[THE NANO NOSE]

BREATHE OUT:

YOU ARE RELEASING A POWERFUL CHEMICAL TRAIL THAT CAN IDENTIFY EARLY SIGNS OF CANCER AND OTHER DISEASES. WELCOME TO MEDICINE'S NEW ERA OF SMELL DIAGNOSIS

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IN OCTOBER 2012, BILLY BOYLE – AN ELECTRONICS ENGINEER –TOOK HIS GIRLFRIEND KATE TO ADDENBROOKE'S HOSPITAL IN CAMBRIDGE, WHEN SHE COMPLAINED OF A STOMACH ACHE. EIGHT HOURS LATER, SHE WAS HAVING EMERGENCY SURGERY TO REMOVE THE LARGE, METASTATIC TUMOUR THAT WAS CHOKING HER COLON. "HER CANCER WAS ADVANCED, IT HAD ALREADY SPREAD TO HER LIVER," BOYLE SAYS, LEANING FORWARD ON A LARGE WOODEN TABLE IN HIS OFFICE AT CAMBRIDGE SCIENCE PARK ON A RAINY APRIL DAY IN 2013. "HER CHANCES OF SURVIVAL WERE FIVE PER CENT."

After the surgery, Boyle tried to think of any early symptoms they might have overlooked, but came up blank. Kate, 34, had been healthy enough to travel around the United States, fundraising for her charity, just days earlier. "I think it comes down to whether or not we could have identified it at an earlier stage, because if we'd spotted it at stage one, her survival rate would have been 90 to 95 per cent," Boyle says. In other words, early diagnosis in such cases are the difference between life and death.

Boyle is a straight-talking Belfast native, with a dimpled smile and light brown hair. At the time Kate was diagnosed, he was running Cambridge-based Owlstone Nanotech, a chemical-sensors startup that he cofounded in 2004. The company's primary focus was to develop technology capable of detecting noxious gases simply by their smell. "This was post-9/11 – you know, hundreds of millions of dollars were being spent on research for detection of explosives and chemical weapons," says Boyle, 34. Owlstone sensors can filter out various dangerous chemicals by their odour – just as dogs sniff out drugs or explosives at airports – for military and domestic security purposes, attracting clients in the oil and gas business. But Boyle's weeks in the sterile corridors of Addenbrooke's oncology wards made him restless. "I realised medical diagnostics is probably a better use of my technology than helping oil companies clean up expensive contaminations," he says. So Boyle began looking at how his military sensors could be adapted for use in medicine.

His extensive reading turned up an unusual pocket of research: the study of how body odours – from breath, skin or urine – can contain clues about health. "It turns out there is this whole new field called 'metabolomics' – how chemical patterns on your breath or off your urine could indicate illness," Boyle says. Published research over the last ten years has shown that diseases such as asthma, chronic obstructive pulmonary disease (COPD), tuberculosis, lung cancer, gastric cancer and colon cancer all have a distinctive "smellprint" – a pattern of volatile molecules that can't be sensed by a human nose, but can be picked up by a mass spectrometer, a large laboratory instrument that separates and identifies chemicals from an air sample. The problem currently faced by engineers is how to shrink a cumbersome, £500,000 machine into a simple table-top device for a GP's office.

STILL LIFE (PREVIOUS SPREAD): CHARLIE SURREY

Far left: Claire Guest, whose breast cancer was detected by her pet, Daisy (left), before a core needle biopsy

Mass spectrometers aren't the only ways to sniff out disease. In 2009, Claire Guest, a clinical psychologist in Milton Keynes, also found out she had cancer. But unlike Boyle's girlfriend, it wasn't her doctor who alerted her; it was her fox-red Labrador, Daisy. "She kept jumping up and pawing my chest; she was strangely anxious around me," says Guest, 49. When she felt that the pawing had made her chest unusually sore, she had a breast-cancer biopsy conducted, which came back negative. When Daisy's strange behaviour continued, Guest had a core needle biopsy. The procedure confirmed her fears – she had breast cancer. "It was a very deep tumour, and could have been quite advanced by the time I noticed it," says Guest, who has since had treatment and is now cancer-free. Guest is a passionate advocate of using dogs as disease sensors; she runs a charity called Medical Detection Dogs that works with researchers, NHS trusts and universities to train specialist dogs to detect the odours of human disease.

But dogs aren't machines – their work is not easily reproducible and they cannot convey nuances. So at the Technion – Israel Institute of Technology, in Haifa, a team has been working to develop an electronic nose. "My inspiration for this work was from dogs, which can naturally smell chemical traces in the range of parts-per-trillion," says Hossam Haick, a chemical engineer at the Technion. Utilising animals for real clinical applications is not realistic, he says, mostly because we don't understand how the data is being processed. "I thought an electronic system that works on similar principles, but where we know what data was fed in and how the results are calculated, could serve just as well," says Haick. "It would be suitable for use outside of specialist settings, and significantly reduce screening costs, because the chip itself is low-cost and treatments would be given earlier."

There are already a handful of electronic breath-tests on the market which have been given regulatory approval by the US Food and Drug Administration (FDA). Besides alcohol breathalysers, there are tests for carbon-monoxide poisoning, heart-transplant rejection and asthma. Tests for breast, prostate, colon and lung cancer, as well as tuberculosis (TB), heart failure and diabetes, are being developed. Such tests would be relatively cheap, painless and quick.

In medical fields such as oncology and TB, where expensive, invasive tests such as biopsies, sputum cultures and blood tests are the norm, a simple smell-test could be transformative in countries with limited resources. "A tuberculosis breath test would be particularly interesting for developing countries where the disease is extremely prevalent, because breath is always available and easy to collect," says Anton Amann, director of the Breath Research Institute of the Austrian Academy of Sciences, and editor-in-chief of the *Journal of Breath Research*, which he founded in 2007. "It could completely change how we screen for life-threatening diseases."

Your every exhalation is a window into your body's inner workings; each breath contains infinitesimal concentrations – in the range of parts-per-trillion – of over 3,000 volatile organic compounds. Did you just breathe out? You just revealed clues about whether you are male or female, young or old, pregnant or sick, what drugs you regularly take (even illegal ones such as cocaine and marijuana), and whether you may have radiation poisoning. This can be identified by the complex mixtures of alkanes, alcohols, aldehydes, oxides and acids which form a redolent pattern based on what's going on inside you.

Israeli microbiologist Mel Rosenberg made a living out of analysing these breath odours, before he recently retired to write children's books about dental hygiene. "I started smelling breath professionally in the 80s and have probably sniffed over 10,000 people for signs of disease, over my career," Rosenberg says. "It took me 30 years, but I can distinguish between bacterial odours from the nose and the back of the tongue, those from kidneys, lungs or gums." In fact, research from ETH Zurich's Department of Chemistry, published in the *PLOS ONE* journal this April, has shown that breath-prints are a lot like fingerprints: although they fluctuate in a single person over the course of a day, each pattern, measured by a mass spectrometer, has a core signature that is unique enough to identify a person.

As Boyle began reading about the role of smell in detecting disease, he realised that his nano-sensors could recognise these signatures, just like a mass spectrometer. "The challenge is how to shrink it and bring it to clinics," he says. "We realised that our sensors would be easier to use within the medical community, and far cheaper than what's currently available." In fact, because the pattern-recognition element would be programmed into the software, "we could just change what we were looking for on the computer, and adapt the sensor for a number of different diseases," he says.

This ability to hack electronics easily is second nature to Boyle. He has been experimenting with DIY electronics since he was 13,

when he spent the summer of 1992 in Charlotte, North Carolina, as part of a programme called Project Children. "It was during the troubles in Ireland and they got a scruffy little Catholic kid – me – and a Protestant kid from Belfast and put us in a house together with an American family," Boyle says, grinning at the memory. Apart from going to barbecues and hanging out at McDonald's, Boyle spent his free time at a nearby electronics retailer, where he experimented with metal detectors, fuses, LEDs and micro-controllers. "When I got home, I started buying lots of chemicals and electronics. My dad was convinced I was a glue sniffer because I had so many weird chemicals," Boyle says. "My brother was like, 'Don't be ridiculous. He's just a geek.'" The teenage Boyle would build data loggers and sensors that he would hook up around the house. "I made little things like, if you left your car's indicator light on for too long, an alarm would go off for 30 seconds," he says.

Although this fascination waned as an engineering undergraduate at Trinity College, Cambridge, where Boyle was much more absorbed by business, economics and the pub, he re-discovered his love for fixing engineering systems when he graduated. To pay his final term's rent, Boyle took a summer job as a research associate for his engineering professor in the Microsystems and Nanotechnology Group, and decided to stay on. That's where he met his two Owlstone cofounders, American PhD student Andrew Koehl and Spanish engineer Dave Ruiz-Alonso. "The three of us would sit together at the same boring-ass Thursday lunchtime talks, go out for drinks after lab and get to know each other. That's when Andrew told us about his idea for the design of this chip," Boyle says. Boyle had experience in manufacturing silicon chips and Ruiz-Alonso knew how to model signal-processing algorithms, so the three decided to start a business. "We wrote a business plan, and thought, 'Ah, we can stick that down on our CVs,' and we went out and tried to raise some money. We got two million dollars from a private New York investor within six weeks," Boyle says.

The result: the field asymmetric ion mobility spectrometry (FAIMS) chip, Owlstone's core product. Although not a mass spectrometer, the 7mm-wide chip acts on a similar principle, as a chemical filter. "What our device does is take a mix of gases from any air sample, and ionise the chemicals, giving each particle a charge," Boyle explains. When a high-voltage electric current is applied to the chip, the different ions will move at different rates that are characteristic to each one, and can be separated from one other. "So it picks out the chemical composition of a gas and displays it as a graph," Boyle says. If the sensor has been measuring your breath, that graph is your breathprint. And people with a certain disease should have breath-graph displays which are entirely different from the graphs of their healthy counterparts.

Far left: Tjip van der Werf of the University of Groningen blows into an eNose Aeonose diagnostic unit. Left: an isolation room for TB patients

The Owlstone sensor has impressed Michael Phillips, CEO of Newark, New Jersey-based Menssana Research. One of the longest-established companies in the field of breath research, it has been running clinical trials of breath samples for around 20 years. Phillips is now using Owlstone sensors in experiments. "We are actively working with Billy [Boyle] to use his chips in lung- and breast-cancer diagnoses," Phillips says. "It's getting the technology from lab to clinic,"

In 2007, Phillips set out to correlate chemical patterns in diseased breath with the source of the illness. "We looked at the breath of patients with pulmonary TB and found that a combination of six to eight biomarkers could accurately predict the disease," he says. "We then tested smells from the TB bacteria itself, and found that several compounds were the same or similar to what we saw in breath." Phillips knew he didn't need a mass spectrometer: "We wanted a cheap instrument to identify these known chemicals and spit out a diagnosis." Although Menssana is testing the Owlstone chip, it has also patented its own system called BreathLink – a cloud application linked to the Breathscanner, a machine that, with the app, can deliver a TB diagnosis in six minutes. The Breathscanner sensor is not a spectrometer, but it makes breath patterns based on the sonic frequency of specific chemicals. A multi-centre trial in London, Mumbai and the Philippines tested the Breathscanner/BreathLink in 2010. "Somebody sat at the machine in Mumbai, India, breathing out – and a couple of minutes later, we could upload data and see the results in New Jersey."

The results of the study, published in the journal *Tuberculosis* in 2012, showed that the Breathscanner was about 85 per cent accurate when diagnosing TB-positive patients. The important thing, though, was its negative predictive value – how sure you could be that a negative result was correct. "Ours was better than 99 per cent. So if [the result is] negative, we can say with full confidence you don't have it," Phillips says. If it's negative, you go home; if it's positive, your doctor does another test to confirm, which, in the case of TB, is a chest X-ray or a sputum culture. "This is not just for TB, but also for lung and breast cancer," Phillips says. "A biopsy is the gold standard, but with a breath test you can minimise the cost and pain."

SMELL SCIENCE: HOW THE SENSORS WORK

Owlstone FAIMS

Type: Field asymmetric ion mobility spectrometry
How it works: The chip is an ion mobility spectrometer. When it encounters a mixture of gases, such as a patient's breath, it gives each particle a charge, making them move at different speeds in an electric field. This way the chip can separate the various components and identify them.
Cost per test: A few pence

eNose Aeonose

Type: Metal-oxide sensor
How it works: Developed in the Netherlands, the eNose Aeonose is a metal-oxide sensor. A volatile chemical in the breath, such as that caused by an infection, can react with the metal oxide when it changes its electrical conductivity. This change can be measured, and ascribed to a certain chemical. A breath pattern is built up by measuring a range of changes in electrical conductivity.
Cost per test: Less than £8

Breathscanner

Type: Gas chromatography/ surface acoustic-wave detector
How it works: The sensor relies on the modulation of surface acoustic waves to sense the gases. The device transduces the affected mechanical wave into an electrical signal. Changes in amplitude, phase, frequency or time delay can be used to detect and measure the presence of specific chemicals.
Cost per test: "It will be cheaper than a TB sputum-test."

The Owlstone chip is also being used at the University of Warwick hospital, in a pilot study conducted by engineer James Covington. "With all the technology and drugs available, people have forgotten about smell," he says. "Doctors in China still sniff their patients." Covington was interested in bowel disorders: irritable bowel syndrome, ulcerative colitis and colon cancer, which are hard to tell apart in their early stages. The diagnostic test for these is a colonoscopy – "a metal tube up the bum"-type procedure, as Boyle describes it, to scrape out a sample of tissue from your bowels. The process is primitive: clinical reports say about one in 5,000 patients die from it; three times that number have their colons perforated – a surgical emergency. There are no widely accepted alternatives to this test.

Covington ran a small trial using Owlstone's and other sensors in late 2012, testing the urine odours of 100 people; some were healthy and others had ulcerative colitis or colon cancer, like Boyle's girlfriend, Kate. "We got the initial results back just before Christmas, right in the middle of her chemotherapy," Boyle says. "The sensor had ID'd every colon-cancer patient in the group." Covington says they were even able to differentiate the cancer patients from those with ulcerative colitis. He is now running a larger trial, adding irritable bowel-disease patients, to see if the smell test can differentiate the three groups, and how advanced each disease has to be before it is detected. "If this works in double-blind trials, it will be huge," Covington says. "The odour reader will first appear in secondary hospitals, but you want to get it into GP and walk-in clinics. For specific disease groups, you could even have home monitoring."

Larger trials are being run in Hossam Haick's lab at the Technion. His sensor, the NA-Nose, and Boyle's FAIMS sensor are built on a similar principle: "You know you're smelling coffee, but your nose doesn't know what chemicals are in it," Boyle explains. It's your brain that interprets this signal. Similarly, the sensors do not distinguish between separate molecules, but transfer the collective pattern to a computer. The sensors first need to be taught to identify a particular smell. These signals are then processed using a pattern-recognition algorithm, and stored, until the sensor encounters an unknown sample that matches this.

Haick's lab has succeeded in identifying lung, breast, colorectal, prostate, head-and-neck, and stomach tumours. The NA-Nose was particularly effective at stomach cancers when tested on a group of 130 patients, and the results, published in March, are now being verified in trials running via a European consortium of eight universities and companies, led by Haick. "The ultimate goal is to use the NA-Nose to detect cancers at the level of a single cell," Haick says.

Far left: Billy Boyle, cofounder of Cambridge-based Owlstone. Below: the Owlstone Tourist – a detector which uses the FAIMS chip

Although diagnosing cancers earlier could save lives, screening for TB could help stanch the unchecked spread of a disease that affects about 8.7 million people every year, according to the World Health Organisation. "I was disappointed by the first generation of breath tools in the 80s, but these new sensors, they are a sort of magic box," says pulmonary physician Tjip van der Werf at the University of Groningen in the Netherlands. Van der Werf is a balding 59-year-old Dutchman who paces up and down as he talks passionately about TB. "Currently, what do we have in the TB toolbox? Very little, to be honest," he says. "We are working with technologies invented over 100 years ago. If you compare to malaria or HIV, there have been many more advances, both in diagnosis and treatment." Van der Werf spent his early years as an infectious-disease doctor, training in Ghana, where TB became a particular obsession for him. "I ended up doing a PhD in it while I was in Ghana, alongside my medical training. I shipped a bunch of sputum samples back to the lab in Groningen and started building up a database of local infections." Now, as the head of the infectious-disease unit at the University of Groningen's hospital, van der Werf wants to tackle TB again – this time using the most modern technology available. In Groningen's hi-tech TB sanatorium, where infectious patients are compulsorily admitted by the government, a Dutch-based company called eNose is training its smell sensor using the ward's in-house patients, and comparing their results to those obtained from standard diagnostic techniques.

The eNose Aeonose is a sky-blue, iPad-sized machine with a funnel, which you grip on either side and directly breathe into for five full minutes. Just as Boyle's sensors were originally used for military and defence purposes, the Aeonose uses technology from a device built for the Dutch police, used to sniff out cannabis. In 2010, the device was tested in Bangladesh on 150 TB patients. "The results from Bangladesh made me puzzled and thrilled," says van der Werf, recalling the moment when he saw the numbers. "An automated self-learning network system was recognising patterns and you can see the two clouds of TB and no-TB widely apart. From a clinical perspective, we never see such a difference." If the breath test could serve as a screening tool before expensive chest X-rays and sputum cultures, unnecessary expense could be prevented, but precious time would also be saved – suspected patients could be isolated to prevent the infection spreading. After the current trial, van der Werf wants to test if the Aeonose can monitor treatment and if human breath can reflect the difference between drug-resistant and susceptible TB. "Drug-resistant TB is becoming incurable. If you could spot it early, that would be world-changing."

The Groningen sanatorium is a large, airy facility, with private rooms for its inhabitants, each equipped with a desk, a bed and a television, plus air filters and negative pressure gauges to ensure TB bacteria can't escape. Most of the patients are either immigrants from endemic countries such as India, Bangladesh or Senegal, or homeless locals, some of whom have drug and alcohol problems. Divya [name changed] is a 32-year-old software engineer from Kanpur, India, who moved to the Netherlands when she married last year. Recently diagnosed with highly infectious drug-resistant TB, she is living in isolation; speaking with Divya requires a special mask that seals off your mouth and nose and continuously filters the air she is raggedly breathing out. This is the second time she has been diagnosed with the disease – the first time was in India, when she took a nine-month course of medicines and was told she was cured: no one checked whether she had a drug-resistant form of the disease. "I tested out the [Aeonose] and it was so easy, much simpler than the X-rays and the sputum," she says. A quick breath-screen before she came to the Netherlands could have shown that she was still positive and she could have stayed in India while being treated with a last-resort drug, which she is taking now. "And then maybe I wouldn't be here on my own," she says, softly.

The idea of diagnosing diseases through smell is not a new one: Nobel Prize-winning chemist Linus Pauling performed an early chemical analysis of breath in the 70s, using gas chromatography to separate out the volatile compounds and identify illnesses. Since then, researchers have used gas chromatography, mass spectrometry and electronic noses, but the technique has not yet made it into mainstream clinical practice. The bottleneck until now, according to researchers, has been the technology itself. "The sensors weren't very accurate – there was a large overlap between people with and without the disease in the readouts," says van der Werf. The process has also been slowed down by clinical trials, which have to be run for each disease group. "Clinical testing and licensing is a long and expensive process; small companies will need the help of large, wealthy medical-diagnostics and marketing companies," says Phillips, of Menssana, who is trying to obtain FDA approval for the TB Breathscanner.

Billy Boyle, however, is optimistic. "I think we are uniquely positioned to launch this commercially," he says. "What you want is two things: raw analytical capability and usability – is this portable and cheap? Our system is both."

In March this year, Boyle's girlfriend Kate had to fly to the US to have seven tumours removed from her liver, before coming home for three more months of chemotherapy. Prior to travelling for her surgery, she and Boyle were married. "The chemo is working and her chances are about 50-50 now," says Boyle. "It's a lot better than five per cent."

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