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Breathing Out

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Decoding the mix of volatile organic compounds within human and animal breath could provide new diagnostic tools for disease.

During a recent exam, my veterinarian sniffed my cat's breath and noted that she was very sick. "Can you smell her halitosis?" she asked.

My vet was drawing on a skill that clinicians have used since ancient times: using their sense of smell to diagnose disease from the overall odor of a patient's breath or bodily fluids. An earlier ultrasound exam had shown that my cat had a bladder tumor that was probably cancerous.

Cancer wards in hospitals don't smell good, says Renato Zenobi of the Swiss Federal Institute of Technology (ETH), Zurich. And dogs have been successfully trained to distinguish the breath samples of cancer patients from those of healthy people by scent. "They're not bad. These animals, they have a 90+ percent hit rate," he says. But noses—whether human or dog—don't reveal the actual mix of volatile organic compounds (VOCs) responsible for these odors, Zenobi adds. Being able to collect a breath sample and decode the chemical mixtures present could be a quick, noninvasive way to diagnose disease, without the mess and special handling needed for blood, urine, or other bodily fluids. Analytical instruments could also detect key molecules before a nose could, and with better accuracy and sensitivity. But to do that, chemists have to be able to work out what the key compounds are and how their relative concentrations form a fingerprint that is characteristic of a disease.

It's not as easy as a sniff. While chemists and engineers now have techniques that can detect critical VOCs in breath at parts per billion (ppb) and even parts per trillion (ppt) levels, exhaled moisture, carbon dioxide, and confounding compounds in the ambient air can mask a disease's footprint. Also, breath composition varies from person to person, making it challenging to draw clear lines between healthy and sick individuals. To address these and other challenges, chemists and engineers are collaborating with clinicians to invent better sampling techniques, adapting analytical methods for the complex VOC mixtures in breath, and developing machine learning techniques that quickly and accurately identify disease signatures.

In 1971 Linus Pauling first probed the contents of breath when his team used gas chromatography (GC) to identify approximately 250 VOCs within breath samples. These compounds are carried on the breath, but they originate in cellular metabolism. Deep within the lungs, volatile molecules produced by metabolism and passed into blood diffuse across capillary walls and are exhaled.

Taking a Deep Breath

The idea of designing devices to efficiently capture breath samples percolated in the back of physician Michael Phillips's mind for years once he learned about breath analysis during his medical fellowship. After reading an early paper in the mid-1980s that looked at lung cancer biomarkers in breath, he began pursuing the idea, eventually forming the biotech company Menssana Research, in Newark, NJ.

Their team's portable breath collection system, called BreathLink, maximizes the amount of alveolar breath in a sample. Alveolar breath is the component of an exhalation

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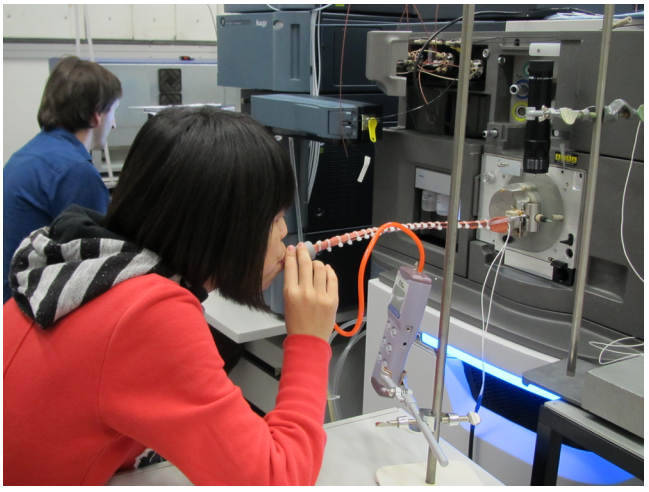
from deep within the lungs where the exchange of compounds with blood takes place, so it has the highest concentration of molecules of interest and best represents a person's blood metabolites. In the approximately half liter of a human exhalation, roughly the first 150 mL is "dead space," gas with little significant interchange within the lungs, Phillips says. Subjects wear a nose clip and breathe through a disposable mouthpiece into a stainless steel cylinder. A pump pulls breath through a Teflon tube into a sorbent trap that concentrates the VOCs of interest. GC-mass spectrometry (GC-MS) analysis of samples collected with the device allowed the company to identify a combination of breath biomarkers for lung cancer that held up in a blind replicated study.

Good sampling is critical for breath analysis, says Donald Blake of the University of California, Irvine, and it's a flaw that has plagued many breath studies over the years. Some researchers will compare samples from healthy subjects, taken outside a hospital, with those from sick patients taken within the hospital, without accounting for the differences in ambient air, he says. "There are a lot of gases in hospitals. Isopropyl alcohol is everywhere." Inevitably breath samples taken in hospitals have huge amounts of that compound, but that doesn't make it a meaningful biomarker.

Blake, an atmospheric chemist, got involved in breath analysis in 2000 after a colleague in the medical school called him, wanting to collaborate. For years, Blake's team had been collecting atmospheric samples from around the world in 2-L canisters and analyzing those mixtures using a self-built, sensitive GC system. Blake and his team took on the work, using the same instruments and techniques that they'd used with outdoor air samples. "Rather than going to the South Pole, we were taking a sample from a person's mouth." To analyze breath samples captured in their cylinders, they use a loop cooled with liquid nitrogen to preconcentrate several hundred milliliters of a breath sample and analyze components in concentrations as low as several parts per trillion.

After a decade working on human breath diagnostics, Cristina Davis of the University of California, Davis, was approached by a researcher at the National Marine Mammal Foundation, in San Diego, who was interested in tapping her expertise to help them find less invasive ways to monitor the health of animals. So her team went to work designing a breath collection system that would be held above a dolphin's blowhole instead of fitting around a human mouth. A valve system then routes exhaled breath to a chilled glass tube. The team could collect samples from trained animals within 5 min. GC-MS provided baseline information about the compounds in dolphin breath and showed apparent differences between wild and managed populations that may reflect differences in diet and life history.



A volunteer exhales into a heated tube attached to the inlet of a high-performance mass spectrometer, which collects and measures volatile organic chemicals in the breath. Credit: Courtesy of Renato Zenobi.

Detecting Compounds

The high moisture and CO_2 levels within breath are issues that all breath analysis groups have to overcome in the standard GC and GC-MS analysis of samples. CO_2 tends to interact with GC columns, broadening the subsequent peaks and sometimes shifting molecules' retention times, which makes concentration measurements less accurate or leads to difficulties in identifying peaks, Blake says. To overcome this problem, Blake's group adds 5% CO_2 to their standards, he says, "so that when you run them, they all look dirty and messy." Breath analysis also presents an interesting research challenge for newer, highly sensitive analytical techniques.

Zenobi's group has focused on ambient MS, an electrospray ionization technique. Breath analysis has been a natural application for this technique because traditional electrospray ionization places samples under high vacuum and dilutes the analytes, a problem his team can avoid while measuring low abundant VOCs within breath. Laser spectroscopy could be a useful complementary technique to MS, says Jerome Faist, Zenobi's colleague at ETH Zurich. With laser spectroscopy, it's possible to monitor the quantity of compounds continuously as they vary over the course of an exhalation, something that is difficult to do with MS, he says.

Hossam Haick at the Technion in Israel has developed a suite of nanosensors that bind various polar and nonpolar functional groups. That chemical binding produces an electrical signal which generates a fingerprint that his team

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can then compare against models. Using the nanosensors, Haick has done breath profiles of cancers, neurodegenerative diseases, asthma, and hypertension, as well as brucellosis, a bacterial disease in cattle.

Sorting Signals

Simple breath tests are currently available to measure blood alcohol and carbon dioxide for monitoring respiration, and the Food & Drug Administration has approved a test for nitric oxide as a measure of inflammation in individuals with asthma. But information about a single compound usually isn't enough information to diagnose disease.

Even though detection of VOCs at ppb or even ppt levels in breath is now possible, it's far from simple to distinguish the chemistry of a healthy exhalation from that of someone who is sick. A variety of algorithms and machine learning techniques should continue to make this process easier, says Zenobi. But breath analysis, as with all diagnostic biomarker research, is fraught with challenges. Factors such as genetic makeup and diet lead to variations in the breath signatures among both healthy and sick individuals, Haick says, which makes it challenging to draw clear lines between the two populations.

In addition, it's difficult for researchers to collect samples from a sufficient number of subjects to get statistically valid clinical results, Phillips adds. A typical GC-MS analysis of a breath sample includes approximately 200 VOCs, he says. As with other biomarker research, if a breath study looks for patterns of that number of compounds in a relatively small number of patients, those results might fit an initial model that then falls apart when researchers attempt to validate it with a different group of patients.

Although a variety of clinical studies are underway to profile the breath signatures of disease, researchers will have to develop devices that meet the high standards of the FDA or other regulatory agencies for diagnostics. For breath analysis to truly enter routine clinical use, researchers say, more companies will need to invest in developing smaller, easy-to-use instruments suitable for a doctor's office.

Even with his decades of work in the field, Phillips is skeptical that breath testing will have a place as a stand-alone diagnostic for cancer. Instead, he thinks breath analysis may find its place as part of a suite of tools for clinicians. He and his team are currently applying for FDA approval for their device to be used as a lung cancer diagnostic in conjunction with CT scanning to reduce the number of false positive and negative results that come from those exams. Eventually he thinks that their device could serve as an initial inexpensive and noninvasive screen for lung cancer, one that picks out patients who might have the disease and would benefit from additional, but more expensive, testing.

"We need cheaper screening tests," he says. Those initial tests don't necessarily have to be extremely accurate at flagging which patients definitely have cancer, he says, but clinicians need to have extremely high confidence (>99%) that an initial test isn't going to flag a sick patient as healthy. "Therefore, you can trust the results," he says.

Sarah Webb is a freelance contributor to Chemical & Engineering News, the weekly newsmagazine of the American Chemical Society.

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