**Q&A**

# Animal Olfactory Detection of Disease: Promises and Pitfalls

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The association of unique odors with disease has a long-standing history in medicine, from the initial detection of inborn errors of metabolism in babies, to ketoacidosis in hyperglycemic patients, to the most recent research field of cancer odor profiles. However, the human capacity to detect odor is far exceeded by the very sensitive olfactory detection systems of some animals, such as dogs and rats. For example, dogs are regularly used in police enforcement to detect narcotics or forensic remains, and rats are used to detect landmines in some regions of Africa. More recently, a variety of animal species have been used in the context of medicine to detect diseases, such as tuberculosis and cancer. The utility of animals for the detection of disease has the greatest appeal in communities where medical funds and accessibility are limiting, and also in the context of serious disease for which no screening programs exist or current biomarkers are not ideal.

Although there are reports of success in the literature regarding animal training and olfactory detection using a variety of disease specimens, quite a few challenges exist to ensure a successful future for this research field. For example, the odor molecules detected by animals are currently unknown. In an attempt to identify these molecules, some research groups are working toward the development of an "electronic nose" to detect both odorous and non-odorous molecules emitted from disease specimens. Such a device would lead to the automated detection of odor profiles or fingerprints that could identify disease for the purposes of screening or diagnosis. In this Q&A, experts from the field of olfactory detection of disease discuss the success of the research field to date, pitfalls to avoid in the future, and future applications of their work.

### Historically, how have animals been used as scent detectors?



**Bart Jan Christiane Weetjens:** Trained animals have been used historically for all kinds of detection applications, some of which are so common that we have forgotten about them. For example, truffles have been detected for many centuries by means of pigs and trained dogs. The oldest scent detection application has probably been hunting dogs, where trained dogs track and trace targeted wild animals.



**Giorgio Pennazza:** The 3 main contexts for which animals have been used as scent detectors are food and prey hunting, drug and explosive screening, and disease detection. The first 2 are well established and normally considered for commercial and security activities. They are also well supported by scientific papers, although practical training is, of course, the predominant factor discussed in the literature.

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**Nanne de Boer:** Dogs have predominantly been used as scent detectors for several purposes, as their sense of smell greatly exceeds the accuracy of the human nose. Perhaps even more importantly, dogs are relatively easy to train and instruct. Certain dogs, for example bloodhounds, are renowned for their ability to track down and rescue humans. Some canines are specifically bred for hunting, like terriers and spaniels. Although dogs have historically been trained to find food, hogs are also able to localize truffles that are located underground. More commonly, a canine nose is used for the protection of houses or property.

**Marije K. Bomers:** Humans have exploited dogs' superior sense of smell for centuries, for instance while hunting for animals like rabbits and foxes. Dogs have also assisted in warfare by tracking fugitives or detecting explosives, weapons, or enemy fighters. Since the early 18th century, monks living in the Alps kept Saint Bernard dogs to guide them on their rescue missions after bad snowstorms. The dogs were not trained, but younger dogs supposedly learned how to perform search missions from the older dogs. Other unique examples include Bedouins' camels that use their sense of smell to find water by detecting geosmin (a bacterial product found in wet dirt) from up to 50 miles away, which is a real asset when travelling through the desert. Also homing pigeons, used for pigeon post since before Christ, are thought to at least partly depend on their sense of smell by navigating through odors they pick up from different wind directions.

*What are some nonmedicinal examples of animal olfactory detection?*

**Bart Jan Christiane Weetjens:** Examples can be divided generally into 4 categories: environment, forensics, customs, and biosecurity. Environmental applications include detection of pollutants in soils and chemical compounds in wastewater (such as mercury, detergents, and DDT). An accredited national protocol in the Netherlands for forensic applications includes the identification of suspected criminals in association with firearms used in criminal acts. Other similar applications include detection of cadavers and blood stains and of accelerants in arson investigations. Customs applications include detection of explosives, narcotics, illicit goods, electronics, cash money, and trafficked humans. Finally, biosecurity control includes fresh fruits, vegetables, dairy products, and particular pest species.

**Giorgio Pennazza:** Some of the historical applications cited earlier are still used today, such as food and prey hunting and drug and explosive screening. The effectiveness of canine olfaction in crucial applications such as explosive and drug detection has been proven and supported by the identification of key compounds.

**Nanne de Boer:** The accurate canine olfactory system has been used for many nonmedical purposes. Dogs can be trained not only to detect illegal drugs and explosives at airports or borders, but also mobile phones in prisons. The police also depend on the canine sense of smell when searching for human remains. Another example includes giant African pouched rats that are trained to accurately detect landmines. Their success as mine detectors is largely based on their superior sense of smell and low bodyweight (so mines will not be activated).

**Marije K. Bomers:** We are most familiar with the use of dogs for detection of narcotics, explosives and weapons, human remains (even under water), agricultural quarantine materials (the "Beagle Brigade" at customs), and search and rescue. However, detection dogs are used for a large variety of additional applications, including detection of insects (gypsy moths, screwworm pupae and larvae, bedbugs, and termites), endangered species, (Pacific water shrews, black-footed ferrets, and Oregon spotted frogs), bears, parasites in sheep feces, brown tree snakes in cargo, gas pipeline leaks, mobile phones in prison cells, blood, mold, and even specific currencies (pounds sterling and euros). Furthermore, there are other detection animals at work besides dogs. Colleague Weetjens and his team have done impressive work training rats for detecting landmines in Southern Africa. Similarly, both mice and honeybees have been trained as an efficient and low-cost means to screen large areas for hidden explosives.

*Which are the best animals to use for scent detection and why?*

**Bart Jan Christiane Weetjens:** Whereas dogs are "man's best friend," socially accepted and widely used for all kinds of detection applications, giant African pouched rats represent an excellent alterna-

tive with many advantages over dogs, apart from the social acceptance (see https://www.flickr.com/photos/42612410@N05/). These animals are capable of performing repetitive tasks endlessly, in return for simple food reinforcement. Also, giant African pouched rats can be trained easily and quickly. For landmine detection, an average of 194 training sessions of 20 min are needed before the animals pass IMAS (International Mine Action Standards) accreditation tests, which is a much shorter training period than for mine-detection dogs. Logistically, giant African pouched rats are very convenient. They require a small space and little care and attention. A relatively large number of rats can be transported in relatively small transport cages, and their kennels can be much smaller than, for example, dog kennels.

Giant African pouched rats are also quite robust and resilient to tropical diseases. They require little medical care. At APOPO headquarters in Tanzania, around 250 animals are checked and taken care of by a veterinarian once a week in less than a half hour. Interestingly, giant African pouched rats form less intense personal bonds with their trainers compared to dogs and their trainers, which allows an easier transfer of trained animals between trainers and operational sites. Finally, in limited-resource settings, giant African pouched rats form a sustainable and renewable resource and many animals are accessible and available.

**Giorgio Pennazza:** Dogs, mice, rabbits, pigs, bears, and many other animals have an excellent sense of smell, supported by $10^8$ sensory neurons and a very complex and efficient fluid dynamics system for sniffing. Among them, dogs seem to express a richer diversity of odorant receptors and express a unique fluid dynamics system, which contributes enormously to enhance their olfaction performance. The spatially separated olfactory stimulus inside the nostril, as demonstrated by magnetic resonance imaging, indicates also that the pattern followed by odorants inside the nasal cavity gives a strong contribution to specific odor discrimination.

**Nanne de Boer:** Two factors are essential in the selection of animals for scent detection purposes in general: the accuracy of detection by their sense of smell and the possibility for training. It is also important to keep in mind the specific goal of training. For instance, bears have an excellent sense of smell and can be trained. However, their potential use in detecting landmines is limited given their large body weight. In addition, the general circumstances (for example, temperature or geographical conditions) in which an animal has to perform its job are vital as every animal has its own specific physical capacities and limitations. Dogs may

well be the best animal scent detectors, as their abilities encompass all the above-mentioned characteristics.

**Marije K. Bomers:** There are several characteristics a scent detection animal should have. An excellent olfactory capacity is one, but the animal also has to be trainable. To the best of my knowledge, no comparative studies between species have been performed. However, different animals have different advantages. For instance, rats are easy to maintain and transport. They also have low bodyweight and therefore will not trigger landmines. On the other hand, bees are less expensive and more quick to train (they actually train each other). But a beehive is not as readily transportable and is not suited for every environment (such as crime scenes, hospitals, or airports). For dogs, a curious nature or drive, combined with a certain level of obedience, is essential. Various breeds of canine are suitable, but golden retrievers, Labrador retrievers, German shepherds, and border collies are all commonly used.

### What are some examples of animal olfactory detection in medicine and healthcare?

**Bart Jan Christiane Weetjens:** There are several examples of dogs being trained for medical applications across the world. In the US, the Pine Street Foundation has trained dogs for the detection of cervical cancer in breath samples. In the UK, the Medical Detection Dogs Group established proof-of-principle detection for other cancers, including prostate cancer in urine samples, bowel cancer in breath and stool samples, breast cancer and lung cancer in breath samples, and bladder cancer and skin cancer in urine samples. For skin cancers and bowel cancers, similar proof of principle has been provided by research in Japan. The same Medical Detection Dogs Group has trained dogs for the detection of diabetes and epileptic surges. Also, researchers in the Netherlands have trained dogs to detect *Clostridium difficile*. Finally, in Tanzania and Mozambique, APOPO vzw has trained giant pouched rats for the detection of pulmonary tuberculosis in sputum samples.

**Giorgio Pennazza:** The use of animal olfaction in the medical field is a challenging opportunity with substantial evidence and a number of unresolved but intriguing questions. The urgent need for disease prevention and the obvious desire for noninvasive sample collection make cancer the most intriguing challenge to be faced by "dog doctors." Thus we can find many reports presenting the feasibility and the advantages and the disadvantages of canine detection of cancer.

**Nanne de Boer:** Several scientific publications conclude that the canine sense of smell can be used in the

detection of cancer (e.g., skin, bladder, lung, breast, colorectal, and ovarian cancer). One of the first reports (1989) describes the case of a dog that persistently sniffed at a mole on the leg of the dog handler. This skin lesion turned out to be a melanoma. The observation was later confirmed by a larger formal study demonstrating that canines are able to correctly detect melanomas. Furthermore, a skilled Labrador retriever from Japan demonstrated that in the detection of colorectal cancer canine scent detection by sniffing at breath and watery stool samples obtained from patients and controls was superior to fecal occult blood testing. In addition to cancer, dogs can detect other diseases. A 2-year old beagle was successfully trained to correctly diagnose *Clostridium difficile* infections in stool samples and hospital patients. The dog was taught to sit or lie down when the infection was detected. Not only have studies shown that dogs are able to smell infectious diseases, but also studies with rats (using human sputum as a specimen) and bees showed that they are able to correctly diagnose tuberculosis.

**Marije K. Bomers:** As mentioned above, in the late 1980s, the first case report appeared of a dog that continuously barked and sniffed at her owner's leg, until eventually the dog actually tried to bite off the mole. The mole was a melanoma. Since then, there have been many anecdotal reports and diagnostic trials involving animals using their sense of smell to detect disease. Diagnostic trials include animals (mainly dogs) detecting malignancies (e.g., melanomas and lung, bladder, prostate, breast, ovarian, and colorectal cancers) and infectious diseases (like rats for tuberculosis, and beagles for *C. difficile* infection). Animals are often as accurate as or even superior to standard diagnostic methods. Dogs are also used to alert humans to oncoming hypoglycemia in diabetes patients and seizures in those with epilepsy, but to the best of my knowledge no formal studies have been performed. It is unclear what triggers the dogs' reaction, but their sense of smell is likely to play a role.

### How are animals trained to detect odors from medical specimens?

**Bart Jan Christiane Weetjens:** There are numerous animal training protocols used for olfactory detection by animals. Though some "avoidance" training protocols have been used in the past by some research groups, in general, "positive reinforcement schedules" provide the most stable and sustainable conditioning patterns.

APOPO has developed training protocols for both direct detection and remote scent tracing applications. Although the training sequence is similar in the early training stages, both are clearly distinct applications: in the former an animal is trained to indicate the source or highest concentration of a particular target odor pattern in ambient air in the environment, and in the latter, samples are used as targets for the animals' discrimination of the headspace vapor on top of the presented samples. Whereas the landmine detection rats are trained in a direct detection mode, the tuberculosis detection animals are trained on medical specimens (sputa) in a remote scent tracing set-up, described in the *International Journal for Tuberculosis* and *Lung Disease* (Weetjens et al. 2009;13:737–43). Protocols for both direct detection and remote scent tracing have been published in detail by APOPO in scientific journals (Poling et al. *Journal of Applied Behaviour Analysis*. 2011;44:351–5; Poling et al. *The Behaviour Analyst*. 2011;34:47–54).

**Nanne de Boer:** In dogs, a reward-based training protocol is usually applied, so correct behavior or sample identification is rewarded with a tennis ball or other treat. This training, which is performed by an expert dog instructor, may take several months. In the first phase of training, the dog has to be familiarized with the studied scent. Over time, the detection of this specific odor has to become more challenging for the dog by using fainter smells and other materials and/or changing environments. Following this step, the canine is finally trained to discriminate between positive and negative samples. After this teaching period, the animal needs to maintain scent detection abilities by regular training.

**Marije K. Bomers:** The universal principle is that animals are conditioned to associate a specific odor (and the response to that odor) with a reward (e.g., a treat for dogs or sugar-water for bees), like a Pavlovian response. For dogs, the scent is first offered in its purest form (if possible). Starting with simple search-and-find games, dogs are taught which scent they are supposed to find. Then, by making the tasks increasingly difficult (by hiding the object or diluting its smell), the training enables the animal to further develop its skills.

### What advantages/disadvantages do animals offer over traditional biomarker assays and automated instrumentation?

**Bart Jan Christiane Weetjens:** One advantage animals offer is high throughput. Whereas a laboratory technician with a microscope can analyze a maximum of 40 sputum samples per day for the detection of tuberculosis, a trained rat can analyze the same number of samples in 7 min. In limited-resource settings, these rats are a cheap, renewable, and resilient resource. Also, while

animal training is labor-intensive, advanced levels of education are not required. Hence, detection rats form a suitable approach for developing nations with a high disease burden and juvenile unemployment.

The main disadvantage is controlling animal stimuli. Animals are living creatures who constantly recalibrate to the targets on which they are reinforced. Therefore, reward samples (confirmed positive samples for reinforcement) and operational or test samples need to be manipulated, processed, stored, and handled in exactly the same way to avoid olfactory cues other than the target odor. With this in mind, experiments that utilize small sample sizes are prone to bias, and therefore lack scientific rigor. Experimental design, including a large variety of both confirmed positive and negative samples and double-blinded and randomized trials are essential to obtain meaningful scientific results. The need to constantly access large sample sets and to continuously monitor performance can also be considered limitations of animal detection technology.

**Giorgio Pennazza:** Disadvantages include difficulties in training and in identifying reproducible and reliable techniques to reveal the dog's "diagnosis." There is also a subjectivity problem with dogs. The main advantage animals afford is the same as for electronic noses: when the specific biomarkers are not known, the combination of biomarkers produces a unique fingerprint, which is very similar to the qualitative synthesis given by an odor signature. However, there is high complexity in sampling techniques with chemical sensors and similar instruments. In this context, animals are of course the most sensitive "tools," while an electronic nose (e-nose) can express this signature in a numerical format.

**Nanne de Boer:** Until now, studies using animal scent detection showed that this technique is superior to biomarker assays when looking at accuracy. However, their training is often time-consuming and, at the end, more expensive. Moreover, a dog can work for merely several hours per day, which makes this technique practical especially for case finding. In national colorectal cancer screening programs, for example, in which thousands of samples or patients are to be screened, an easy-to-use, inexpensive, and high-throughput technique is preferred above a low-throughput canine scent detection technique, despite the fact that the canine nose is more accurate than currently used screening tests like fecal immunochemical tests or endoscopic assessment.

**Marije K. Bomers:** Animal noses are unbeatable in their olfactory capacity when compared to any other currently available technique. Their sense of smell is exceptionally sensitive and specific, an ability that has proven difficult to replicate artificially. It is hard to imagine a technical device detecting a mobile phone, wrapped in plastic and submerged in a toilet water tank, on its own initiative.

Nonetheless, the use of detection animals has major drawbacks. Their training takes time and expertise. Since we are dealing with animals, we are also dealing with behavioral and mood variations. Study results are not easily generalizable and all trained animals need an individual assessment of performance (or calibration) and regular practice to maintain their skills. This makes them difficult to "mass produce." For hygienic purposes there are obvious restrictions with regard to allowing animals into healthcare facilities. Furthermore, medical societies often have difficulty accepting new methods that are unconventional or are not included in current standard operating procedures commonly used for the introduction of new techniques into daily practice.

***Do you think animals will supplement automated instruments in the laboratory or are there other applications for this research field?***

**Bart Jan Christiane Weetjens:** Animals can greatly complement automated instruments and reduce costs of screening operations, especially in high-risk populations in developing countries. In Tanzania, APOPO has developed a decision algorithm for tuberculosis screening combining the detection of rat results with fluorescence microscopy and GeneXpert for confirmation. This approach allows for large-scale systematic screening and active case finding, which would be extremely expensive and logistically quasi impossible by means of automated instruments only. For tuberculosis screening purposes, for which the standard diagnostic tool in developing countries is sputum microscopy, complementing detection rats (featuring a high sensitivity) and microscopy (featuring a high specificity) can greatly enhance tuberculosis case finding, as demonstrated by APOPO in Tanzania and Mozambique.

**Giorgio Pennazza:** I think that a good starting point could be the experimental demonstration of a correlation between e-nose and canine olfactory results.

**Nanne de Boer:** The olfactory potential of animals in general (medical and nonmedical) is undervalued and as a result understudied. Their superior sense of smell should inspire researchers to undertake more scientific projects on the detection of volatile organic compounds (VOC) or disease-specific smellprints, and should stimulate the further development of better

sensors and scent-detecting devices (like e-noses). Given the limitations and impracticalities of animal scent detection in daily laboratory practice and the innovations in the field of VOC detection, over time the (underused) need for animal scent detectors will decrease.

**Marije K. Bomers:** In my opinion, animal scent detection is especially suited for screening purposes. For diseases like prostate, lung, and colorectal cancers, currently used diagnostics either have limited diagnostic accuracy or are of an invasive nature. I can imagine detection animals screening urine, breath, or fecal samples, followed by confirmatory diagnostic testing (bronchoscopy, colonoscopy) in positive results. In low-resource settings, the use of detection animals could also be a relatively quick and low-cost means to screen a large number of samples (for example, using rats for the diagnosis of tuberculosis on sputum samples).

Unfortunately, despite very promising results, most animal detecting studies have not been confirmed in independent trials. More uniform training and testing methods for these different applications are needed to build a convincing body of evidence for optimum use of this unique diagnostic ability.

*What is an "electronic nose" and how can this technology be used to detect disease?*

**Bart Jan Christiane Weetjens:** I have no expertise in e-noses, but what I have learned from those working in this field is that the big challenge is not the sensors for particular compounds, but the artificial neural networks to categorize complex compositions of VOC. It will be challenging to beat billions of years of evolution!

**Giorgio Pennazza:** E-nose is a bioinspired term used to name an array of nonselective gas sensors, which is of course "electronic," but not "nose." The large number of natural olfactory receptors (a thousand different members organized in a total number of about $10^6$) is mimicked by a smaller number (up to $10^2$) of functionalized sensors arranged in a measuring cell. Each of these sensors consists of a transducer (based on conductivity, acoustics, optics, or electrochemical working principles) coupled with a chemical interactive material (organic or inorganic films) with a range of ppb to ppm resolution to a large spectrum of VOC. When certain biomarkers have been identified as disease specific, their concentrations are evaluated against significant thresholds. Otherwise, disease diagnosis, phenotyping, and monitoring can be performed by the analysis of VOC mixtures as registered by the e-nose. These records consist of fingerprints which, when an-

alyzed by pattern recognition techniques, can be associated with certain diseases revealing a specific disease signature. The classification process is a work flow with 3 crucial steps: (1) a reproducible and a minimally invasive and effective VOC sampling protocol; (2) the collection of a huge number of clinical parameters to be correlated with sensor outputs; and (3) an explorative analysis to study sensor correlation with specific (target) parameters, followed by a dedicated supervised analysis oriented to the building of a classification/predictive model.

**Nanne de Boer:** Humans are not able to distinguish a single VOC; however, we are capable of detecting a certain scent, being a mixture of several VOC (referred to earlier as a smellprint). These mixtures can also be measured by a device with pattern recognition sensor arrays and compared with other control smellprints. This specific technique bears a resemblance to our human olfaction, and for that reason is referred to as an e-nose.

No individual VOC can be determined by an e-nose, which can be done by complex and costly techniques like GC-MS. An e-nose measures profiles of VOC rapidly and at low costs, which makes it an ideal technique for daily clinical desktop practice where information on individual VOC is not needed. So a disease-specific smell can be directly determined by using an e-nose that may assess different VOC profiles emanating from potentially diseased individuals (in urine, feces, or exhaled breath specimens). For example, in a patient presenting with a shortness of breath and coughing, instant analysis of exhaled breath by an e-nose may lead to a potentially more rapid diagnosis of lung cancer or pneumonia. E-nose technology also seems an attractive candidate for large-scale colorectal cancer screening programs, for which more accurate, high-throughput, and inexpensive fecal-based tests are awaited.

**Marije K. Bomers:** E-noses are designed to mimic the unique biological olfactory system. As indicated earlier, their objective is pattern recognition of gaseous mixtures, by using a variety of sensors that detect the VOC emanating from a sample. Some variations more resemble GC-MS and depend on the VOC mass and charge. Other traditional techniques rely on molecular affinity to bind to a specific electrochemical receptor.

E-noses are also increasingly being studied as diagnostic tools, especially for malignancies and pulmonary diseases like tuberculosis, asthma, and chronic obstructive pulmonary disease. Drifting of sensors after calibration, a requirement of expensive and large equipment, and, again, lack of independent confirmatory studies are important drawbacks here. But obvious

benefits are the noninvasive character of e-nose examinations, and compared to animals, their better reproducibility and freedom from variations in temperament and mood. So far, animals simply have the superior sense of smell. To assess whether their use is cost-effective and improves our current diagnostic arsenal, this type of research should receive higher priority in terms of research effort and funding.