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## Diagnostics to take your breath away

After decades of research and development, devices for detecting infectious agents in breath are finally maturing, with SARS-CoV-2 galvanizing progress. Carrie Arnold reports.

Carrie Arnold

Rather than swabbing your nose, imagine taking a simple breath test for COVID-19. Since the beginning of the pandemic, efforts have been underway to find chemical hallmarks of SARS-CoV-2 infection in exhaled breath. On 14 April, the US Food and Drug Administration (FDA) announced the first [emergency use authorization \(EUA\)](#) for a test to detect COVID-19 in breath; [according to study data submitted to the FDA](#), InspectIR Systems' COVID-19 Breathalyzer has a sensitivity and specificity to rival the gold standard: PCR tests.

InspectIR joins a handful of other breath-testing devices under development, a few of which have passed muster with regulators (Table 1). Advances in molecular identification and detection, combined with improvements in computer algorithms and artificial intelligence (AI), are moving these devices closer to the goal of identifying

thousands of organic compounds from exhalations.

But the field needs a device of proven reliability that works in many settings and across populations to gain widespread commercial adoption. In 2021, the market launch of Amsterdam-based Breathomix's SpiroNose was put on hold after less than a week when the device missed 25 positive COVID-19 cases. Still, Cristina Davis, a mechanical and aerospace engineer at the University of California, Davis, says that payoffs are just around the corner. "It's a really exciting time for the entire field because everyone sees the promise of what rapid diagnostics can do," she says. Jane Hill, a bioengineer at the University of British Columbia agrees: "Breath is the new blood."

### A nose for disease

Every day, we breathe an average of 22,000 times. Most of what leaves our lungs is

atmospheric gases: oxygen, carbon dioxide and nitrogen. But a small fraction of this air — less than 1% — consists of small, carbon-based molecules called volatile organic compounds (VOCs) that rapidly spread through the air. More than just the aftereffects of a garlic-laced lunch, these VOCs also contain valuable clues about the body's metabolism. Air from deep in our lungs — so-called alveolar breath — is expelled at the tail end of each breath and contains information-rich VOCs that can provide clues to health. Microscopic water droplets present in all parts of breath contain their own array of nucleic acid, protein and other polar molecules separate from VOCs, collectively known as condensates or aerosols.

To the ancient Greeks and other cultures, VOCs in breath contained a bonanza of information. Hippocrates asserted that no medical exam was complete if the physician

**Table 1 | Breath analyzers under development**

Company	Device	Technology	Stage	Specifications
Breathe Biomedical (New Brunswick, Canada)	SohnoXB for breath collection; Picomole Exhaled Breath Sampler for analysis	IR plus AI, cavity ringdown	Preclinical	Depends on molecule being measured
Breathonix (Singapore)	BreFence Go COVID-19 Breath Test System	MS plus AI	Provisional in Singapore	Less than 60 seconds, 85.7% sensitivity, 97% specificity
Breathomix (Amsterdam)	SpiroNose	DART-MS (metal oxide semiconductors)	CE mark (suspended)	Less than 1 minute, 87.8–100% sensitivity, 87.5–100% specificity
Canary (Canada)	DigiGene COVID-19 test	Nanosensor AI	CE mark	5–20 minutes, 95% sensitivity, 98% specificity
Deep Sensing Algorithms (Finland)	BreathPass	Nanosensor array plus cloud-based AI	CE mark	45 seconds, sensitivity and specificity not available
Ohio State University	COVID-19 Breathalyzer	Nanosensors	Applied for EUA	15 seconds, 88% sensitivity
Owlstone	ReCIVA Breath Sampler	GC/MS or FAIMS	CE mark	For cancer detection
Silver Factory Technology (Singapore)	TracieX	Sensor chip	Approved in Singapore	2 minutes, 85.3% sensitivity, 97.0% specificity
Sotech Health, University of Texas	E-nose	Detects nitric oxide	Seeking approval in US, EU, Canada, UK	Less than 1 minute

DART, direct analysis in real time; FAIMS, field asymmetric ion mobility spectrometry.

did not sniff a person's breath. It wasn't just an eccentricity, either. Excess chloride produced by babies with cystic fibrosis gives them a slightly salty flavor when kissed. And a diabetic's inability to use glucose forces their body to rely on ketones. The body ultimately breaks these down into acetone, reflected in the characteristically fruity scent of those with uncontrolled blood sugar.

Sifting through the molecules in someone's breath is not unlike a private investigator rummaging through someone's rubbish bin, says Stephen Graham, CEO of Breathe BioMedical, a Canadian biotech developing breath tests. Just as most household waste has to exit in compost, recycling or trash bins, cellular waste also has a limited number of escape routes. Besides obvious excreta like urine and feces, the body can remove unwanted chemicals via sweat, vomit and breath. Deep in the lungs, VOCs dissolved in blood can cross into alveoli and then be exhaled. Because all parts of the body are connected via the bloodstream, breath can contain clues not just to respiratory conditions, but to a multitude of diseases.

The first push for breath-based testing, however, came from the legal profession, not the medical one. The end of Prohibition in the United States in 1933 dovetailed with the rise of the automobile. The surge of new drivers on the road and their recently unfettered access to alcohol meant a dramatic rise in drunk driving deaths. To make drinking and driving illegal, however, police needed a way to measure a person's level of intoxication.

Scientists and engineers jumped on the problem, building all manner of Rube Goldberg devices. Though ingenious, the machines were persnickety to operate and often too difficult and bulky for routine use. That changed in the 1950s with the invention of the Breathalyzer. Robert Borkenstein built a device that detected a color change when ethanol reacted with potassium dichromate, turning it from red to green. What truly made the device revolutionary was its portability and ease of use, such that they have become standard police issue.

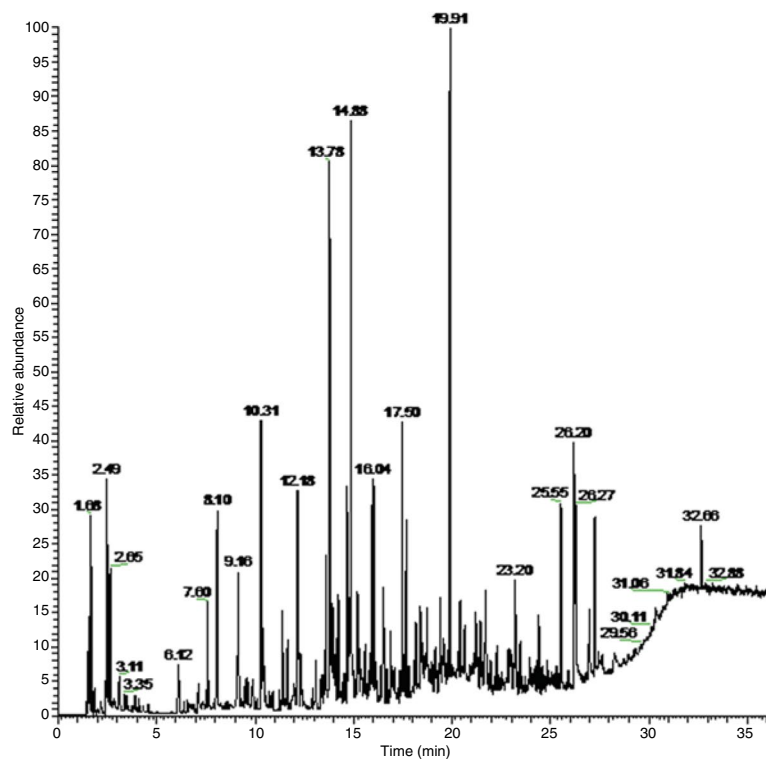
Although the Breathalyzer indicated that breath tests were possible, physicians trying to develop tests for disease rather than intoxication faced major hurdles. Ethanol is easy to detect because it is not normally found at high concentrations in breath and does occur at high concentrations when an intoxicated person exhales. What's more, scientists only needed to identify a single molecule out of the countless other chemicals in the average human breath. Initial hopes of linking a specific molecule with a specific condition were quickly dashed by early breath scientists in the 1960s and 1970s — with two exceptions: *Helicobacter pylori* infections (the cause of gastric ulcers) and small intestinal bacterial overgrowth. The former can be detected by drinking a solution containing <sup>13</sup>C-labeled urea. *H. pylori* produces urease, which breaks down the urea into ammonia and carbon dioxide. The labeled CO<sub>2</sub> can be detected in exhaled breath by infrared (IR) spectroscopy. Breath testing for small

intestinal bacterial overgrowth relies on the presence of elevated levels of methane. For most diseases, however, finding a breath signature of molecules linked to cellular metabolism “is the real needle in the haystack,” says Graham.

### Becoming mainstream

To identify a range of compounds that are present in concentrations of parts per billion, the field very early turned to mass spectrometry as the tool of choice — and it remains the gold standard, according to Davis (Fig. 1). Separating the VOCs with a gas chromatography step before mass spec analysis provides greater resolution. But separating the spectra of a complex mixture isn't easy, especially when molecules are very similar. Mass spectrometers can also be temperamental to operate, and conventional instruments are too large and slow to be used in the field.

For breath diagnostics to become mainstream, engineers needed to build a device more like a Breathalyzer. To do that, scientists took their inspiration from mammalian olfactory systems. Our brains recognize an odor not by singling out a specific chemical but by analyzing patterns of how olfactory receptors are activated. To replicate that in a device, engineers built a machine that was filled with electrochemical sensor arrays, often coated with metal oxides. When the VOCs from breath bound to a sensor, it would change the electric current in the sensor. The electronic nose would then ‘read’ the patterns of current changes created by someone's breath without



**Fig. 1 | Breath-print of VOCs by gas chromatography.** With gas chromatography (GC) technology, exhaled breath is collected and VOCs are trapped. They are then released for analysis by solvent or thermal desorption. Individual molecular components can be assessed by GC, usually followed by mass spectrometry (GC-MS) or flame ionization detection (GC-FID). (Reprinted from K. D. van der Kant et al., *Respir. Res.* 13, 117 (2012), [CC BY 2.0 Generic](#)).

identifying the molecules themselves. Just as humans can recognize the characteristic aroma of a chocolate chip cookie or spraying skunk without evaluating the scent molecule by molecule, so can an electronic nose. The principle was sound, says Oliver Gould, an analytical chemist at the University of the West of England, Bristol, but the early devices were “rubbish.”

Multiple failures forced breath scientists to go back to the drawing board — and back from electrochemical sensors to mass spectrometry. From a scientific standpoint, says Lieuwe Bos, a physician and breath researcher at the University of Amsterdam, mass spectrometry has the advantage of being an untargeted approach. “You don’t have to know beforehand what kind of molecules are in the breath to identify and quantify them,” Bos says.

First attempts to measure the chemicals in breath often were thwarted by an array of chemicals that could shift with everything from time of day to someone’s last meal. What’s more, this large variability often swamped the subtler changes that were associated with disease. The exquisite

sensitivity and detail of mass spectrometry analyses of human breath provided clues to help the extraction of very small signature signals from an almost deafening amount of noise. Davis says that engineers experimented with different sorbents (materials that preferentially absorb particular molecules) to preferentially capture VOCs and other molecules of interest, along with various types of inert plastic to capture breath for later analysis, not unlike a very fancy balloon. But it has been a wave of miniaturization in the past decade that has allowed researchers to develop portable breath analyzers based on mass spec.

Singapore-based Breathonix, spun out from the National University of Singapore by Zhunan Jia and her colleagues, has developed a device that relies on mass spectrometric analysis for much the same reasons as Hill and Davis. “A lot of molecules in the breath have very similar structures and chemical properties, and they can be viewed as having the same pattern on sensors,” Jia says.

The Breathonix device stands waist-high, about the size of a mini fridge, and connects

to a long mobile arm capped by a disposable plastic cover that captures an individual’s breath. The gas undergoes direct analysis in real time mass spectrometry (DART-MS), which can quantify a range of chemicals. The machine compares the test-taker’s pattern of breath VOC to profiles taken from patients known to have COVID-19 and can provide results in less than 60 seconds. Breathonix has tested their device on >50,000 people, reporting 85.7% sensitivity and 97% specificity. Jia says that their large database of breath samples is helping them to refine their algorithms and providing valuable data for the development of breath tests for lung cancer and other diseases.

Cambridge, UK’s Owlstone Medical, for their part, have miniaturized their sampler, but not the mass spectrometer. Their ReCIVA Breath Sampler is a handheld device that captures VOCs and condensates from breath samples on adsorbent tubes. Researchers subsequently analyze the compounds using GC/MS or field asymmetric ion mobility spectrometry (FAIMS), which uses a fluctuating electric field to separate and identify ions. Because the device doesn’t provide on-the-spot results, it can’t be used for screening large crowds, but co-founder and CEO Billy Boyle says that the tradeoff is in superior analysis and accuracy for conditions like cancer, where an accurate result is more important than portability or speed.

### Beyond mass spectrometry

Today, mass spectrometry isn’t the only analytic method being used for breath screening and diagnosis. IR spectroscopy uses infrared radiation to excite the covalent bonds in organic molecules and measures the change in rotational and vibrational states to identify a compound. Though it’s not considered the ‘gold standard’, Davis says that many companies find the smaller size profile of most IR spectrometers appealing for point-of-care use.

Breathe Biomedical, based in Moncton, New Brunswick, Canada, uses a type of IR spectroscopy called cavity ring-down spectroscopy. The company got its start with a technology developed at the University of Alberta to analyze emissions and gas leaks in oil sands fields. Breath poses a similar problem. The company designed a tabletop device that can capture alveolar breath on sorbent tubes. The tubes are then analyzed using IR-CRDS (cavity ring-down spectroscopy) at the company’s headquarters. The company has remained focused on screening for breast and lung cancer, as well as beginning work to predict the development of long COVID-19. Oncologist Anthony Reiman at Saint John

Regional Hospital, who has field-tested a prototype of this system, says that even if Breathe's technology functions best as a screening tool, it will still be incredibly valuable. Low-dose computed tomography (LDCT) is currently recommended to screen individuals at high risk of lung cancer, but the tests are expensive and require exposure to low doses of radiation, which may explain why only 12.7% of smokers at high risk in the United States have had the test. Switching to a cheaper, safer test could help increase the uptake of such screenings.

"To me, it seems one of the biggest opportunities for breath testing is cancer screening," Reiman says. Even if breath tests would just narrow down the number of people who needed more expensive and invasive screenings, they would create value.

Other groups, such as Dallas-based Sotech Health; BreathPass by Tampere, Finland's Deep Sensing Algorithms; and the NA-NOSE, developed at the Israel Institute of Technology, are taking a fresh look at e-noses. All these devices are portable and handheld, around the size of two television remotes stuck back-to-back, and all promise rapid, accurate results for COVID-19 or other diseases. All e-noses consist of gas sensors (often a type of metal oxide semiconductor or carbon sensor embedded with gold nanoparticles whose electrical conductivity changes in the presence of certain types of molecules) coupled to pattern recognition software. Advances in microelectronics have allowed engineers to shrink the size, cost and power needs of electronic noses, making them suitable for use outside of research labs.

Kade France, chief technology officer at Sotech Health, says that an e-nose is ideal for this since the tests are detecting shifts in the ratio of VOCs produced by the body and not pinpointing a specific biomarker, so the devices' ability to sense patterns is perfectly suited.

"We're detecting with our sensor trillionths of an ampere. It's very, very small and patterns can be mistaken for noise," says France. "We're really in the infancy of what we can do." Bringing a device to market means working out these remaining kinks, he says.

Recent advances in machine learning have opened new opportunities for e-noses, which require sophisticated algorithms. This is why Deep Sensing Algorithms CEO Pekka Rissanen says his company, which

specializes in machine learning, first got involved. "We train our algorithms a bit like you would train a detection dog," he says. "We take healthy people, we take sick people, we earmark them for the algorithm, and then we ask what makes them different."

### Not all smooth sailing

Although all these methods have theoretical strengths, in practice the results have been less than stellar. Breath-based diagnostics, whether for COVID-19, cancer or any other condition, mean testing hundreds or thousands of people to identify almost infinitesimal shifts in a VOC profile. In the field, they have faced setback after setback.

### "Breath is the new blood." — Jane Hill, University of British Columbia

In 2021, the Dutch government abandoned trials of Breathomix's SpiroNose after the device returned a small but concerning number of false negatives. (The company maintains that the problems were caused by user error, not the device itself.) The recent FDA EUA of the InspectIR COVID-19 Breathalyzer indicates that breath tests are attaining greater reliability, sensitivity and selectivity, although Owlstone's Boyle will remain skeptical until he can see the data submitted to the FDA. "I know a few of us in the breath community who would be keen to see more data," he says.

Perhaps the biggest challenge for breath-based detectors has been in validating small studies in larger, more diverse populations. The innate variability of human breath is testing's biggest strength and its Achilles' heel. The same variability that makes breath an information-rich source for diagnostics also means that extra steps need to be taken to detect real differences between health and disease, and not just factors like the room where the breath was sampled or what people may have had to eat just before the test.

"If you stopped to get some gas on the way to the hospital [for the test], then this will increase the alkanes in your breath," Bos says. This makes it hard for scientists to identify what chemicals are shifting because of disease and what are changing on the basis of other factors.

That's why an Israeli team from the Weizmann Institute of Science in Israel has begun testing air exhaled from the nostrils rather than the mouth. Their work, published in *PLoS ONE*, showed that the PEN3 eNose by Airsense Analytics, which uses ten different metal oxide sensors to detect compounds exhaled through the nose into a disposable plastic cartridge slightly larger than a cigarette lighter, could reliably detect SARS CoV-2 infection from air exhaled through the nose. "We're getting pretty impressive results, the kind of results that make it plausible to hope for medical applications," says first author Kobi Snitz.

To Boyle, what the field needs more than another nifty gizmo is some basic science work. Researchers still don't have an idea of what can be found in 'normal' breath, and until they have that answer, any breath-based test is going to be fraught with uncertainty, he says. But money for such experiments is hard to come by, leading to clinical validation in groups of people that are too small.

"People have been trying for 40 years and not one has gone from an initial pilot observation to something that's been deployed," Boyle says. "It's quite easy to think you've found the signal, but actually what you've found is just some chemical noise." Having said that, Boyle hopes the new InspectIR device may prove an exception; he looks forward to seeing published results.

The ongoing challenges of developing a reliable breath test have led others to rethink the role of breath tests in medicine. Reiman sees a great potential for breath tests as a quick and cheap cancer screening. If researchers could identify a breath signature that was not associated with cancer, that could be used to reduce the number of more invasive cancer screenings, such as colonoscopies and computed tomography scans.

The pandemic has created a new chance for breath test developers to show how their products can shine, says France. "COVID-19 was an easy win for the short term, and I think that in just a few more years' time, we're going to be able to display an impressive suite of products." □

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