Testing, Further Algorithm Validation/Two Expert Testing) and PCR result.

Abbreviations: AI = artificial intelligence, NA = not applicable, PCR = polymerase chain reaction, SD = Standard Deviation.

*Does not include 3 confounders added to the third set and presented to the experts as new patient data.

respective presence or absence of one specific molecule type, or a specific molecule combination.

Patients

Patients were selected in two leading Israeli hospitals based on the following criteria. All patients were seen in the various hospitals' emergency departments and selected for SARS-CoV-2 testing due to possible exposure to SARS-CoV-2 infected persons. Selected patients were asymptomatic for COVID-19. Patients were excluded from the study if they had any of the following symptoms: difficulty breathing or cough with shortness of breath, along with at least two of the following COVID-19 symptoms: fever, chills, muscle pain, headache, sore throat, or a new loss of taste or smell. Since this was an initial proof-of-concept study, data was not collected regarding subject co-morbidities, medical background, SARS-CoV-2 vaccination status, or other symptomatology. Formal power analysis was not performed since the study involved the initial AI algorithm creation such that it was impossible to perform a preemptive power analysis. Sample size was determined by the number of patients recruited during the data collection period. The study was approved by the respective institutional review boards at the participating hospitals. The study was registered in the Israeli Ministry of Health database for clinical studies (Protocol #: MOH_2020−10−15_009403). Signed informed consent was obtained from all patients prior to study participation. Patients were coded and study investigators were blinded to study participant identities. Patient recruitment and sample collection occurred from February through April 2021.

Sample collection

All patients were sampled via SARS-CoV-2 PCR nasal/throat swabs and exhaled breath. PCR testing was done using routine respective hospital laboratory machinery (Hadassah Ein Karem Hospital: BGI Genomics Real-Time Fluorescent RT-PCR Kit for SARS-CoV-2; Sheba Tel HaShomer: Seegene, Inc. RT-PCR). Exhaled breath samples were obtained as follows: Subjects were asked to exhale, then inhale deeply and then exhale into a recyclable plastic (Tedlar®) bag (catalog number SKC232 −945A) 0.7-liter air by volume. Tedlar® bags were used since they have been shown to be suitable for diagnostic breath sampling due to minimal pollutants and breath product decay.[36,37] The exhale tube's diameter size was carefully selected to just allow a medium exhalatory effort into the bag. Exhaled breath samples were analyzed via FTIR spectroscopy utilizing the Breath of Health Ltd. (BOH) Merkava 1.1 device. All breath samples were discarded following testing.

Subject demographics

There were 297 patients sampled in total (96 from Hadassah Ein Karem Hospital, Jerusalem and 201 from Sheba Tel HaShomer Medical Center, Ramat Gan), of which 96 were PCR-positive and 201 were PCR-negative, respectively. All samples were used and there were no outliers removed from the study. Samples were divided into three sets. One-hundred samples were used for AI learning and algorithm development, 100 were used for preliminary algorithm validation, and 97 samples with 3 additional confounders (100 in total) were used for further validation via two-expert testing. Subject gender and age demographics, sub-grouped based on PCR-results are presented in Table 1. Patients were 156 males, 139 females and 2 unrecorded gender/age with an age range of 18 − 95 years (Mean 57·08 ± SD 18·86). The AI learning/algorithm development set included 55 males and 45 females: age range 18 − 95 (Mean 60·62 ± SD 19·60). The first validation: single-expert testing set included 48 males and 52 females: age range 22−91 (Mean 57·30 ± SD 17·55). The second validation set included 53 males, 42 females and 2 unknowns: age range 19 − 91 (Mean 53·14 ± SD 18·63). Overall, age ranges were similar and showed significant overlap.

The process

Phase 1 − Algorithm Development:

The initial step served to develop the BOH system artificial intelligence (AI) algorithm's points of distinction between SARS-CoV-2 PCR-positive and negative samples. One-hundred samples (50 PCR-positive; 50 PCR-negative) were randomly selected and used for AI learning and algorithm development. The model found all the wave number signal intensity peaks and nadirs for each sample. These represented the maximum and minimum signal intensity (Y-axis) values. The algorithm model created a composite of all the wave numbers (X-values) for which there were peaks and nadirs in the signal intensity (Y-values). It then classified the peaks and nadirs into PCR-positive and negative sample groups, thus creating a raw PCR-positive and PCR-negative wave number profile, respectively. It then subtracted the PCR-negative from the PCR-positive raw profile. The differences represented a set of wave number changes relatively unique to the PCR-positive profile ("positivity distinguishing profile"). Lastly, it selected the wave numbers within the positivity distinguishing profile with the highest probability of selectivity for SARS-CoV-2 positivity (i.e., highest probability of correlating with PCR-positivity). This selection was performed through a set of probability rules derived from AI training.

Phase 2 Step 1 − Initial Algorithm Validation/Proof-of-Concept Testing:

Firstly, 10 of these samples in the first set (5 PCR-positive; 5 PCR-negative) were used to train a medical

expert (IBS) in distinguishing between algorithm-generated positive and negative results. Subsequently, 100 samples (34 PCR-positive; 66 PCR-negative), were used for proof-of-concept and algorithm validation testing by the expert. In an asymptomatic cohort, only a few positive cases are anticipated. This was mirrored by having negative sample predominance (66%). However, the number of positive cases (34%) is high, relative to a real-life asymptomatic cohort, to allow for more robust validation. The medical expert was blinded to each sample's PCR results and to the ratio of PCR-positive:PCR-negative results, and determined SARS-CoV-2 exhaled breath results solely based on the algorithm. Subsequently, expert determinations were correlated with PCR results.

Phase 2 Step 2 — Further Algorithm Validation/Inter-expert Correlation:

A third set of 100 samples (12 PCR-positive; 85 PCR-negative; 3 faux confounders) was used to test for inter-expert differences in algorithm-based result determination and for further algorithm validation. This set included only 12 positive samples to render it more representative of an asymptomatic cohort screening where only a few positive cases are anticipated. A second medical expert (HF) was trained in determining algorithm-based results using the same 10 training samples as the first expert. The primary researchers created 3 confounding sample results. These samples were PCR-positive but were presented to the experts with intentionally altered FTIR/AI wave number results that were SARS-CoV-2 negative per algorithm rules. This precaution was taken due to the first expert testing yielding 1:1 breath:PCR result correlation. Its purpose was to demonstrate expert blinding. The two medical experts (IBS and HF) were blinded to each sample's PCR results and to the ratio of PCR-positive:PCR-negative results, and determined SARS-CoV-2 exhaled breath results solely based on the algorithm. Both experts were also blinded to the addition of confounders to the sample set and were only informed of these after submitting their respective results.

Role of the funding source

RESULTS

Algorithm AI result and sample interpretation

The AI model identified three distinguishing wave numbers. Wave number #1 (X-value 2808.44) had a signal intensity (Y-value) that was peaked in the PCR-positive patients relative to the signal intensity in both its flanking wave numbers but was lower than at least one flanking wave number in PCR-negative patients. In contrast, two other distinguishing wave numbers [Wave numbers #2 (X-value 3230.31) and #3 (X-value 3404.36)] were higher in signal intensity (Y-value) than at least one flanking wave number in PCR-positive patients but were at a nadir relative to both flanking wave numbers in PCR-negative patients (Figure 1.a-f). According to the algorithm, a sample is determined SARS-CoV-2 positive or negative based on at least 2 of 3 wave numbers indicating the same result.

Initial algorithm validation: single expert testing results

For each sample in the second set, each distinguishing wave number was assessed, in isolation, by the medical expert. As per algorithm rules, the expert determined the composite SARS-CoV-2 positive or negative status based on at least 2 of 3 wave numbers indicating the same result. In 97 of 100 proof-of-concept samples, all three distinguishing wave numbers yielded concordant results. In only three cases, one wave number result was discordant with the other two wave numbers. A different wave number was discordant in each of the three. All discordant wave numbers had the following commonalities: 1) All occurred in PCR-negative samples, 2) all indicated SARS-CoV-2 positivity while the other two wave numbers indicated negativity.

PCR correlation data for this single-expert testing phase is presented in Table 2. All 34 PCR-positive samples were diagnosed by the expert as SARS-CoV-2 positive based on algorithm criteria. All 66 PCR-negative samples were diagnosed by the expert as SARS-CoV-2 negative based on the algorithm criteria. In comparison with PCR, the algorithm's sensitivity and specificity were both 100% and algorithm/AI based SARS-CoV-2 determination correlated 1:1 with PCR results.

Further algorithm validation: two expert testing results

In 95 of 100 samples tested by two experts; all three distinguishing wave numbers yielded concordant results. In only two non-confounder cases, one wave number
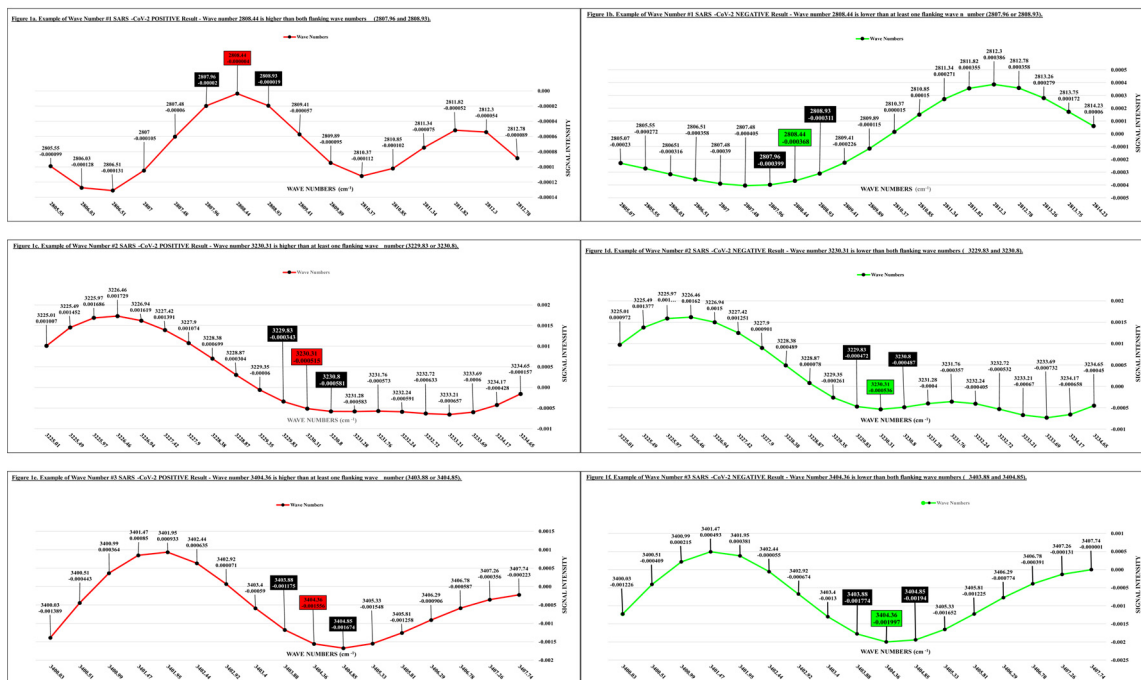
Figure 1. (a-f) present graphic examples of each of the three FTIR AI algorithm generated distinguishing wave number as they appear in SARS-CoV-2 Positive and Negative results, respectively. Each Figure presents a graph of Wave Numbers (cm^{-1}) vs. Signal Intensity second derivative values. SARS-CoV-2 Positive graphs appear in red and SARS-CoV-2 Negative graphs appear in green.

result was discordant with the other two wave numbers, and the experts' SARS-CoV-2 status determinations were based on the majority. The remaining three discordant samples were confounders and experts determined SARS-CoV-2 status based on the majority.

PCR correlation data for this two-expert testing phase is presented in Table 3. All 12 non-confounder PCR-positive samples were diagnosed by both experts as SARS-CoV-2 positive based on the algorithm criteria. All 85 non-confounder PCR-negative samples were diagnosed by both experts as SARS-CoV-2 negative based on the algorithm criteria. All three confounders were diagnosed by both experts as SARS-CoV-2 negative, as per algorithm criteria. For all 97 non-confounder

results, expert final diagnosis had an inter-evaluator correlation of 1:1. Expert individual wave number determination for each sample also had an inter-evaluator correlation of 1:1.

In terms of PCR correlation, FTIR algorithm/AI based SARS-CoV-2 determination of non-confounder samples correlated 1:1 with PCR results and the algorithm's sensitivity and specificity were 100% each, respectively. For all three confounders, FTIR algorithm/ AI based SARS-CoV-2 determination had null correlation with PCR results, though inter-evaluator

Total = 100	Expert Determination Positive	Expert Determination Negative
PCR-positive	34	0
PCR-negative	0	66

Table 2: PCR Correlation − Algorithm Validation: Single Expert Testing. Presents correlation data between PCR result and single-expert determination of SARS-CoV-2 positive/negative status based on FTIR test results interpretation using the AI derived algorithm criteria.
Abbreviations: AI = artificial intelligence, NA = not applicable.
PCR = polymerase chain reaction.

Total = 100	Expert Determination Positive	Expert Determination Negative
PCR-positive	12	3 (confounders)
PCR-negative	0	85

Table 3: PCR Correlation − Further Algorithm Validation: Two Expert Testing. Presents correlation data between PCR result and two-expert determination of SARS-CoV-2 positive/negative status based on FTIR test results interpretation using the AI derived algorithm criteria. Experts' determination is presented as unified values there was 1:1 agreement in all their determinations.
Abbreviations: AI = artificial intelligence, FTIR= Fourier-transform infra-red spectroscopy,.
NA = not applicable, PCR = polymerase chain reaction.

	Total	Wave Number #1 (2808.44)	Wave Number #2 (3230.31)	Wave Number #3 (3404.36)	Final Determination
PCR-positive	46	46	46	46	46
PCR-negative	151	150	148	150	151
PCR-positive% Correlation	NA	100%	100%	100%	100%
PCR-negative% Correlation	NA	99.34%	98.01%	99.34%	100%

Table 4: **Distinguishing Wave Number Correlation with PCR**. Presents PCR-status correlation data for FTIR/AI algorithm-based SARS-CoV-2 status determination. Results are divided based on PCR status. Results are subdivided to demonstrate PCR-result correlation for each of the three distinguishing FTIR Wave Numbers as per AI-algorithm criteria. Results are presented in absolute numbers and as percent correlation for PCR-positive and negative samples, respectively.

Abbreviations: AI = artificial intelligence, FTIR= Fourier-transform infrared spectroscopy.
NA = not applicable, PCR = polymerase chain reaction.
**Confounder samples not included in table data.

correlation was maintained at 1:1 for final expert diagnosis and individual wave number determination.

Table 4 presents a composite breakdown of distinguishing wave number correlation with PCR. All three wave numbers demonstrated 1:1 PCR correlation (100% specificity) for SARS-CoV-2 positivity. All three wave numbers had instances of false positive results though 1:1 PCR correlation remained high at 99.34%, 98.01% and 99.34%, respectively.

Discussion

In this study FTIR technology combined with artificial intelligence (FTIR/Algorithm) was compared to PCR in its ability to identify and distinguish asymptomatic SARS-CoV-2 positive and negative patients, respectively. The FTIR technology combined with the AI based algorithm demonstrated a 1:1 correlation with SARS-CoV- 2 PCR findings with a sensitivity and specificity of 100%. This is the first usable, rapid, point-of-care breath analysis technology that has demonstrated such strong correlation with PCR in SARS-CoV-2 screening of asymptomatic individuals. This opens important possibilities for rapid, mass, point-of-care screening, such as at airports and large sports arenas.

It is rare to find 100% correlation between two testing methodologies. This, therefore, requires further elaboration. Firstly, the algorithm is based on the findings of three, rather than one, wave numbers. Although each wave number demonstrated less than 100% sensitivity, the additive effect of all three wave numbers, each with >95% sensitivity (Table 4), can easily yield 1:1, or near 1:1 correlation. To that effect, the study is limited by the relatively small sample size of 297 samples and the lack of a formal power analysis. The BOH FTIR/AI system requires further validation via studies involving a larger number of samples with a formal power analysis. None the less, the following factors should have increased the odds of discordancy; the absence of which increases the strength of our findings:

1. The FTIR/AI algorithm was tested in two phases, each of which demonstrated 1:1 PCR correlation, in isolation.
2. Samples were obtained from two different hospitals,
3. PCR testing was conducted in two separate laboratories,
4. A second evaluator tested the second set,
5. Confounding samples were added to the second set.

On the one hand, SARS-CoV-2 PCR has a near 100% specificity, which suggests that the FTIR/AI system can provide equal specificity with 1:1 PCR correlation. However, the "gold-standard" SARS-CoV-2 PCR has its own limitations and can require repeated testing due to a possible initial false negative rate of up to 54%.[38]

The 1:1 PCR correlation suggests that the FTIR/AI system has the same limitations and provides false negative results in the same patients. The reasons for this may be multi-factorial. The PCR false negative rate has several etiologies. The first is sampling error, though this can be reduced, if not eliminated, when sampling is performed by experienced professionals. In this study, since PCR samples were obtained by experienced emergency room staff, they may be void of sampling error. Secondly, PCR false negative rate is highest when viral loads are low, specifically, when obtained within the first few days of, or 1−1.5 weeks following, infection.[39] This is expected to parallel disease associated metabolic processes that produce organic compounds. Early in the infective period the individual has a low viral load, is asymptomatic and has barely commenced these metabolic processes.[39] The individual can therefore evade both PCR and organic compound testing. The disease specific metabolic processes increase in tandem with rising viral loads and both viral RNA and distinguishing organic compounds rise to detectable levels.[33,39,40] Further testing will be required to determine whether repeated testing or a more complex AI algorithm could reduce the false negative rate and identify infected

individuals prior to PCR detectability. Testing subjects serially with tandem FTIR/AI, PCR, and antigen testing could further elucidate FTIR/AI sensitivity and specificity relative to PCR and antigen testing throughout the disease time-course.

The study has several limitations surrounding sampling and subject-related conditions. Firstly, although this method does not attempt to identify the distinguishing compound, it is likely that the distinguishing wave numbers represent products of innate immune system activation.[41] It is conceivable, therefore, that FTIR breath analysis could miss sub-populations of immunosuppressed SARS-CoV-2 positive patients in whom these production processes are subdued. Similarly, the study's design as a proof-of-concept with limited sample size and lack of subject comorbidity data leads to additional questions regarding sampling . The subject sample had a male predominance overall, with more males testing negative in all groups. Prior studies repeatedly demonstrated male gender as a risk factor for COVID-19 morbidity and mortality.[42-50] This includes gender differences in immune response.[48,50] Recently, Liangou and colleagues demonstrated age differences in the concentrations of various VOC's of individuals with COVID-19.[51] It is possible that similar age and gender differences exist in organic product production in the asymptomatic phase. Additionally, sampling occurred in the winter and early spring, yet subjects were not tested for possible infection, or co-infection, with other respiratory pathogens. Furthermore, it is conceivable that the subject group recruited in the emergency department was more homogenous and less representative of the general population screened in a non-medical public venue. Lastly, subject SARS-CoV-2 vaccination status was unknown and SARS-CoV-2 variant type was not determined in SARS-CoV-2 positive patients. The latter requires further clarification since the public health risk of the SARS-CoV-2 evolving genome demands that any potential SARS-CoV-2 diagnostic test undergo mutation and vaccine-specific validation. This is highlighted by the rapid global spread of the Omicron (B.1.1.529) variant at the time of this study's publication.[52] Overall, any of the aforementioned factors could skew results, either by altering subject immune response, or by limiting generalizability.

Despite the above, study results and epidemiological data suggest some important hypotheses. The 1:1 FTIR to PCR correlation raises the important possibility that the wave number distinguishing profile targets a set of disease process products specific to SARS-CoV-2 infection, largely uninfluenced by other factors. Alternatively, the 1:1 PCR correlation may suggest the absence of such factors in the study sample. For example, the 2021 winter/spring seasons in Israel, as in other geographic regions, experienced an atypically low incidence of respiratory pathogens other than SARS-CoV-2.[53] Therefore, it is less likely that study subjects suffered from non-SARS-CoV-2 respiratory pathogens. Further research is needed to determine whether SARS-CoV-2 induces a disease specific wave number signature in the presence of other respiratory pathogens even early in the disease course when the relatively non-specific innate immune response predominates. In terms of SARS-CoV-2 variants, Israel Ministry of Health data indicate that during the study recruitment period, SARS-CoV-2 variants Alpha (B1.1.7), Beta (B.1.351) and Gamma (P.1) predominated as disease producing variants in Israel.[54] The first cases of variant Delta (B.1.617.2) appeared in Israel in mid-April 2021 and likely had little, if any, representation in the study population. It is likely, therefore, that all three variants (Alpha, Beta and Gamma) were represented in the SARS-CoV-2 positive subjects and that FTIR results correlated with PCR findings in all three variants. However, the above explanations are speculative and further research is needed to determine BOH FTIR/AI performance in various sub-groups including the immunosuppressed, during concurrent infection, in vaccinated and unvaccinated individuals and for current and future variants.

Several prior studies incorporated AI algorithms with breath analysis technologies to yield a COVID-19 specific diagnostic breath print. Ruszkiewicz and colleagues[31] and Chen and colleagues[29] used gas chromatography ion mobility spectrometry combined with machine learning to develop COVID-19 VOC diagnostic prediction models. Steppert and colleagues developed a diagnostic prediction model using VOC breath analysis via multicapillary column ion mobility spectrometry coupled with machine learning.[35] More recently, Liangou and colleagues utilized proton transfer reaction time-of-flight mass spectrometry coupled with AI to also generate a VOC disease distinguishing profile.[51] Unlike these studies, we posit that distinguishing light frequency/intensity patterns are sufficient for accurate disease state diagnosis without having to identify the underlying molecules or molecular combinations. Additional FTIR data analysis could likely reach such identification, which could add scientific value. However, from a diagnostic perspective, the primary importance lays in finding a method with strong sensitivity and specificity that can accurately diagnose the disease state, regardless of whether the method identifies a specific compound. The FTIR system aims to provide this accuracy via distinguishing wave patterns, whereas other methods aim to achieve the same goal by identifying distinguishing compounds. Our study results demonstrated a 1:1 correlation with PCR. No study to date successfully demonstrated this degree of PCR correlation. Furthermore, this study focused on SARS-CoV-2 detection in asymptomatic individuals due to its importance for mass screening. In contrast, all the identified studies focused on, or included, symptomatic individuals.[26,29-32,34,35,51] Symptomatic individuals have higher viral loads and increased immune response activation. It

is not surprising then, that they have higher quantitative VOC or RNA loads that are detectable by breath analysis. Without further testing, this cannot be extrapolated to mass screening of asymptomatic individuals.

Additionally, the BOH FTIR/AI-based system possesses several attractive qualities in terms of its usability. It is ready-to-use in its current point-of-care form, is mobile, user-friendly, and requires minimal training to operate. It has a self-contained purification and decontamination system. As such, it can be deployed in any location with a reliable source of electric power. In terms of cost effectiveness, each BOH FTIR/AI machine costs less than $100,000 and efforts are underway to further reduce costs. Lastly, it has a full cycle time (testing and purging) of only 2 min 15 s per sample. In contrast, all the studies to date, fall short in these points. Some are presented as preliminary feasibility studies.[24,30,33] All the researched technologies to date are lacking in their ability to provide a result turnaround that enables rapid human throughput. The need for rapid throughput is of prime importance in providing mass screening in venues demanding mass rapid turnover, such as airports and sports arenas. PCR based breath testing shares the time limitations of current PCR technology, requiring at least 30 min for result turnaround. Many gas chromatography based systems require about 10 min or more for testing and purging.[26,29] For example, ion mobility gas spectrometry (GC-IMS) requires 2−10 min for testing and 5 min to clean the machinery (purge time) between tests. Multi-capillary column coupled ion mobility spectrometry (MCC-IMS) technology requires a 6-minute testing window and 10-minute purge time. Both technologies allow for only 3−4 tests per hour. Some studies did not present the time required for a full cycle of testing and purging.[31,35] Liangou and colleagues recently reported a proton transfer reaction time-of-flight mass spectrometry/AI model with a rapid test run time of 200 s but did not report the purge time or time for result output.[51] The most rapid point-of-care technology was reported by Maniscalco, et al., utilizing rapid antigen detection of exhaled breath condensate.[34] Although this study showed strong sensitivity and specificity, it was limited by its case-control design with inclusion of symptomatic patients. Additionally, the six-minute turnaround, though better than other technologies, almost triples the turnaround time when compared to the 2 min 15 second cycle time (including testing and purging) of the BOH FTIR/AI system.

The BOH FTIR/AI system's practical usability also depends on the rate of anticipated crowd throughout per unit time. In venues such as airports, where only a few hundred individuals need to be screened for each flight, it is practical to provide on-site testing. However, in very large venues such as 50,000 or 100,000 attendee sports arenas, most attendees enter the arena within a short time interval. In such circumstances, even a 2 min 15 second result turnaround coupled with numerous on-site screening kiosks would result in significant crowd bottlenecks. A practical solution demands spreading screening machines throughout accessible off-site locations. Most attendees need to be tested within a certain time window prior to arriving at the venue of interest. Though this is akin to current worldwide PCR or antigen testing strategies, its advantage is that it provides a much more rapid result turnaround, less discomfort, and results that are, preliminarily, as accurate as PCR and more accurate than rapid antigen tests.

The BOH FTIR/AI system presents one other significant advantage. Other breath analysis methodologies, such as PCR or antigen testing, focus on identifying one molecule, whether nucleic or antigenic. Even VOC testing, such as rapid gas chromatography techniques require focusing the test on a predetermined narrow frequency window.[55,56] In contrast, the BOH FTIR technology, currently deploys the lowest FTIR resolution of 4 cm^{-1} with 32 screenings, which yields 7882 data points. The machine can reach a resolution of 0.5 cm^{-1}, which can generate millions of data points, each representing a specific or combination of organic compounds. This expansive range coupled with the system's AI-generated open-ended subtraction analysis yields an incredibly vast wave number/intensity profile. We project that coupling this vast profile with appropriately powerful AI technologies will be capable of generating an array of disease distinguishing profiles far beyond those offered by other breath analysis technologies. This may broaden its diagnostic range, both in terms of identifying SARS-CoV-2 in the presence of other co-infections, and in its ability to identify the distinguishing breath profiles of a broad array of diseases. Further research is needed to determine the extent of the Breath of Health FTIR/AI system's diagnostic potential.

References

1 Cohen J, Kupferschmidt K. Mass testing, school closings, lockdowns: countries pick tactics in 'war' against coronavirus. *Science.* March 18, 2020;2020.
2 Scholz N. *Coronavirus Testing Contributing to Efforts to Stem the Second Wave.* European Parliament; 2020:1–8. In: Service EPR, editor.
3 Control ECfDPa. *Population-wide Testing of SARS-CoV-2: Country Experiences and Potential Approaches in the EU/EEA and the UK Stockholm.* ECDC; 2020:1–12.
4 Prevention CfDCa. Overview of Testing for SARS-CoV-2 (COVID-19). In: Prevention CfDCa, editor.; 2021.
5 Organization WH. *Recommendations For National SARS-CoV-2 Testing Strategies and Diagnostic Capacities.* 2021:1–16. June 25, 2021.
6 HealthIMo. *International Travel.* Health IMo; 2022.
7 Prevention CfDCa. U.S Citizens, U.S. Nationals, U.S. *Lawful Permanent Residents, and Immigrants: Travel to and from the United States.* 2022.
8 Commission E. *Council Recommendation on a Coordinated Approach to Facilitate Safe Free Movement During the COVID-19 Pandemic and Repealing Recommendation (EU) 2020/1475.* Brussels; 2021:1–27. November 25, 2021.
9 Prevention CfDCa. *CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel In: Diseases DoV.* 7 ed. Atlanta; 2021:31.
10 Pray IW, Ford L, Cole D, et al. Performance of an Antigen-Based Test for Asymptomatic and Symptomatic SARS-CoV-2 Testing at Two University Campuses - Wisconsin. September-October 2020 *MMWR Morb Mortal Wkly Rep.* 2021;69(5152):1642–1647.
11 Prince-Guerra JL, Almendares O, Nolen LD, et al. Evaluation of Abbott BinaxNOW Rapid Antigen Test for SARS-CoV-2 Infection at Two Community-Based Testing Sites - Pima County, Arizona, November 3-17, 2020. *MMWR Morb Mortal Wkly Rep.* 2021;70 (3):100–105.
12 Drain P, Sulaiman R, Hoppers M, Lindner NM, Lawson V, Ellis JE. Performance of the LumiraDx Microfluidic Immunofluorescence Point-of-Care SARS-CoV-2 Antigen Test in Asymptomatic Adults and Children. *Am J Clin Pathol.* 2021.
13 Dinnes J, Deeks JJ, Adriano A, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev.* 2020. http://europepmc.org/abstract/MED/32845525.
14 Dinnes J, Deeks JJ, Berhane S, et al. Rapid, point-of-care antigen and molecular-based tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database of Systematic Reviews.* 2021;3.
15 Chernov VI, Choynzonov EL, Kulbakin DE, et al. Non-Invasive Diagnosis of Malignancies Based on the Analysis of Markers in Exhaled Air. *Diagnostics (Basel).* 2020;10(11).
16 Dharmawardana N, Woods C, Watson DI, Yazbeck R, Ooi EH. A review of breath analysis techniques in head and neck cancer. *Oral Oncol.* 2020;104: 104654.
17 Ghosh C, Singh V, Grandy J, Pawliszyn J. Recent advances in breath analysis to track human health by new enrichment technologies. *J Sep Sci.* 2020;43(1):226–240.
18 Bruderer T, Gaisl T, Gaugg MT, et al. On-Line Analysis of Exhaled Breath Focus Review. *Chem Rev.* 2019;119(19):10803–10828.
19 Hjoberg J, Shore S, Kobzik L, et al. Expression of nitric oxide synthase-2 in the lungs decreases airway resistance and responsiveness. *J Appl Physiol (1985).* 2004;97(1):249–259.
20 Boots AW, Bos LD, van der Schee MP, van Schooten FJ, Sterk PJ. Exhaled Molecular Fingerprinting in Diagnosis and Monitoring: validating Volatile Promises. *Trends Mol Med.* 2015;21(10):633–644.
21 Ibanez-Martinez E, Ruiz-Gaitan A, Peman-Garcia J. Update on the diagnosis of invasive fungal infection. *Rev Esp Quimioter.* 2017;30 (Suppl 1):16–21.
22 Shende P, Vaidya J, Kulkarni YA, Gaud RS. Systematic approaches for biodiagnostics using exhaled air. *Journal of Controlled Release.* 2017;268:282–295.
23 Ryan DJ, Toomey S, Madden SF, et al. Use of exhaled breath condensate (EBC) in the diagnosis of SARS-COV-2 (COVID-19). *Thorax.* 2021;76(1):86–88.
24 Sawano M, Takeshita K, Ohno H, Oka H. RT-PCR diagnosis of COVID-19 from exhaled breath condensate: a clinical study. *J Breath Res.* 2021;15(3).
25 Li X, Li J, Ge Q, et al. Detecting SARS-CoV-2 in the Breath of COVID-19 Patients. *Front Med (Lausanne).* 2021;8: 604392.
26 Duan C, Buerer L, Wang J, et al. Efficient Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) from Exhaled Breath. *J Mol Diagn.* 2021.
27 Maiti KS, Lewton M, Fill E, Apolonski A. Human beings as islands of stability: monitoring body states using breath profiles. *Sci Rep.* 2019;9(1):16167.
28 Selvaraj R, Vasa NJ, Nagendra SMS, Mizaikoff B. Advances in Mid-Infrared Spectroscopy-Based Sensing Techniques for Exhaled Breath Diagnostics. *Molecules.* 2020;25(9).
29 Chen H, Qi X, Zhang L, et al. COVID-19 screening using breath-borne volatile organic compounds. *J Breath Res.* 2021;15(4).
30 Shan B, Broza YY, Li W, et al. Multiplexed Nanomaterial-Based Sensor Array for Detection of COVID-19 in Exhaled Breath. *ACS Nano.* 2020;14(9):12125–12132.
31 Ruszkiewicz DM, Sanders D, O'Brien R, et al. Diagnosis of COVID-19 by analysis of breath with gas chromatography-ion mobility spectrometry - a feasibility study. *EClinicalMedicine.* 2020;29.
32 Ibrahim W, Cordell RL, Wilde MJ, et al. Diagnosis of COVID-19 by exhaled breath analysis using gas chromatography-mass spectrometry. *ERJ Open Res.* 2021;7(3).

33 Grassin-Delyle S, Roquencourt C, Moine P, et al. Metabolomics of exhaled breath in critically ill COVID-19 patients: a pilot study. *EBioMedicine*. 2021;63: 103154.

34 Maniscalco M, Ambrosino P, Ciullo A, et al. A Rapid Antigen Detection Test to Diagnose SARS-CoV-2 Infection Using Exhaled Breath Condensate by A Modified Inflammacheck(R) Device. *Sensors (Basel)*. 2021;21(17).

35 Steppert C, Steppert I, Sterlacci W, Bollinger T. Rapid detection of SARS-CoV-2 infection by multicapillary column coupled ion mobility spectrometry (MCC-IMS) of breath. A proof of concept study. *J Breath Res*. 2021.

36 Steeghs MM, Cristescu SM, Harren FJ. The suitability of Tedlar bags for breath sampling in medical diagnostic research. *Physiol Meas*. 2007;28(1):73–84.

37 Beauchamp J, Herbig J, Gutmann R, Hansel A. On the use of Tedlar(R) bags for breath-gas sampling and analysis. *J Breath Res*. 2008;2:(4) 046001.

38 Arevalo-Rodriguez I, Buitrago-Garcia D, Simancas-Racines D, et al. False-negative results of initial RT-PCR assays for COVID-19: a systematic review. *PLoS ONE*. 2020;15(12). e0242958-e.

39 Mercer TR, Salit M. Testing at scale during the COVID-19 pandemic. *Nature Reviews Genetics*. 2021;22(7):415–426.

40 Yu PJ, Cassiere H, Bocchieri K, DeRosa S, Yar S, Hartman A. Hypermetabolism in critically ill patients with COVID-19 and the effects of hypothermia: a case series. *Metabol Open*. 2020;7: 100046.

41 Ahmed WM, Lawal O, Nijsen TM, Goodacre R, Fowler SJ. Exhaled Volatile Organic Compounds of Infection: a Systematic Review. *ACS Infect Dis*. 2017;3(10):695–710.

42 Ancochea J, Izquierdo JL, Soriano JB. Evidence of Gender Differences in the Diagnosis and Management of Coronavirus Disease 2019 Patients: an Analysis of Electronic Health Records Using Natural Language Processing and Machine Learning. *Journal of Women's Health*. 2020;30(3):393–404.

43 Gomez JMD, Du-Fay-de-Lavallaz JM, Fugar S, et al. Sex Differences in COVID-19 Hospitalization and Mortality. *Journal of Women's Health*. 2021;30(5):646–653.

44 Khan M, Khan H, Khan S, Nawaz M. Epidemiological and clinical characteristics of coronavirus disease (COVID-19) cases at a screening clinic during the early outbreak period: a single-centre study. *J Med Microbiol*. 2020;69(8):1114–1123.

45 Raimondi F, Novelli L, Ghirardi A, et al. Covid-19 and gender: lower rate but same mortality of severe disease in women—An observational study. *BMC Pulm Med*. 2021;21(1):96.

46 Bwire GM. Coronavirus: why Men are More Vulnerable to Covid-19 Than Women? *SN Compr Clin Med*. 2020:1–3.

47 Gebhard C, Regitz-Zagrosek V, Neuhauser HK, Morgan R, Klein SL. Impact of sex and gender on COVID-19 outcomes in Europe. *Biol Sex Differ*. 2020;11(1):29.

48 Takahashi T, Ellingson MK, Wong P, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*. 2020;588(7837):315–320.

49 Jin JM, Bai P, He W, et al. Gender Differences in Patients With COVID-19: focus on Severity and Mortality. *Front Public Health*. 2020;8:152.

50 Haitao T, Vermunt JV, Abeykoon J, et al. COVID-19 and Sex Differences: mechanisms and Biomarkers. *Mayo Clin Proc*. 2020;95 (10):2189–2203.

51 Liangou A, Tasoglou A, Huber HJ, et al. A method for the identification of COVID-19 biomarkers in human breath using Proton Transfer Reaction Time-of-Flight Mass Spectrometry. *EClinicalMedicine*. 2021;42: 101207.

52 Organization WH. *Weekly epidemiological update on COVID-19 - 28*. December 2021:5. 72 ed; 2021.

53 State of Israel Ministry of Health Corona National Information and Knowledge Center Daily Report 30 April 2021, 2021.

54 Abdel Latif A, Mullen J, Alkuzweny M, et al. *Israel Variant Report*. 2021.

55 Paglia G, Astarita G. Ion Mobility-Mass Spectrometry Methods and Protocols: methods and Protocols; 2020.

56 Eiceman GA, Karpas Z, Hill HH. *Ion Mobility spectrometry. Third Edition*. Boca Raton: CRC Press; 2014.