Philippine COVID-19 Living Clinical Practice Guidelines
Institute of Clinical Epidemiology, National Institutes of Health, UP Manila
In cooperation with the Philippine Society for Microbiology and Infectious Diseases
Funded by the Department of Health

EVIDENCE SUMMARY

Should breath tests be used to diagnose COVID-19 infection?

Update by: Mar Christopher F. Epetia, MD, Howell Henrian G. Bayona, MSc, Leonila F. Dans, MD, MSc
Initial review by: Christopher G. Manalo, MD, Cary Amiel G. Villanueva, MD, & Howell Henrian G. Bayona, MSc

RECOMMENDATION

There is insufficient evidence to recommend the use of breath test in detecting COVID-19 infection. *(Low certainty of evidence)*

Consensus Issues
Despite the addition of five new studies since the previous recommendation, insufficient evidence remains to recommend for or against breath tests. The diagnostic accuracy of breath tests cannot be ascertained due to the heterogeneity across studies. The panel also raised concerns on the availability and accessibility of the test, its cost, and ease of use.

PREVIOUS RECOMMENDATION

There is insufficient evidence to recommend the use of breath test in detecting COVID-19 infection. *(Low quality of Evidence)*

Previous Consensus Issues
No recommendation was made as there was only one study found that used a technology that is not accessible at the moment.

What's new in this version?

- Five new studies were included, featuring three cross-sectional studies and two prospective studies.
- Breath test analysis utilizing spectrometry and rapid antigen were included in addition to olfactory technology.

Key Findings

This review included three cross-sectional and three prospective studies on the use of breath tests in the diagnosis of COVID-19 infection. The overall accuracy of breath tests was high, with sensitivity of 97% (95% CI 0.90-0.99) and specificity of 85% (95% CI 0.72-0.92). However, the overall certainty of evidence was low due to significant heterogeneity. This heterogeneity may be attributed to the different mechanisms of the devices despite using the same idea of breath testing. Further evidence is recommended. The technology is not yet sold locally and information about its cost and resource requirements are limited.

Introduction

Reverse transcription polymerase chain reaction (RT-PCR) remains to be the gold standard for SARS-COV2 testing. Collection of specimens for RT-PCR requires time-consuming invasive procedures which also entail biohazard exposure to health personnel acquiring the sample.[1,2] Breath testing, a novel method, addresses these concerns as samples are obtained via non-invasive sampling and results are usually rapidly acquired with a turnover time of 60 seconds to 10 minutes.[3] Testing usually requires the individual blowing or breathing into a disposable mouthpiece that is connected to a breath sampler. The information is fed into an analyzer, which then produces the result.

Breath testing analyzes the composition of either volatile organic compounds (VOCs) or antigen present in exhaled breath [3,4] to confirm the presence of an infection. Metabolic changes from respiratory viral infection leads to changes in breath profiles, suggesting that infection-associated VOCs may be used to develop non-invasive diagnostic modalities through breath analyzers using sensor arrays or electronic noses. At present, the US, Finland, Singapore, India, and Israel are testing whether this technology can be used as a mass screening tool for COVID-19.[5,6] This study updates the previous evidence reviewed by Manalo et al. 2021 on the diagnostic accuracy of breath tests.[7]

Review Methods

Literature search was done for articles that investigated the utility of breath tests in diagnosing COVID-19 among COVID-19 suspects. A systematic literature search until 4 October 2021 was performed in online databases (MEDLINE and Cochrane CENTRAL Database), trial registries (ClinicalTrials.gov and WHO International Clinical Trials Registry Platform) and pre-print servers (MedRxiv, BioRxiv, and chinaRxiv).

The search terms “COVID-19”, “SARS-COV2”, “Breath Test”, “Volatile Organic Compounds”, “Sensitivity” and “Specificity” were used. No language restrictions were applied. Narratives, commentaries, case report and case series articles, and case-control studies were excluded in the analysis. Summary estimates for sensitivity and specificity were derived using a bivariate mixed-effects binary regression modeling through the *midas* command in Stata 14.0. Subgroup analysis was performed according to the mechanism of breath testing (VOCs using spectrometry, VOCs using olfactory technology and rapid-antigen using Inflammacheck® technology).

Results

Characteristics of included studies

Five additional studies were added to the previous version of this review, bringing the total to six studies. Three were prospective studies [8,9,10] and three were cross-sectional studies.[3,4,11] The population included in the studies were all suspects for SARS-CoV-2 infection ranging from asymptomatic, symptomatic, to severely ill individuals. Most of the studies included a mix of these range of symptoms. Diagnosis was confirmed using RT-PCR as the reference standard. All of the studies utilized a breath test as the index test. However, the device and the composition of breath (VOCs or antigen) used for analysis differed across the included studies. VOCs were analyzed more commonly across the studies.

Methodological quality

The overall methodological quality of the studies were moderate to high. Two studies [4,10] presented with low risk of bias. Most of the studies rated as moderate methodological quality were unclear on how the index test and/or reference standard avoid influencing the other.

Diagnostic accuracy of breath tests

A. Overall diagnostic accuracy

Breath testing showed an overall pooled sensitivity of 97% (95% CI 0.90-0.99) and specificity of 85% (95% CI 0.72-0.92) based on 6 studies. Although diagnostic accuracy appeared moderate to high, substantial heterogeneity was noted for both the summary sensitivity (I^2=88.32) and specificity (I^2=94.18) estimates. The composition used for analysis (VOCs or antigen) and the device used were identified as possible sources of heterogeneity.

Table 1. Subgroup analysis for sensitivity and specificity of breath testing

Variable	SENSITIVITY			SPECIFICITY		
	Current Review		Previous Review	Current Review		Previous Review
	Studies (number of samples)	Sensitivity (95%CI)	Sensitivity (95%CI)	Studies (Samples)	Specificity (95%CI)	Specificity (95%CI)
OVERALL	**6 (493)**	**0.97 (0.90-0.99)**	**0.99 (0.97-1.00)**	**6 (4,553)**	**0.85 (0.72, 0.92)**	**0.79 (0.78, 0.81)**
Method of Breath Testing						
VOCs using Spectrometry	3 (75)	0.90 (0.77-0.96)	-	3 (137)	0.88 (0.74-0.95)	-
VOCs using Olfactory Technology	2 (405)	0.99 (0.93-1.00)	-	2 (4,524)	0.74 (0.64-0.82)	-
Rapid-antigen using exhaled breath condensate	1 (13)	0.92 (0.64-1.00)	-	1 (92)	0.99 (0.94-1.00)	-
Symptomatology						
Symptomatic	4 (396)	0.95 (0.86-0.98)	1.00 (0.98-1.00)	4 (2,663)	0.73 (0.63-0.82)	0.80 (0.78-0.82)
Mixed	2 (29)	0.92 (0.64-1.00) to 1.00 (0.79-1.00)	-	2 (150)	0.97 (0.88-1.00) to 0.99 (0.94-1.00)	-
Asymptomatic	1 (68)	1.00 (0.95-1.00)	0.98 (0.90-0.99)	1 (1,740)	0.78 (0.76-0.80)	0.78 (0.75-0.81)

B. Subgroup analysis

By method of breath testing

Subgroup analysis by method of breath testing showed that VOCs using olfactory technology had the highest sensitivity (Sn 0.99, 95% CI 0.93-1.00), followed by rapid antigen using Inflammacheck® technology (Sn 0.92, 95% CI 0.64-1.00), and VOCs using spectrometry (Sn 0.90, 95% CI 0.77-0.96). The highest specificity was demonstrated by rapid antigen using Inflammacheck® technology (Sp 0.99, 95% CI 0.94-1.00), followed by VOCs using spectrometry (Sp 0.88, 95% CI 0.74-0.95), and VOCs using olfactory technology (Sp 0.74, 95% CI 0.64-0.82).

While breath tests that analyze VOCs through spectrometry demonstrate high sensitivity, its diagnostic accuracy for SARS-COV2 cannot be ascertained completely because of inconsistencies in the specificities across included studies in this subgroup. Additionally, the pooled number of patients became significantly low from the subgrouping, thereby affecting the test sensitivity and specificity.

Likewise, the diagnostic accuracy of breath tests that analyze VOCs through olfactory technology cannot be ascertained. Current evidence shows serious heterogeneity of both sensitivity and specificity which may be attributed to the differences in the devices used across the studies despite using the same mechanism of breath testing.

Rapid antigen testing using Inflammacheck® on exhaled breath condensate included only one study. As such, the subgroup was assessed to have serious imprecision from the low sample size. Furthermore, publication bias is a concern as there is only one study in this subgroup.

B. By symptomatology
When used for testing symptomatic individuals, four studies showed that breath tests had high sensitivity at 0.95 (95% CI 0.86-0.98) and moderate specificity at 0.73 (95% CI 0.63-0.82). One study provided evidence that breath tests had comparable sensitivity and specificity estimates for asymptomatics (Sn = 1.00 [95% CI 0.95-1.00], Sp = 0.78 [95% CI 0.76-0.80]). Two studies that evaluated breath tests on mixed populations showed excellent sensitivity and specificity estimates.

Recommendations from Other Groups

Currently, there are no published recommendations on the use of breath tests in the diagnosis of COVID-19 infection from the World Health Organization, US National Institutes of Health, and the Centers for Disease Control. The Malaysian Health Technology Assessment Section (MaHTAS) [7] of the Ministry of Health Malaysia recognized the good sensitivity and specificity of breath test analysis to discriminate and screen for COVID-19 infection among COVID-19 confirmed patient and healthy controls. However, further evaluation and validation studies with larger sample size are required to ascertain its effectiveness and safety.

Research Gaps

Information about the cost of breath testing, its resource requirements, and its cost-effectiveness in the local and international setting are limited. In May 2021, it was announced that breathalyzer tests were done in a deployment trial in Singapore involving incoming travelers from Malaysia. Individuals who tested positive in the breath test underwent confirmatory PCR swab. No results were published. The price of the breath tests were listed as S$5 (Php 190) to S$20 (Php 735).

One non-randomized clinical trial on breath test in Israel (n=4,000) was estimated to be done by July 2021 but still on actively recruiting status. Three observational studies (n=500) from the US and Canada are currently investigating the diagnostic accuracy of breath test in COVID-19 infection among asymptomatic and symptomatic individuals. The estimated completion is at the end of 2021 for the study from the US, and March to September 2022 for the remaining two studies.

References

[1] Shan B, Broza Y, Li W, Wang Y, Wu S, Liu Z et al. Multiplexed Nanomaterial-Based Sensor Array for Detection of COVID-19 in Exhaled Breath. ACS Nano. 2020;14(9):12125-12132. doi: 10.1021/acsnano.0c05657

[2] Rodríguez-Aguilar M, Díaz de León-Martínez L, Zamora-Mendoza B, Comas-García A, Guerra Palomares S, García-Sepúlveda C et al. Comparative analysis of chemical breath-prints through olfactory technology for the discrimination between SARS-CoV-2 infected patients and controls. Clinica Chimica Acta. 2021;519:126-132. doi: 10.1016/j.cca.2021.04.015

[3] Ruszkiewicz D, Sanders D, O'Brien R, Hempel F, Reed M, Riepe A et al. Diagnosis of COVID-19 by analysis of breath with gas chromatography-ion mobility spectrometry - a feasibility study. EClinicalMedicine. 2020;29-30:100609. doi: 10.1016/j.eclinm.2020.100609

[4] Maniscalco M, Ambrosino P, Ciullo A, Fuschillo S, Valente V, Gaudiosi C et al. A Rapid Antigen Detection Test to Diagnose SARS-CoV-2 Infection Using Exhaled Breath Condensate by A Modified Inflammacheck® Device. Sensors. 2021;21(17):5710. doi: 10.3390/s21175710

[5] National University of Singapore. 60-second breath test to detect COVID-19. National University of Singapore; 2020 October 20 [cited 2021 May 01]. Available from https://news.nus.edu.sg/60-second-breath-test-to-detect-covid-19_20201026094346785/

[6] Malaysian Health Technology Assessment Section. COVID-19 Breathanalyser Screening Tools. [Internet]. Ministry of Health Malaysia; 2020 November 26 [cited 2021 May 01]. Available from http://covid-19.moh.gov.my/kajian-dan-penyelidikan/mahtas-covid-19-rapid-evidence-updates/10_COVID-19_Breathanalyser_screening_tools.pdf.

[7] Manalo CG, Villanueva CA & Bayona HB. Should Breath Tests Be Used to Detect COVID-19 Infection? Philippine COVID-19 Living Clinical Practice Guidelines; 26 May 2021.

[8] Wintjens A, Hintzen K, Engelen S, Lubbers T, Savelkoul P, Wesseling G et al. Applying the electronic nose for pre-operative SARS-CoV-2 screening. Surgical Endoscopy. 2020;. doi: 10.1007/s00464-020-08169-0

[9] Grassin-Delyle S, Roquencourt C, Moine P, Saffroy G, Carn S, Heming N et al. Metabolomics of exhaled breath in critically ill COVID-19 patients: A pilot study. EBioMedicine. 2021;63:103154. doi: 10.1016/j.ebiom.2020.103154

[10] de Vries R, Vigeveno R, Mulder S, Farzan N, Vintges D, Goeman J et al. Ruling out SARS-CoV-2 infection using exhaled breath analysis by electronic nose in a public health setting. 2021;. doi: 10.1101/2021.02.14.21251712

[11] 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. 2020;. doi: 10.1101/2020.06.30.20143347

Appendix 1. Evidence to Decision (n=11)

FACTORS	JUDGEMENT					RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Problem	No	Yes (11)				RT-PCR currently being used as gold standard requires time-consuming and invasive specimen collection which is also hazardous to health personnel acquiring the sample.
Benefits	Large (5)	Moderate (6)	Small	Uncertain		Breath testing will decrease the need for significant health personnel contact during specimen collection and time needed for analysis will be greatly reduced. Breath testing does not require invasive nasopharyngeal and/oropharyngeal collection.
Harms	Large	Moderate	Small (5)	Uncertain (6)		
Balance of Benefits and Harms	Favors the use of breath test (1)	Probably favors the use of breath test (6)	Does not favor the use of breath test (4)			
Certainty of Evidence	High	Moderate (2)	Low (8)	Very low (1)		Pooled results showed sensitivity of 0.97 (0.90-0.99) and specificity of 0.85 (0.72-0.92). However, significant heterogeneity is apparent with I2 = 88.32 and I2 = 94.18 respectively. Subgroup analysis was done to the identified of heterogeneity, but the heterogeneity was not addressed in all subgroups. As the sample size became smaller and the studies were distributed to different subgroups in the process, concern regarding imprecision and publication bias became apparent.
Accuracy	Very Accurate	Accurate (3)	Inaccurate (1)	Very Inaccurate (1)	Uncertain (6)	
Values	Important uncertainty or variability (4)	Possibly important uncertainty or variability (4)	Possibly NO important uncertainty or variability (3)	No important uncertainty or variability		No evidence found.

FACTORS	JUDGEMENT						RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Resources Required	Uncertain	Large cost	Moderate Cost (5)	Negligible cost (1)	Moderate savings (2)	Large savings (3)	Breath testing requires minimal personnel and requires less time. A COVID-19 Breath Testing device from Singapore, BreFence Go COVID-19 Breath Test, costs around 5 to 20 USD.
Certainty of evidence of required resources	No included studies (4)	Very low (1)	Low (4)	Moderate (2)	High		No evidence found.
Cost effectiveness	No included studies (9)	Favors comparator (1)	Does not favor either breath tests or the comparator (1)	Favors breath tests			No evidence found.
Equity	Uncertain (6)	Reduced (1)	Probably no impact (1)	Increased (3)			No evidence found.
Acceptability	Uncertain (5)	No (1)	Yes (5)				No evidence found.
Feasibility	Uncertain (5)	No	Yes (6)				No evidence found.

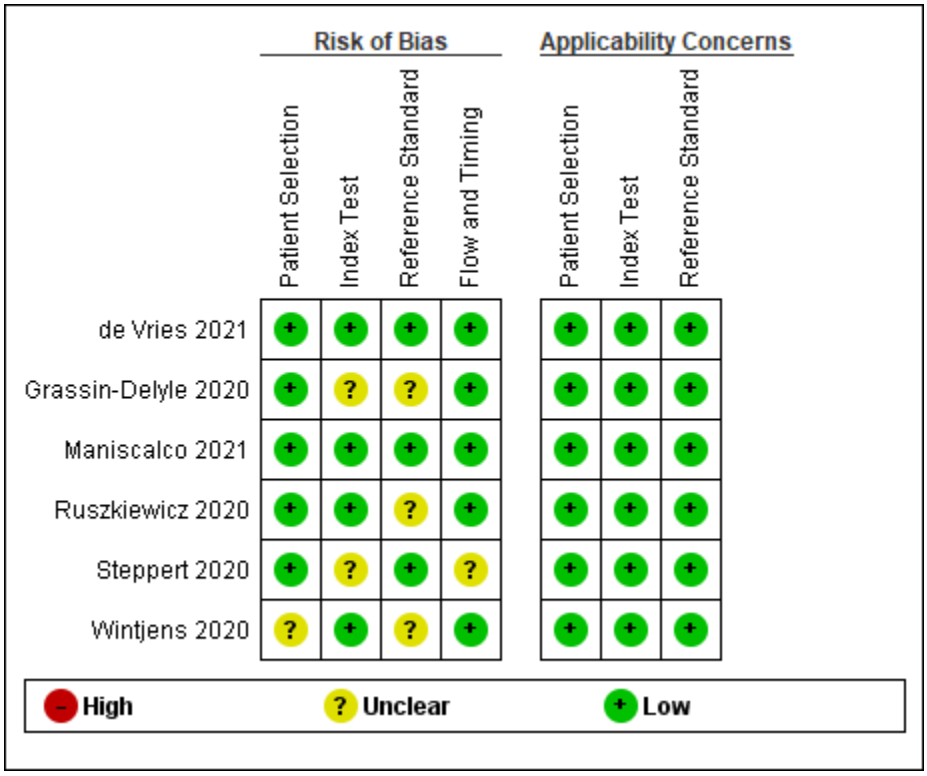
Appendix 2. Search Yield and Results

Database	Search Strategy	Results	Eligible Studies
PubMed	((“Sensitivity”) OR (“Specificity”)) AND ((COVID-19) OR (SARS-COV-2)) AND ((Breath Test) OR (Volatile Organic Compounds))	36	6
Cochrane	((“Sensitivity”) OR (“Specificity”)) AND ((COVID-19) OR (SARS-COV-2)) AND ((Breath Test) OR (Volatile Organic Compounds))	64	1 review, 63 trials
medRxiv	((“Sensitivity”) OR (“Specificity”)) AND ((COVID-19) OR (SARS-COV-2)) AND ((Breath Test) OR (Volatile Organic Compounds))	947	2
bioRxiv	((“Sensitivity”) OR (“Specificity”)) AND ((COVID-19) OR (SARS-COV-2)) AND ((Breath Test) OR (Volatile Organic Compounds))	258	0
chinaRxiv	All Fields:(Breath Test) AND All Fields:(Covid-19)	5	0
Clinicaltrials.gov	Condition or Disease: COVID-19 | Other Terms: Breath Test	44	4 ongoing research of interest
WHO ICTRP	* *Updated 28 September 2021*	11791 registered trials	0
ChiCTR	Target Disease: COVID-19 | Intervention: Breath Test	0	0
HERDIN Plus	All Fields: COVID-19 AND All Fields: Breath Test	1	0

Appendix 3. Table of Included Studies

Study (Sample)	Study Design	Population	Index Test	Gold Standard	Outcome
De Vries 2021 [10] (n=4,510)	Prospective real-world study	Individuals 18 and above with symptoms suggestive of COVID-19 and/or who had been in contact to a known case	Breath testing for VOCs (eNose)	RT-PCR	*Validation Set:* (n=1,808) Sn: 1.00(0.95, 1.00) Sp: 0.78(0.76, 0.80) *Replication Set:* (n=1,948) Sn: 1.00(0.98, 1.00) Sp: 0.80(0.78, 0.82) *Asymptomatic Set:* (n=754) Sn: 0.98(0.89, 1.00) Sp: 0.78(0.75, 0.81)
Grassin-Delyle 2020 [9] (n=40)	Prospective Observational Study	Adult patients above 18 years old in the intensive care unit requiring mechanical ventilation	Breath testing for VOC2 (mass spectrometry [Ionicon Analytic GmBH])	RT-PCR	Sn: 0.89(0.72, 0.98) Sp: 0.92(0.62, 1.00)
Maniscalco 2021 [4] (n=105)	Cross-sectional multicenter study	Adult patients above 18 years old with clinical suspicion of COVID-19	Breath testing utilizing rapid-antigen on exhaled breath condensate (Inflammacheck®)	RT-PCR	Sn: 0.92(0.64, 1.00) Sp: 0.99(0.94, 1.00)
Ruszkiewicz 2020 [3] (n=98)	Independent observational prevalence study	Patients presenting with respiratory at the emergency room	Breath testing for VOCs (gas chromatography-ion mobility spectrometry [BreathSpec])	RT-PCR	*Edinburgh Set:* (n=33) Sn: 0.81(0.58, 0.95) Sp: 0.75(0.43, 0.95) *Dortmund Set:* (n=65) Sn: 0.90(0.55, 1.00) Sp: 0.80(0.67, 0.90)
Steppert 2020 [11] (n=74)	Proof of concept study	Adults with suspected COVID-19	Breath testing for VOCs (multi-capillary-coupled ion mobility spectrometry [STEP IMS NOO])	RT-PCR	Sn: 1.00(0.79, 1.00) Sp: 0.97(0.88, 1.00)
Wintjens 2020 [8] (n=219)	Prospective proof of principle study	Employees with COVID-19 symptoms	Breath testing for VOCs (Aenose)	RT-PCR	Sn: 0.86(0.74, 0.94) Sp: 0.54(0.46, 0.62)

Appendix 4. Detailed Study Appraisal



Appendix 5. GRADE Evidence Profile

Should breath test be used to diagnose COVID-19 in clinically suspected individuals?

Pooled sensitivity: 0.97 (95% CI: 0.90 to 0.99)
Pooled specificity: 0.85 (95% CI: 0.72 to 0.92)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 9.4%	
True positives (patients with COVID-19)	6 studies (493 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[a]	not serious	none	91 (85 to 93)	⊕⊕◯◯ Low
False negatives (patients incorrectly classified as not having COVID-19)								3 (1 to 9)	
True negatives (patients without COVID-19)	6 studies (4,553 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[b]	not serious	none	770 (652 to 834)	⊕⊕◯◯ Low
False positives (patients incorrectly classified as having COVID-19)								136 (72 to 254)	

Should breath test for VOCs by spectrometry be used to diagnose COVID-19 in clinically suspected individuals?

Pooled sensitivity: 0.90 (95% CI: 0.77 to 0.96)
Pooled specificity: 0.88 (95% CI: 0.74 to 0.95)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 32%	
True positives (patients with COVID-19)	3 studies (75 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	serious[a]	none	288 (246 to 307)	⊕⊕⊕◯ Moderate
False negatives (patients incorrectly classified as not having COVID-19)								32 (13 to 74)	
True negatives (patients without COVID-19)	3 studies (137 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	serious[b]	serious[a]	none	598 (503 to 646)	⊕⊕◯◯ Low
False positives (patients incorrectly classified as having COVID-19)								82 (34 to 177)	

Should breath test for VOCs by olfactory technology be used to diagnose COVID-19 in clinically suspected individuals?

Pooled sensitivity: 0.99 (95% CI: 0.93 to 1.00)
Pooled specificity: 0.74 (95% CI: 0.64 to 0.82)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 8.4%	
True positives (patients with COVID-19)	2 studies (405 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[a]	not serious	none	83 (78 to 84)	⊕⊕◯◯ Low
False negatives (patients incorrectly classified as not having COVID-19)								1 (0 to 6)	
True negatives (patients without COVID-19)	2 studies (4,324 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[b]	not serious	none	678 (586 to 751)	⊕⊕◯◯ Low
False positives (patients incorrectly classified as having COVID-19)								238 (165 to 330)	

Should breath test utilizing rapid-antigen on exhaled breath condensate be used to diagnose COVID-19 in clinically suspected individuals?

Pooled sensitivity: 0.92 (95% CI: 0.64 to 1.00)
Pooled specificity: 0.99 (95% CI: 0.94 to 1.00)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 11.4%	
True positives (patients with COVID-19)	1 study (13 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	very serious[a]	Publication bias strongly suspected[b]	105 (73 to 114)	⊕◯◯◯ Very low
False negatives (patients incorrectly classified as not having COVID-19)								9 (0 to 41)	
True negatives (patients without COVID-19)	1 study (92 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	very serious[a]	Publication bias strongly suspected[b]	887 (833 to 886)	⊕◯◯◯ Very low
False positives (patients incorrectly classified as having COVID-19)								9 (0 to 53)	

Should breath test be used to diagnose COVID-19 in clinically suspected symptomatic individuals?

Pooled sensitivity: 0.95 (95% CI: 0.86 to 0.98)
Pooled specificity: 0.73 (95% CI: 0.63 to 0.82)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 12.36%	
True positives (patients with COVID-19)	4 studies (396 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[a]	not serious	none	117 (106 to 121)	⊕⊕◯◯ Low
False negatives (patients incorrectly classified as not having COVID-19)								7 (3 to 18)	
True negatives (patients without COVID-19)	4 studies (2,663 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	very serious[b]	not serious	none	640 (552 to 719)	⊕⊕◯◯ Low
False positives (patients incorrectly classified as having COVID-19)								236 (157 to 324)	

Should breath test be used to diagnose COVID-19 in clinically suspected individuals regardless of symptomatology?

Pooled sensitivity: 0.92 (95% CI: 0.92 to 1.00)
Pooled specificity: 0.77 (95% CI: 0.97 to 0.99)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 8.9%	
True positives (patients with COVID-19)	2 studies (29 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	very serious[a]	none	82 to 89	⊕⊕◯◯ Low
False negatives (patients incorrectly classified as not having COVID-19)								0 to 7	
True negatives (patients without COVID-19)	2 studies (150 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	serious[b]	none	884 to 902	⊕⊕⊕◯ Moderate
False positives (patients incorrectly classified as having COVID-19)								9 to 27	

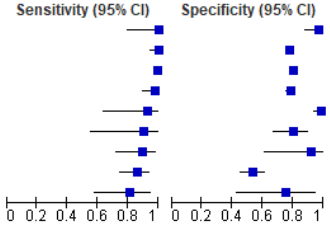
Should breath test be used to diagnose COVID-19 in clinically suspected asymptomatic individuals?

Pooled sensitivity: 1.00 (95% CI: 0.95 to 1.00)
Pooled specificity: 0.78 (95% CI: 0.76 to 0.80)

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 3.8%	
True positives (patients with COVID-19)	1 study (68 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	very serious[a]	publication bias strongly suspected[b]	38 (36 to 38)	⊕◯◯◯ Very Low
False negatives (patients incorrectly classified as not having COVID-19)								0 (0 to 2)	
True negatives (patients without COVID-19)	1 study (1,740 patients)	Cross-sectional (cohort type accuracy study)	not serious	not serious	not serious	not serious	publication bias strongly suspected[b]	750 (731 to 770)	⊕⊕⊕◯ Moderate
False positives (patients incorrectly classified as having COVID-19)								212 (192 to 231)	

Appendix 6. Forest plots

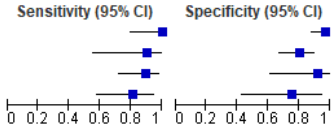
Study	TP	FP	FN	TN	Breath Test Diagnosis Method	Breath Test Device	Sensitivity (95% CI)	Specificity (95% CI)
Steppert 2020	16	2	0	56	Volatile Organic Compounds	Multicapillary Column Coupled - Ion Mobility Spectrometry (STEP IMS NOO)	1.00 [0.79, 1.00]	0.97 [0.88, 1.00]
de Vries 2021 (VS)	68	383	0	1357	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	1.00 [0.95, 1.00]	0.78 [0.76, 0.80]
de Vries 2021 (RS)	229	347	1	1371	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	1.00 [0.98, 1.00]	0.80 [0.78, 0.82]
de Vries 2021 (AS)	49	152	1	552	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	0.98 [0.89, 1.00]	0.78 [0.75, 0.81]
Maniscalco 2021	12	1	1	91	Rapid-Antigen	Inflammacheck (Exhalation Technology LTD)	0.92 [0.64, 1.00]	0.99 [0.94, 1.00]
Ruszkiewicz 2020 (D)	9	11	1	44	Volatile Organic Compounds	Gas Chromatography-Ion Mobility Spectrometry (BreathSpec)	0.90 [0.55, 1.00]	0.80 [0.67, 0.90]
Grassin-Delyle 2020	25	1	3	11	Volatile Organic Compounds	Mass Spectrometry (Ionicon Analytic GmbH)	0.89 [0.72, 0.98]	0.92 [0.62, 1.00]
Wintjens 2020	49	75	8	87	Volatile Organic Compounds	Aenose (The Aeonose Company)	0.86 [0.74, 0.94]	0.54 [0.46, 0.62]
Ruszkiewicz 2020 (E)	17	3	4	9	Volatile Organic Compounds	Gas Chromatography-Ion Mobility Spectrometry (BreathSpec)	0.81 [0.58, 0.95]	0.75 [0.43, 0.95]



Pooled Sn: 0.97 (0.90, 1.00) with I^2=88.32; Pooled Sp: 0.85(0.72, 0.92); I^2=94.18

Figure 1. Overall sensitivity and specificity of breath test

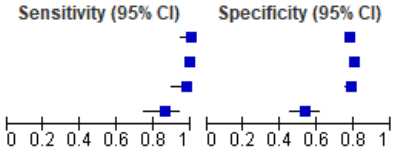
Study	TP	FP	FN	TN	Breath Test Diagnosis Method	Breath Test Device	Sensitivity (95% CI)	Specificity (95% CI)
Steppert 2020	16	2	0	56	Volatile Organic Compounds	Multicapillary Column Coupled - Ion Mobility Spectrometry (STEP IMS NOO)	1.00 [0.79, 1.00]	0.97 [0.88, 1.00]
Ruszkiewicz 2020 (D)	9	11	1	44	Volatile Organic Compounds	Gas Chromatography-Ion Mobility Spectrometry (BreathSpec)	0.90 [0.55, 1.00]	0.80 [0.67, 0.90]
Grassin-Delyle 2020	25	1	3	11	Volatile Organic Compounds	Mass Spectrometry (Ionicon Analytic GmbH)	0.89 [0.72, 0.98]	0.92 [0.62, 1.00]
Ruszkiewicz 2020 (E)	17	3	4	9	Volatile Organic Compounds	Gas Chromatography-Ion Mobility Spectrometry (BreathSpec)	0.81 [0.58, 0.95]	0.75 [0.43, 0.95]



Pooled Sn: 0.90(0.77, 0.96) with I^2=27.69; Pooled Sp: 0.88(0.74, 0.95) with I^2=67.55

Figure 2. Sensitivity and specificity of breath test for VOCs using spectrometry

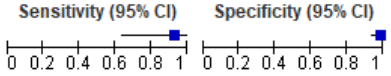
Study	TP	FP	FN	TN	Breath Test Diagnosis Method	Breath Test Device	Sensitivity (95% CI)	Specificity (95% CI)
de Vries 2021 (VS)	68	383	0	1357	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	1.00 [0.95, 1.00]	0.78 [0.76, 0.80]
de Vries 2021 (RS)	229	347	1	1371	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	1.00 [0.98, 1.00]	0.80 [0.78, 0.82]
de Vries 2021 (AS)	49	152	1	552	Volatile Organic Compounds	eNose (SpiroNose - Breathomix)	0.98 [0.89, 1.00]	0.78 [0.75, 0.81]
Wintjens 2020	49	75	8	87	Volatile Organic Compounds	Aenose (The Aeonose Company)	0.86 [0.74, 0.94]	0.54 [0.46, 0.62]



Pooled Sn: 0.99(0.93, 1.00) with I^2=91.63; Pooled Sp: 0.74(0.64-0.82) with I^2=96.90

Figure 3. Sensitivity and specificity of breath test for VOCs using olfactory technology

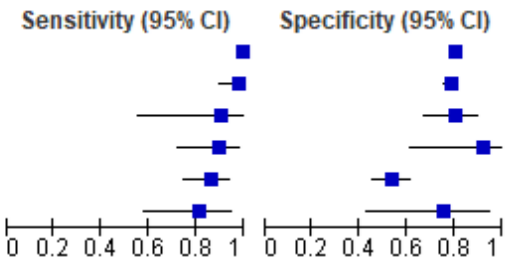
Study	TP	FP	FN	TN	Breath Test Diagnosis Method	Breath Test Device	Sensitivity (95% CI)	Specificity (95% CI)
Maniscalco 2021	12	1	1	91	Rapid-Antigen	Inflammacheck (Exhalation Technology LTD)	0.92 [0.64, 1.00]	0.99 [0.94, 1.00]



Sn: 0.92 (0.64, 1.00); Sp: 0.99 (0.94, 1.00)

Figure 4. Sensitivity and specificity of breath test for rapid-antigen on exhaled breath condensate

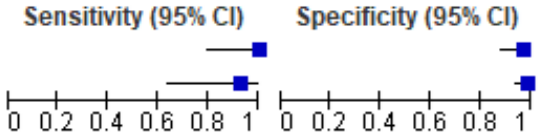
Study	TP	FP	FN	TN	Symptomatology	Sensitivity (95% CI)	Specificity (95% CI)
de Vries 2021 (RS)	229	347	1	1371	Symptomatic	1.00 [0.98, 1.00]	0.80 [0.78, 0.82]
de Vries 2021 (AS)	49	152	1	552	Symptomatic	0.98 [0.89, 1.00]	0.78 [0.75, 0.81]
Ruszkiewicz 2020 (D)	9	11	1	44	Symptomatic	0.90 [0.55, 1.00]	0.80 [0.67, 0.90]
Grassin-Delyle 2020	25	1	3	11	Symptomatic	0.89 [0.72, 0.98]	0.92 [0.62, 1.00]
Wintjens 2020	49	75	8	87	Symptomatic	0.86 [0.74, 0.94]	0.54 [0.46, 0.62]
Ruszkiewicz 2020 (E)	17	3	4	9	Symptomatic	0.81 [0.58, 0.95]	0.75 [0.43, 0.95]



Pooled Sn: 0.95 (0.86, 0.98) with I^2=84.88; Pooled Sp: 0.73 (0.63, 0.82) with I^2=94.58

Figure 5. Sensitivity and specificity of breath test in symptomatic individuals

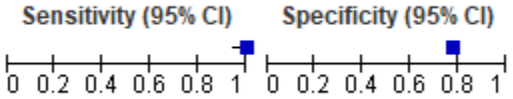
Study	TP	FP	FN	TN	Symptomatology	Sensitivity (95% CI)	Specificity (95% CI)
Steppert 2020	16	2	0	56	Mixed	1.00 [0.79, 1.00]	0.97 [0.88, 1.00]
Maniscalco 2021	12	1	1	91	Mixed	0.92 [0.64, 1.00]	0.99 [0.94, 1.00]



Pooled Sn: 0.96 (0.74, 1.00) with I^2=4.24; Pooled Sp: 0.98 (0.94, 0.99) with I^2=66.89

Figure 6. Sensitivity and specificity of breath test in symptomatic and asymptomatic individuals

Study	TP	FP	FN	TN	Symptomatology	Sensitivity (95% CI)	Specificity (95% CI)
de Vries 2021 (VS)	68	383	0	1357	Asymptomatic	1.00 [0.95, 1.00]	0.78 [0.76, 0.80]



Sn: 1.00 (0.95, 1.00); Sp: 0.78 (0.76, 0.80)

Figure 7. Sensitivity and specificity of breath test in asymptomatic individuals

Appendix 7. Table of Ongoing Studies

Study ID Design	Design	Sample Size	Population / Setting	Intervention/s	Gold Standard
NCT04602949 (Israel)	Non-randomized open-label clinical trial	4000	COVID-19	Breath Test Analysis	RT-PCR
NCT04867213 (Canada)	Prospective Cohort (Observational)	200	COVID-19	Breath Test Analysis	RT-PCR
NCT04341012 (United States of America)	Prospective Cohort (Observational)	200	COVID-19 & Liver Disease	Breath Test Analysis	RT-PCR
NCT04760639 (United States of America)	Feasibility study (Observational)	100	COVID-19	Breath Test Analysis	RT-PCR