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Assessing individual performance and maintaining breath sample integrity in biomedical detection dogs

Catherine Reeve[a,*], Peter Wentzell[b], Bjorn Wielens[c], Cortney Jones[d], Kim Stehouwer[e], Simon Gadbois[a]

[a] Canid Behaviour Research Lab, Department of Psychology and Neuroscience, Dalhousie University, Halifax, N.S., B3H 4R2, Canada,
[b] Department of Chemistry, Dalhousie University, Halifax, N.S., B3H 4R2, Canada,
[c] 908 Devices Inc., Boston, MA, USA
[d] Atlantic Veterinary College, U.P.E.I., Charlottetown, P.E.I.,C1A 4P3 Canada,
[e] Maastricht University, Netherlands

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ABSTRACT

In empirical tests of biomedical detection dogs, exhaled breath samples are often used because breath contains volatile organic compounds that can signal metabolic states, infection, or disease. However, in studies that present dogs with breath samples, results show a notable degree of variability both between and within studies. Differing protocols for the collection and storage of exhaled breath samples may contribute to this observed variability. The goal of the current study was therefore to test whether there was a difference in the detectability of breath samples collected using silicone-coated versus uncoated cotton balls. This was tested in two experiments. In the first experiment, breath samples were prepared using both silicone-coated and uncoated cotton balls, which were then left exposed to the surrounding air. Four dogs' detection of the samples was tested using a cued, three alternative forced choice (3AFC) procedure at regular intervals up to two hours after the samples were prepared. The results of Experiment 1 showed that the dogs' performance was above chance and there was no significant difference in the dogs' detection of the breath samples across conditions. In the second experiment, a series of breath samples were prepared and stored for one, two, three, and four week periods. The same four dogs' ability to detect the breath samples was tested each week using the same cued 3AFC procedure. The results of Experiment 2 showed that when silicone-coated cotton balls were used, all four dogs could detect the breath samples at above chance levels after the samples were stored for three weeks, and two dogs could detect the samples that were stored for four weeks. When the dogs were tested on their ability to detect the breath samples prepared using uncoated cotton, two dogs' performance fell to below chance levels at one week of storage time, while the other two dogs could detect the breath samples at above chance levels after the samples were stored for four weeks. Taken together, the results of the two experiments illustrate that silicone-coated cotton balls do not improve detectability of breath samples within two hours, but can greatly improve the detectability of breath samples stored over longer periods of time. Since the use of silicone-coated cotton balls only improved the detectability of the breath samples for two of the four dogs, these results highlight the importance of examining individual differences in dogs' performance. Furthermore, we argue that, given the inherent differences in olfactory ability across dogs, widespread use of silicone-coated cotton balls for the collection of breath samples would increase the pool of testable dogs for biomedical detection studies and would decrease the degree of variability both within and between studies.

There is a great need for simple, non-invasive screening and diagnostic techniques to effectively prevent disease and disease complications. The effective management and treatment of disease is dependent upon early diagnosis. For example, throughout the 20th century, deaths due to most types of cancer have declined as a result of emphasis on early detection (DeSantis et al., 2014; Siegel et al., 2014; Siegel et al., 2016). However, deaths due to lung and pancreatic cancer do not show increased survivability because diagnoses are often made at late stages of the disease (Siegel et al., 2016). Current screening and diagnostic tools for disease often involve subjecting patients to painful and invasive procedures such as biopsies, laparoscopies, and blood tests (Amann and Smith, 2013; Burak and Liang, 1987; Jezierski et al., 2015;

* Corresponding author.
E-mail address: reevecat@dal.ca (C. Reeve).

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Wilson, 2015). The invasive nature of these procedures may prevent some individuals from seeking out screening tests due to fear of the procedures themselves (Burack and Liang, 1987) thereby leading to late diagnoses. Furthermore, many diagnostic tools involve the use of equipment that requires specially trained individuals to operate, analyze, and interpret the results. Therefore, these procedures are not only invasive, but can also be very expensive.

The analysis of exhaled breath has been proposed as a simple and non-invasive alternative to current diagnostic tools (Schmidt and Podmore, 2015). Breath serves as a promising channel for diagnostic purposes because cells emit compounds that, when dissolved in the blood, become volatilized and exhaled in the breath during pulmonary circulation (Amann et al., 2014) in concentrations of parts per billion (nmol/mol) and parts per trillion (pmol/mol) (Schmidt and Podmore, 2015). These volatile organic compounds (VOCs) therefore provide a window to the metabolic processes of the body (Amann et al., 2014). Donation of a breath sample typically involves an individual exhaling into a breath collection bag, or breathing onto an absorbent material that is then contained for later analysis. This approach is less invasive than traditional diagnostic tools, permits easy, repeat donations of breath samples for most individuals, allows sampling in the hospital or at home, and the process is very inexpensive (Solga and Risby, 2013).

Currently, the most common tools for analyzing the VOC content of exhaled breath samples involve specialized extraction techniques such as solid-phase microextraction (SPME), followed by analytical techniques such as gas chromatography-mass spectrometry (GC–MS) or the use of specialized VOC sensors (Buszewski et al., 2012b; Sun et al., 2016). Using GC–MS, Phillips et al. (1999) examined the VOCs in breath samples from 50 “normal” individuals. In total, Phillips et al. (1999) found over 3400 different VOCs across the participants, with individual samples averaging 204.2 VOCs. Although individuals differ greatly in the number and type of VOCs emitted in their breath, specific diseases and physiological conditions may present specific VOC profiles (Phillips et al., 1999; Wilson, 2015). The analysis of disease-specific VOCs in exhaled breath has been proposed as a method for the detection of a wide range of physiological conditions (Schmidt and Podmore, 2015), including cancers (Balseiro and Correia, 2006; Buszewski et al., 2012b; Rudnicka et al., 2014; Sun et al., 2016; Szulejko et al., 2010), lung inflammation and disease (Corradi and Mutti, 2013), liver function (Modak, 2013) and diabetic hypoglycemia (Minh et al., 2012; Neupane et al., 2016; Smith et al., 2011) to name a few. Despite progress developing VOC disease profiles, current technologies are only able to detect VOCs in concentrations of parts per billion (nmol/mol) (Schmidt and Podmore, 2015), and disease conditions are often marked by numerous different VOCs or simply by *changes* in VOCs (Solga and Risby, 2013), making analysis even with the most sensitive technologies difficult (Buszewski et al., 2012b; Wilson, 2015). Moreover, these techniques can be expensive and require highly trained individuals to perform the tests and analyze the results (Ross and Esarik, 2013).

Alternatively, trained domestic dogs have been proposed as a cheaper and more accessible VOC analysis technique. A dog’s nose is physiologically perfected to take in and process volatile compounds. When a dog sniffs, the shape of the nostrils efficiently direct airborne molecules into the nasal cavity where the molecules contact the olfactory epithelium (Craven et al., 2010; Buszewski et al., 2012b). The dog’s genome contains a minimum of 1094 functional olfactory receptor genes (Quignon et al., 2005) coding for the olfactory receptors located in the olfactory epithelium. This translates into the ability to detect some odours at 1 part per trillion (ppt, Pearsall and Verbruggen, 1982; Walker et al., 2006). Furthermore, dogs are highly trainable, and using the principles of operant conditioning, can be trained to identify specific odours (Gadbois and Reeve, 2014). Using an olfactometer, Waggoner et al. (1998) tested four dogs’ ability to detect a target odour in the presence of an extraneous odours as the concentration of the extraneous odours increased. All of the dogs could successfully detect a target odour in the presence of extraneous odours, and one dog could detect the target odour even when the extraneous odour increased to a concentration 100 times stronger than the target odour. Furthermore, Walker et al. (2006) reported that two dogs were able to detect n-amyl acetate at parts per trillion; concentrations significantly lower than those detectable by current technologies (Schmidt and Podmore, 2015). These results illustrate the incredible sensitivity of dogs’ noses, and their ability to identify specific odours. It further suggests that complex mixtures of many odours, such as would be expected in a breath sample, do not necessarily impede the ability of dogs to detect specific target odours. Applied to biomedical detection, dogs’ incredible olfactory abilities combined with their trainability make them promising diagnostic assistants. Empirical studies of dogs’ ability to detect disease and physiological states from breath samples present promising results, but a careful examination of the literature shows inconsistencies both within and between studies.

Empirical tests of dogs’ efficacy as biomedical detection tools have focused primarily on dogs’ ability to detect a variety of cancers, but also on physiological states such as diabetic hypoglycemia. Here we will briefly review those studies that present dogs with breath samples specifically.

To the best of our knowledge, McCulloch et al. (2006) have conducted the only study to examine dogs’ ability to detect breast cancer from breath samples. McCulloch et al. (2006) obtained breath samples from individuals with biopsy-confirmed breast cancer as well as breath samples from healthy controls, and then tested five dogs’ ability to identify a cancerous sample amongst control samples. McCulloch et al. (2006) reported that the dogs could identify cancerous breath samples with 88% sensitivity and 98% specificity. Likewise, Sonoda et al. (2011) have published the only study to examine dogs’ ability to detect colorectal cancer from breath samples. Sonoda et al. (2011) presented one dog with breath samples from individuals with colorectal cancer against breath samples from healthy controls and reported the dog’s detection sensitivity was 91% and specificity was 99%. Taken together, these results are impressive and encouraging with respect to the effectiveness of biomedical detection dogs.

To date, there has been a greater focus on the empirical examination of dogs’ ability to detect lung cancer, and the results have been more inconsistent. A review of the current studies shows more inconsistencies in the dogs’ performance than those reported for breast and colorectal cancer detection. Buszweski et al. (2012a,b) and McCulloch et al. (2006) presented trained dogs with breath samples from individuals with lung cancer and healthy controls. Buszweski et al. (2012a,b) reported the dogs (the number of dogs was not reported) tested detected the cancerous samples with detection sensitivity and specificity of 82.2% and 82.4% respectively, and McCulloch et al. (2006) reported that the five dogs tested identified the lung cancer samples with 99% sensitivity and 99% specificity. Similarly, Ehmann et al. (2012) and Rudnicka et al. (2014) presented dogs with breath samples from individuals with lung cancer and control samples, but here the control samples included breath samples from healthy individuals as well as individuals with asthma (Rudnicka et al., 2014), individuals with Chronic Obstructive Pulmonary Disease (Ehmann et al., 2012; Rudnicka, 2014), or synthetic samples (Rudnicka et al., 2014). Ehmann et al. (2012) reported that the four dogs tested could indicate the lung cancer sample against the controls with detection sensitivity of 71% and specificity of 93%, while Rudnicka et al. (2014) reported that the two dogs tested had overall detection sensitivity of 86% and specificity of 72%. Rudnicka et al. (2014) pointed out, however, that when examining each dogs’ performance individually, it was apparent that one dog detected lung cancer better than the other. Finally, Amundsen et al. (2014) attempted to determine whether dogs could distinguish between malignant and benign conditions. To begin, Amundsen et al. (2014) trained dogs to identify lung cancer by presenting the dogs with cancerous tissue samples and breath samples from healthy controls; a task that the dogs completed with a high degree of sensitivity and specificity. However, when Amundsen et al. (2014) subsequently presented