Methodologies for training detection dogs and scent sample storing

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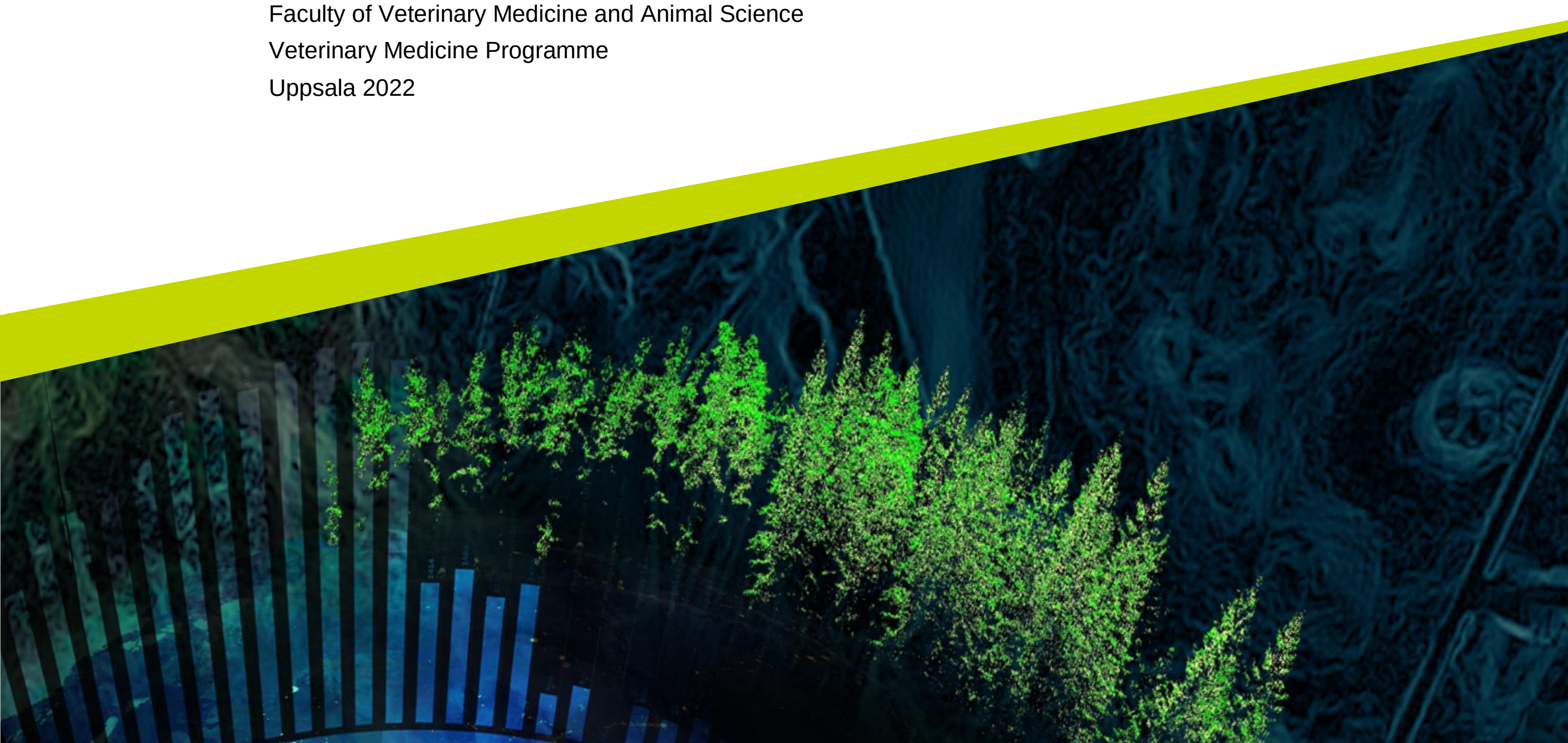
Degree project/Independent project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Veterinary Medicine and Animal Science
Veterinary Medicine Programme
Uppsala 2022



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Credits:	30 credits
Level:	A2E
Course title:	Independent project in Veterinary Medicine
Course code:	EX0869
Programme/education:	Veterinary Medicine Programme
Course coordinating dept:	Department of Clinical Sciences
Place of publication:	Uppsala
Year of publication:	2022
Keywords:	detection dogs, scent training, canine scent detection, search dogs, scent samples

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Abstract

Dogs' spectacular sense of smell has been used as a detection tool by humans in many areas. Their olfaction was first used as a hunting tool, but dogs' detection skills have been shown to also be applicable in, for example, detection of drugs, explosives, and firearms as well as disease detection. Different types of disease detection are possible due to the body's ability to produce volatile organic compounds (VOCs), which reflect the metabolic state of the body. Many illnesses have been shown to cause the body to produce a distinct pattern of VOCs, and these molecules together make up a specific smell. Sweat is a major source of VOCs, therefore sweat samples are used for disease detection by dogs.

The first aim of this study was to examine what track design is most beneficial for detection dogs' learning. This was done through evaluating the performance of two trained detection dogs in three different track shapes, when training the ability to detect individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through sweat samples. All tracks consisted of six consoles. In Track 1 the consoles were positioned in a straight line, Track 2 was a circular track, and in Track 3 the six consoles were placed in a slightly curved line. The track design was changed when the trainers discovered risk factors that could potentially lead to false results. The design of the straight track caused the dogs to have a high level of curiosity which subsequently made them skip the first couple of consoles in many searches. The design of the circular track made it possible for the dogs to pick up on visual cues the handlers were unaware of.

Both dogs' sensitivity was the highest in the first track (96.4% and 97.8% respectively), and the lowest in the third track (62.9% and 82.6%). The sensitivity for Track 2 was 78.9% and 85.1%. Similarly, the specificity was also the highest in the first track (99.3% and 99.8%) and the lowest in the third track (94.3% and 98.5%). The specificity for Track 2 was 96.6% and 99.2%. However, the results may have been influenced by prerequisites that differed between the tracks, which makes the results difficult to interpret.

The second aim of the study was to examine if Falcon centrifuge tubes are suitable as storage containers for scent samples. This was tested through keeping 50 sealed Falcon tubes together in a freezer for one week, out of which three contained pieces of natural rubber that give off a particular smell. Three detection dogs trained to detect that smell searched through all sealed tubes. The results of the study indicate that the scent did not escape the sealed containers as no dog could detect the scent. Falcon tubes are therefore a promising alternative for storing scent samples since scent contamination between samples would not be possible if the containers do not leak the scent. Further studies would be valuable as this could potentially facilitate storing scent samples in a space-efficient way.

Keywords: detection dogs, scent training, canine scent detection, search dogs, scent samples

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1. Introduction

In December 2019 a novel coronavirus, later known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was identified in China (Gorbalenya *et al.* 2020; World Health Organization 2020c). The virus caused a respiratory disease by the name coronavirus disease 2019 (COVID-19) (World Health Organization 2020b). The outbreak rapidly spread to several countries and on January 30, 2020 the World Health Organization (WHO) declared a Public Health Emergency of International Concern (World Health Organization 2020a, 2021b). On March 11, 2020, WHO officially classified COVID-19 as a pandemic (World Health Organization 2020d, 2021b).

With a spreading fatal pandemic, the world needed tools to detect who was infected to minimize the spreading of the virus to prevent further sickness and death. One tool that has previously been used for various types of disease identification is trained detection dogs (Moser & McCulloch 2010; Catala *et al.* 2019; Rooney *et al.* 2019; Maa *et al.* 2021). It has long been known that dogs have a well-developed sense of smell, and dogs' olfaction has therefore been used by humans in many areas. Historically dogs have been of great use in hunting, and in more recent times dogs have become a common sight together with police, military, and customs officers. Studies have shown that many different diseases are reflected in the body odor, and detection dogs can be trained to distinguish these (Ehmann *et al.* 2012; Stone & Waugh 2014; Catala *et al.* 2019; Rooney *et al.* 2019). It seems as it is possible to train dogs to detect anything that has a specific smell. It was not long after the pandemic was declared that "COVID dogs" were being trained in different parts of the world (Grandjean *et al.* 2020, 2021; Jendrny *et al.* 2020, 2021b; Vesga *et al.* 2020; Eskandari *et al.* 2021; Essler *et al.* 2021).

In Sweden, the first project to train dogs to detect COVID-19 positive individuals was commenced by Uppsala University and Region Uppsala in early 2021 (Malmberg 2021; Mellgren 2021). In the initial phase of planning for the study, several questions arose that were hard to find answers to in previous research. One of these being how to store the scent samples in a space-efficient way, preferably in a freezer, without them being contaminated by each other. With knowledge from a similar project that had recently been made in Finland, the Swedish team knew not to store the samples in zip-lock bags close together due to the risk of scent contamination. It was suspected that Falcon centrifuge tubes (Corning Incorporated 2013), a type of container that is standard laboratory equipment, commonly used to store different types of samples, could be suitable in this case.

Another area lacking sufficient amount of published research was methodology to use when training the dogs. There are many studies of what detection dogs can be used for (Sonoda *et al.* 2011; Willis *et al.* 2011; Ehmann *et al.* 2012; Stone & Waugh 2014; Taverna *et al.* 2015; Kitiyakara *et al.* 2017; Rooney *et al.* 2019; Grandjean *et al.* 2020; Vesga *et al.* 2020), but in most cases it is not possible to mimic the training of the dogs after reading the published paper. One factor that may be of great importance for the end result is how the track that the dogs are being trained in is designed. Since detection dogs are used professionally in many different fields, scientific evidence for how to train dogs optimally is needed. Hopefully, the results of this study can facilitate future training of COVID-19 detection dogs and other types of detection dogs.

The two questions this study aims to answer are:

- What track shape is most beneficial for the detection dogs' learning?
- Does scent contamination occur between samples stored in Falcon centrifuge tubes?

2. Literature Review

2.1. The olfactory sense

Animals have always benefitted greatly from having a good sense of smell in different areas of their natural lives. It is often a major part of foraging, finding a suitable partner, and avoiding predators (Young *et al.* 2002; Mombaerts 2004). Having a well-developed sense of smell can therefore be crucial for the survival of an individual, or even an entire species. With that being said, the sense of smell varies greatly between species (Sjaastad *et al.* 2016). While dogs have a spectacular sense of smell, humans, other primates, and most avian species have a poor sense of smell, and toothed whales completely lack an olfactory sense (Kishida *et al.* 2015; Sjaastad *et al.* 2016).

The science behind the olfactory system has not always been clear. The details that explain how mammals' sense of smell works were first described in the early '90s by Linda B. Buck and Richard Axel (Buck & Axel 1991). They were awarded ***The Nobel Prize in Physiology or Medicine 2004*** for their discoveries of odorant receptors and the organization of the olfactory system (The Nobel Assembly at Karolinska Institutet 2004). The scientists discovered a large gene family that gives rise to many kinds of olfactory receptors (Buck & Axel 1991). Each fragrant substance is recognized by a particular combination of these receptors. This combinatorial coding makes the system particularly fine-tuned and explains why humans can differentiate tens of thousands of scents although we only have about 400 different kinds of scent receptors (Glusman *et al.* 2001; Zozulya *et al.* 2001; Fredholm 2004; Niimura & Nei 2007; Olender *et al.* 2012; Trimmer *et al.* 2019).

The olfactory sense of a dog is estimated to be 10,000 to 100,000 times more sensitive than that of a human (Jenkins *et al.* 2018; Sarkis *et al.* 2021). When comparing the olfactory capabilities of different mammals several elements must be taken into consideration. The range of detectable odors is reflected both by the area of the olfactory epithelium, the total number of olfactory receptors expressed therewithin, the amount of different kinds of olfactory receptors, and the size of the olfactory bulb (Buck & Axel 1991; Quignon *et al.* 2003, 2012; Sjaastad *et al.* 2016).

As previously mentioned, humans have about 400 different kinds of olfactory receptors, which is less than half of the types of scent receptors dogs have (Niimura & Nei 2007; Quignon *et al.* 2012). These olfactory cells are located in a sensory epithelium that outlines the ethmoidal turbinate bones in the nasal cavities (Buck & Axel 1991; Sjaastad *et al.* 2016). Different species have different number of turbinate bones and different degree of folding of these, which makes the area of the epithelium differ too. It is about 150 cm^2 in dogs and 5 cm^2 in humans (Sjaastad *et al.* 2016), containing about 300 million and 5-6 million scent receptors, respectively (Else 2020).

The olfactory message is transmitted through nerves to the olfactory bulb (Quignon *et al.* 2012), which is a part of the rhinencephalon, also known as "the smell brain" (de Lahunta & Glass 2009). The development of the olfactory bulb differs extensively among species of mammals, which is reflected in its size. Humans and other primates are microsmatic, they lack a well-developed olfactory system and have a very small olfactory bulb compared to any domestic animal. Dogs are a macrosmatic species (Olender *et al.* 2004), they have a highly developed sense of smell both functionally and anatomically. Bears, sea lions, and opossums have a remarkably large olfactory bulb, suggesting that these species have a well-developed olfactory system (de Lahunta & Glass 2009).

2.1.1. Historical use of detection dogs

Dogs do not only have a spectacular sense of smell. They are also loyal, devoted to man, and easy to train compared to other species. These factors combined make dogs suitable as tools for human use. Hunters have benefitted greatly from dogs sense of smell for centuries (Furton & Myers 2001). The police and military started utilizing dogs' special abilities in the early 1900s, forming the first "canine (K-9) units" or "police dogs" (Gialamas 1996). The military began using dogs as mine detectors during World War II (Lemish 1999 see Fjellanger *et al.* 2002). In 1984 initial tests established the feasibility of training a dog to detect ignitable fluids (Gialamas 1996). The first field operational accelerant detection canine was introduced two years later, in 1986. The dog was trained to detect a variety of accelerants, and within her first year in use, she had searched over 40 fire scenes, some of which resulted in arrests and conviction of suspects.

The use of detector dogs has become widespread and is currently a familiar sight in airports, where they search for drugs, explosives, and firearms (Else 2020). Dogs are now also a natural part of the routine in search and rescue of people (Furton & Myers 2001).

2.1.2. Disease detection

Dogs are being used for numerous types of disease detection. What makes this possible is that many illnesses cause the body to produce a distinct pattern of volatile organic compounds (VOCs) (Buljubasic & Buchbauer 2015). These molecules give off a scent that trained detection dogs can distinguish. The major sources of VOCs include breath, sweat, skin, urine, feces, and vaginal secretions, but what is most suitable to sample depends on what disease one is looking for (Shirasu & Touhara 2011). In a study aiming to determine whether canine scent detection could become an effective tool in colorectal cancer screening, breath and watery stool were compared as sources of VOCs (Sonoda *et al.* 2011). The study showed that the stool samples were superior to the breath samples as the dogs had a higher sensitivity in the stool sample searches – 97%, compared to 91% in the breath samples searches (the specificity was 99% in both cases).

Although it is not always the best source of VOCs, sweat or breath samples are often the most practical types of samples when screening a large number of people (Shirasu & Touhara 2011). This is applicable at major events or airports for example, but the effectiveness, high diagnostic accuracy, and the fact that it is less costly than conventional methods also make it suitable in countries where other screening methods are lacking due to a shortage of access to the required technology (Jendrny *et al.* 2021a).

Dogs have been proven to be able to detect multiple types of cancers, including that of the bladder, liver, lung, prostate, ovaries, and large intestine (Sonoda *et al.* 2011; Willis *et al.* 2011; Ehmann *et al.* 2012; Stone & Waugh 2014; Taverna *et al.* 2015; Kitiyakara *et al.* 2017). Several studies indicate that using detection dogs is a good method for detection of early stages of cancer (McCulloch *et al.* 2006; Sonoda *et al.* 2011; Ehmann *et al.* 2012). One example where early detection is crucial for a better prognosis is lung cancer (Ehmann *et al.* 2012; McCulloch *et al.* 2012). According to global cancer statistics for 2020, lung cancer is the leading cause of cancer-related death (Sung *et al.* 2021), and 75% of lung cancer patients have incurable locally advanced or metastatic disease when first diagnosed (Aberle *et al.* 2011). The five-year survival rate is less than 5% when diagnosed with advanced lung cancer, but can be over 70% for patients whose disease is identified early when the lesion is small and localized (Aberle *et al.* 2011). Since early detection of cancer can be very difficult but is of great importance for the prognosis, this means that dogs potentially could prolong many cancer patients' lives (Buljubasic & Buchbauer 2015). One study even found detection dogs to be a superior alternative, in terms of specificity and sensitivity, compared to conventional methods such as x-ray for detection of early-stage lung cancer and mammography for breast cancer detection (McCulloch *et al.* 2006).

Some detection dogs are of importance for people in their everyday lives. One example of this is diabetic alert dogs, who are reported to greatly improve the quality of life of owners living with Type 1 diabetes. It was recently shown that trained diabetic alert dogs are generally very reliable in alerting their owners to dropping glucose levels (Rooney *et al.* 2019). The median sensitivity for this was 83% among the 27 dogs in the study. However, the performance varied greatly even though all the dogs initially were trained to an equivalent high standard (75% sensitivity). The authors explained this as an effect of the environmental factors of working in a home.

COVID-19 detection dogs

Many sniffer-dog scientists turned their attention to COVID-19 early in the pandemic since it has previously been suggested that viral infections also cause the body to produce specific VOCs (Aksenov *et al.* 2014; Angle *et al.* 2015; Rochford *et al.* 2016; Else 2020). During 2020, research could confirm their beliefs: COVID-19 positive individuals produce VOCs with a specific odor, and dogs can be trained to detect that odor and thereby detect people infected with SARS-CoV-2 (Grandjean *et al.* 2020; Jendrny *et al.* 2020; Vesga *et al.* 2020). A recent study demonstrated that even asymptomatic SARS-CoV-2 infected people, and those with mild symptoms, have a distinct odor that trained dogs can identify with a high degree of accuracy (Guest *et al.* 2021). Sweat is the most commonly sampled source of VOCs, but urine and saliva have been proven to be similarly suited for reliable detection of SARS-CoV-2 infected individuals (Jendrny *et al.* 2021b).

Dogs are already being used to detect people with COVID-19 in airports, for example in Finland and Lebanon (Else 2020; World Health Organization 2021a). In both of these airports, sweat is used as the source of VOCs, and in both places dogs have been able to detect infected people days before a conventional test (Else 2020). According to a UK study (Guest *et al.* 2021), trained dogs can detect COVID-19 positive people with similar accuracy as reverse transcription-polymerase chain reaction (RT-PCR). Using dogs as a tool in mass screening could save a lot of time, inconvenience and money compared to RT-PCR testing the same amount of people (Guest *et al.* 2021; Jendrny *et al.* 2021b). Implementing dog screening and only using PCR test on those selected by the dogs could detect up to 91% of SARS-CoV-2 infected individuals, which would avert up to 2.2 times as much spread of the virus in comparison to solely isolating symptomatic individuals (Guest *et al.* 2021).

2.2. Track design

When training scent detection dogs a construction is needed for holding the scent samples and guiding the dog in its search. There are different types of equipment available on the market (Central Engineering Design Ltd. n.d.; Kynoscience n.d.), but to date no scientific article has been published comparing them to each other or comparing different methods of using the equipment. In some studies where scent detection training is involved, the training method is described (Fjellanger *et al.* 2002; McCulloch *et al.* 2006; Angle *et al.* 2014, 2015; Grandjean *et al.* 2020; Jendrny *et al.* 2020, 2021b; Essler *et al.* 2021; Sarkis *et al.* 2021), but to the best of my knowledge there are no scientific articles describing why a certain method has been chosen over another.

One device that is reoccurring in scent detection training is the scent wheel, also known as a scent carousel. This device comprises of a center stand with steel arms sticking out, making it possible to place a container holding a scent sample at the end of each arm. The dog is then supposed to go around the device to search all scent carriers. This type of training apparatus is described as the method of choice in some studies (Fjellanger *et al.* 2002; Angle *et al.* 2014, 2015; Essler *et al.* 2021), while others have chosen to present the sample carriers on stands placed in a straight line (McCulloch *et al.* 2006; Grandjean *et al.* 2020; Sarkis *et al.* 2021). Two studies by the same authors (Jendrny *et al.* 2020, 2021b) used the Detection Dog Training System (DDTS) instead, which is a device that presents the samples to the dog in an automated and randomized way, without human interference. Some studies do not mention what type of equipment has been used, and out of those who do neither discusses the track design and the alternatives (Fjellanger *et al.* 2002; McCulloch *et al.* 2006; Angle *et al.* 2014, 2015; Grandjean *et al.* 2020; Jendrny *et al.* 2020, 2021b; Essler *et al.* 2021; Sarkis *et al.* 2021). Therefore, it is hard to know why a particular method has been chosen. It can only be suspected that the choices are based on experience, tradition, the curiosity of the trainers, and the funding available for example.

The number of scent carriers used in each search also varies between different studies. In one study (Grandjean *et al.* 2020), a line of three or four stands were used, whereas another (Ehmann *et al.* 2012) used five stands placed in a circle. The DDTS has seven holes for the dog to sniff (Jendrny *et al.* 2020, 2021b), and there are scent wheels with eight (Angle *et al.* 2014, 2015) and twelve arms (Essler *et al.* 2021). One study had a scent wheel with twelve arms but only used six in the initial training phase (Fjellanger *et al.* 2002).

Since detection dogs are used to training with their handlers and being responsive to their cues, the degree of interference by the dog handler in the searches is of relevance for the dog's performance. There have been findings suggesting that the stress level of the handler may influence the dog's performance (Jezierski *et al.* 2014). To mitigate that risk, the handler should be positioned out of sight of the dog during the searches (Johnen *et al.* 2017). Different methods to achieve this have been described. In one study where a scent wheel was used, the room had a one-way window and a blind for trainers to hide behind (Fjellanger *et al.* 2002). A similar method was used in a recent study of COVID-19 detection dogs (Guest *et al.* 2021). A one-way screen was used during the blind-testing, to prevent the dog from receiving visual cues from its handler. Another method was used by Ehmann *et al.* (2012) who chose to keep the handler in the room with the dog, but remove the person who knew the placement of the target odor from the room before the dog and the trainer entered. One of the previously mentioned studies went from having zero, one or two trainers in the room either hidden or out in the open, to instead remove or hide all personnel for all searches (Fjellanger *et al.* 2002). It is not mentioned why the researchers chose to make that decision, but it probably raised concerns that having people visible to the dog would affect its performance.

2.3. Scent sample storing

When scent training dogs, a large number of scent samples are needed since each sample should only be used once. If samples are used several times, there is an imminent risk that the dogs recognize the individual samples rather than the target odor (Johnen *et al.* 2017). The reason for this is that every person emits a wide array of VOCs that reflects the metabolic condition in the body, which gives a unique body odor that can be considered as an individual's "odor-fingerprint" (Shirasu & Touhara 2011). The target odor that the dog is trained to detect is therefore only a part of the scent in a sample.

Storing a large number of scent samples can be a challenge since it is also important that scent contamination does not occur between the samples (Furton & Myers 2001; Johnen *et al.* 2017; Goss 2019). No study has been found that compares different types of storing of scent samples, although it has been warranted (Johnen *et al.* 2017). However, one scientific article has been published that describes the physico-chemistry of scents and its relevance for scent dog trainers (Goss 2019). According to the author, glass or metal containers are much preferred over plastic when storing odor. The main reasons for this are that plastic absorb scent to a greater extent and plastic is more penetrable, which makes the samples susceptible to cross-contamination. However, the risk of cross-contamination depends on the type of plastic and its thickness. Bags made of polyethylene, such as zip-lock bags leak odor within hours and are therefore considered worthless for scent sample storing.

Some studies involving scent detection training describe the method for scent sample storing, and it differs a lot between different studies. For example in a recent study about using dogs for COVID-19 detection, sweat samples were put in sterile containers (not specified what kind of containers) which were then disinfected and placed in a plastic envelope (Sarkis *et al.* 2021). Similarly, McCulloch *et al.* (2006) had lung and breast cancer patients exhale through cylindrical plastic tubes which were then sealed with caps and put in ordinary grocery store zip-lock bags. Ehmann *et al.* (2012) also collected breath samples in cylindrical tubes, but made of glass, and then stored in a light-tight cabinet until testing. A different approach was used by Guest *et al.* (2021), where both breath and sweat samples were wrapped in aluminum foil and packaged in plastic bags. This method is described as a suitable way to keep scent samples and minimize the risk of scent contamination (Goss 2019).

Scent sample storing also lacks a standardized storing temperature. In a recent study where both breath and sweat samples were collected, all samples were stored at minus 20°C (Guest *et al.* 2021). In some studies, breath samples have been stored at room temperature (McCulloch *et al.* 2006; Ehmann *et al.* 2012), and in one study sweat samples were stored at 6°C (Sarkis *et al.* 2021). In a study by Jendrny *et al.* (2021b) sweat, saliva and urine samples were collected, and all samples were stored at minus 80°C.

3. Material and Methods

The main material for this retrospective study was a database based on films of dogs in scent detection training for a research project led by the Uppsala University and Region Uppsala. The filmed material was used to verify the data. About eight dogs are part of the research project with the goal to detect people infected with SARS-CoV-2 using trained detection dogs. This study focused on two of those dogs, and additionally three dogs that were used to test if Falcon centrifuge tubes are suitable storing containers for scent samples.

Literature has been collected from scientific articles and books in veterinary medicine suitable to the subject. Some information has been collected from credible organizations such as the World Health Organization. The search for relevant literature was made in databases such as the Web of Science and PubMed, often through search engines as Primo or Google Scholar.

3.1. The dogs

In this study, two dogs were observed, a three-year-old female Belgian Malinois (hereinafter referred to as “Dog 1”) and a two-year-old male Labrador Retriever (hereinafter referred to as “Dog 2”). They were selected for this project due to their strong desire to search, great stamina, and high ability to focus on the task. Both dogs are privately owned, trained similarly, and are accustomed to scent training with Kong (a dog toy made from natural rubber).

To test if Falcon centrifuge tubes are suitable storing containers for scent samples three privately owned dogs trained to detect Kong were used. The dogs were two Welsh Springer Spaniels, a six-year-old female and an eight-year-old male, and a three-year-old female Belgian Malinois.

3.2. Track design

During the observation period (March 10 to August 4, 2021) the dogs were trained in three different track shapes: Track 1 was straight, Track 2 was circular and Track

3 was curved. Every track had six consoles which kept the same design throughout the study, it was only the position of each console that varied. Each console was made up of a stand with a metallic cone at the top, which the dogs were trained to sniff. A container could be connected to each cone, which is where the scent samples were placed.

The dogs were first presented with a straight track along a wall (Figure 1). This design was used for six weeks, from March 10 to April 21. The stands were then placed in a circle with the cones facing outwards (Figure 2), similar to a scent wheel. The shape of Track 2 made it so the dogs had to go around the circle in order to search every console. This design was used for five weeks, from May 5 to June 11. Finally, the consoles were positioned in a slightly curved line (Figure 3) for the final seven weeks of the observed training, from June 16 to August 4.

Figure 1. Linear arrangement of consoles for scent detection (Track 1)

Figure 2. Circular arrangement of consoles for scent detection (Track 2)

Figure 3. Curved arrangement of consoles for scent detection (Track 3)

3.2.1. Training evolvement

The degree of difficulty was adjusted as the dogs' searching skills evolved with the training. Up until April 21 (during the use of Track 1), the target used was a piece of Kong. The other consoles were either empty or contained gauze that was either clean, with hand sanitizer, or with sweat from a person who had tested negative for COVID-19 using PCR. When Track 2 was used the target was a piece of gauze with sweat from a COVID-19 positive individual, and the other consoles mainly contained gauze with sweat from a COVID-19 negative individual, but initially clean gauze and gauze with hand sanitizer were used. During the final phase, when Track 3 was used, the consoles only contained gauze with sweat from either a COVID-19 positive or a COVID-19 negative individual. In Track 3 each sample was only used once, whereas the samples were reused in Track 1 and Track 2. There was never more than one target in each search, but in some searches there was no target, in which case the dogs were rewarded for not marking any of the consoles.

Higher demands could be placed on the dogs as they developed their searching abilities throughout the training period, so the handlers were more permissive in the initial phase compared to at the end stage of the training. Therefore, the definition for a correctly marked console shifted during the observed period. Initially the dogs were allowed to search all consoles several times before deciding which one to mark, whereas that behavior later on counted as not being able to detect the target. If the dogs seemed unfocused, for example by skipping the first consoles, they were interrupted by the handlers and given a second chance. The dog trainers also assessed how certain the dogs were when marking a console. In Track 1 an insinuation from the dog was enough for the handler to reward the dog and it was considered a correct marking. In Track 2 and 3 a doubtful marking did not count as a correct marking.

3.2.2. Statistical analysis

A statistical analysis was made to compare the dogs' performance in the different tracks. Sensitivity and specificity in a 95% confidence interval were calculated for each dog and each track, as well as positive and negative predictive values. The calculations were made through Epitools Epidemiological Calculator for test evaluation against a gold standard. The gold standard in this case was PCR, or the presence of Kong.

3.3. Scent sample storing

A test was made to evaluate whether Falcon centrifuge tubes (Figure 4) could serve as a suitable container for scent sample storing. Kong Classic, a dog toy made from natural rubber (KONG Company 2014) which is frequently used in canine detection training due to its strong scent, was used as the source of scent in the test. Three cubes of different sizes were cut out of the toy. The side of these cubes were 0.5 cm, 1 cm, and 2 cm respectively. Each of the cubes were then placed into separate tubes. The containers were then sealed with the associated screw caps and all three containers were put in a plastic bag together with 47 empty Falcon centrifuge tubes. The plastic bag was stored at minus 18 °C for one week, after which the tubes were taken out without being opened up.

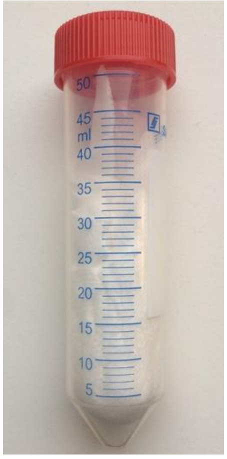

Figure 4. A Falcon centrifuge tube containing gauze

Three dogs trained to detect Kong were used to investigate if Falcon centrifuge tubes containing scent samples can be stored close together without scent contamination occurring. This was tested in a track with six consoles in a linear arrangement. Each console was designed so that the dogs could not see the tubes and their content, only sniff their outside. One tube with Kong inside was used in each search, and the remaining five consoles contained empty tubes. All the tubes (n=50) that had been in the freezer were used in the test and searched by all three dogs. The tubes were thawed before the search was performed. Each dog did 10 searches, and the dog handlers did not know which consoles contained Kong and which did not.

4. Results

4.1. Track design

In Track 1, Dog 1 performed 115 searches, of which seven were falsely marked. The sensitivity was 96.4% (95% confidence interval [CI]: 89.9-99.3%) and the specificity was 99.3% (95% CI: 98.3-99.8%), see Table 1 and Table 2. Dog 2 had similar results as 116 searches were performed, where three were falsely marked. The sensitivity was calculated to 97.8% (95% CI: 92.1-99.7%) and the specificity was 99.8% (95% CI: 99.1-100.0%). The positive predictive values for Track 1 were 95.3% for Dog 1 and 98.9% for Dog 2 (Table 3), and the negative predictive values were 99.5% and 99.7%, respectively (Table 4).

Track 1 was replaced by Track 2 when the dog trainers noticed a problem with the straight design. The placement of the consoles was noticeably giving the dogs curiosity which led to the dogs skipping the first one or two consoles in many cases. After this discovery, the consoles were placed in a circle with the cones facing outward, similar to a search wheel.

In Track 2, Dog 1 performed 168 searches, of which 37 were falsely marked. The sensitivity was 78.9% (95% CI: 71.9-84.9%) and the specificity was 96.6% (95% CI: 95.1-97.7%), see Table 1 and Table 2. Dog 2 did 156 searches, where 23 were falsely marked. The sensitivity was calculated to 85.1% (95% CI: 78.4-90.3%) and the specificity was 99.2% (95% CI: 98.3-99.7%). The positive predictive values for Track 2 were 81.9% for Dog 1 and 95.6% for Dog 2 (Table 3), and the negative predictive values were 95.9% and 97.1%, respectively (Table 4).

Similar to the previous change of track shape, Track 2 was replaced by Track 3 as the dog trainers noticed a possible risk factor for false results. As Track 2 had a circular design, it made it possible for the dog to receive visual cues from the handler during its search. For this reason, the consoles were instead placed in a slightly curved line.

In Track 3, Dog 1 performed 174 searches, of which 67 were falsely marked. The sensitivity was 62.9% (95% CI: 54.3-70.9%) and the specificity was 94.3% (95% CI: 92.5-95.7%), see Table 1 and Table 2. Dog 2 did 141 searches, where 26 were falsely marked, the sensitivity was calculated to 82.6% (95% CI: 74.7-88.9%) and the specificity was 98.5% (95% CI: 97.3-99.2%). The positive predictive values for Track 3 were 62.9% for Dog 1 and 90.1% for Dog 2 (Table 3), and the negative predictive values were 94.2% and 97.1%, respectively (Table 4).

Table 1. Sensitivity of two detection dogs in three different track designs, with a 95% confidence interval

	Dog 1, %	**Dog 2, %**
Track 1	96.4 (89.9-99.3)	97.8 (92.1-99.7)
Track 2	78.9 (71.9-84.9)	85.1 (78.4-90.3)
Track 3	62.9 (54.3-70.9)	82.6 (74.7-88.9)

Table 2. Specificity of two detection dogs in three different track designs, with a 95% confidence interval

	Dog 1, %	**Dog 2, %**
Track 1	99.3 (98.3-99.8)	99.8 (99.1-100.0)
Track 2	96.6 (95.1-97.7)	99.2 (98.3-99.7)
Track 3	94.3 (92.5-95.7)	98.5 (97.3-99.2)

Table 3. Positive predictive values of two detection dogs in three different track designs

	Dog 1, %	**Dog 2, %**
Track 1	95.3	98.9
Track 2	81.9	95.6
Track 3	62.9	90.1

Table 4. Negative predictive values of two detection dogs in three different track designs

	Dog 1, %	**Dog 2, %**
Track 1	99.5	99.7
Track 2	95.9	97.1
Track 3	94.2	97.1

4.2. Scent sample storing

A total of 30 searches were performed by three detection dogs trained to detect Kong. As each search had six consoles the dogs sniffed Falcon tubes a total of 180 times. None of the three trained dogs were able to detect the Kong scent in any of the searches. As each search contained one sealed tube with a piece of Kong inside and five sealed empty tubes that had been stored together with the Kong containing tubes, this suggests that the dogs could not detect the Kong scent through the thick plastic of the Falcon tubes, and the Kong scent had not contaminated the empty tubes.

5. Discussion

5.1. Track design

The training methodology is key to effectively educating detection dogs. Since disease detection dogs are used as diagnostic tools and therefore are compared to other diagnostic methods, it would be beneficial to have a standardized training protocol. Developing useful detection dogs should be done in a structured and economical fashion to be able to compete with the conventional methods. A scientifically based training protocol could upgrade the detection dog industry which could lead to many more dogs being available to perform different types of searches. One aspect that could be standardized in detection dog training is the track shape. To the author's knowledge, there is no scientifically based description of what track design is most beneficial for the detection dogs' learning. A study like this could therefore be of great importance for the evolvement of detection dog training.

The results from the statistical analysis suggest that Track 1 is where the dogs performed their best, both according to sensitivity, specificity, positive predictive values, and negative predictive values. However, as the prerequisites varied in several aspects (source of scent, criteria for correct marking, and visibility of the handler) between the different tracks, it is doubtful whether the statistical results from the tracks are comparable.

The reason for the switch from Track 1 to Track 2 was the fact that the dog handlers noticed that the dogs had a high level of curiosity with the straight track, which made the dogs skip the first one or two consoles in many of the searches. However, as the trainers were more permissive in the initial phase of the training, this is not reflected in the statistical results of Track 1. If a dog skipped the first couple of consoles, the handler could summon the dog and restart the search, and the dog would often do a more thorough search on the second try. Because of the high level of skipped consoles due to curiosity, the consoles were instead put in a circular shape. In Track 2 the dogs did not skip any platforms, suggesting that the circular design made the dogs do a more thorough search compared to the straight track.

Another aspect that complicates the comparison of the track designs is the fact that the dogs were exposed to different types of scents in the different tracks. This is a natural way of scent training dogs as the level of difficulty needs to be adjusted after the dogs' capabilities, which evolve during the training. However, it is hard to know whether the adjustments made were corresponding to the dogs' capabilities or if the dogs experienced a higher degree of difficulty after the adjustments. The accuracy of the dogs was higher initially, and dropped as the level of difficulty was adjusted. It can be assumed that the scent from a sweat sample from a COVID-19 positive individual is more similar to a sweat sample from a COVID-19 negative individual compared to a clean gauze for example. Many of those who test negative for COVID-19 may have other types of viral infections that could make the body produce VOCs with a similar scent to those who test positive for COVID-19. Therefore, it is very important that the dogs are trained to distinguish the target smell from similar smells. A study about using canine scent detection in the diagnosis of lung cancer (Ehmann *et al.* 2012) involved scent samples from lung cancer patients, healthy controls, and from patients with non-malignant lung disease for this reason.

The scent samples used in the final period of training in Track 2 were only sweat samples, either from COVID-19 positive or COVID-19 negative individuals. In some of those searches the dogs had issues with detecting the COVID-19 related smell. Since the searches were not blinded this could lead to the handlers getting stressed. Previous findings suggest that the stress level of the handler may influence the dog's performance (Jezierski *et al.* 2014). The trainers in this study also had those concerns, as they noticed that the handlers would change their body language as they got stressed, for example by adjusting their glasses. Since the handler and the dog were kept in the same room, without blinds, and Track 2 was circular, it is possible that the dogs picked up on the visual cues the handlers were unaware of. As this was considered a risk for false results being obtained in the study, the track was once again redesigned.

Lessons had been learned from the first two track shapes: the third track had to be designed in a way so that the dogs would do a thorough search and not skip any consoles, while keeping the handler out of sight from the dog. This resulted in Track 3, where the consoles were placed in a slightly curved line. Despite this, the statistical analysis for Track 3 shows lower rates for both dogs compared to the other tracks. This may seem as if using Track 3 would be the worst choice out of the three observed track shapes, but as previously mentioned there were different prerequisites for the different tracks. The risk factor for falsely obtained results for Track 2 (the risk of visual cues affecting the dogs' performance) is a potential confounder that could affect the numbers from the statistical analysis so that the track appears better than it is. It has previously been suggested that the handler should be positioned out of sight of the dog during the searches (Johnen *et al.* 2017). This can be achieved through different methods, for example by using a one-way screen in the room like Guest *et al.* (2021). Fjellanger *et al.* (2002) also took this into consideration and used both a one-way window and a blind for trainers to hide behind.

A new addition in Track 3 was that each sweat sample was only used once. There were two reasons for this decision: first, there is a risk that the dogs remember the scent of specific samples rather than being able to detect the COVID-19 scent; second, it is likely that the dogs put scent into a sample when sniffing. Reusing samples can therefore lead to unwanted guidance.

Considering how large the detection dog industry is, it is remarkable that the search training methodology is not further researched. The descriptions of methods used in scientific articles regarding canine scent detection are also oftentimes inadequate. By sharing experiences, the industry could evolve, and the education of detection dogs could improve greatly. It would be more efficient and resourceful since fewer dog trainers would hit the same difficulties in the training. Without published research on the subject, the training methodology is often based on the trainers' previous experiences, which makes the training very personal and not standardized. If more published research existed, dogs could be trained in a more efficient way and more people could benefit from their incredible detection capabilities. This would, of course, be applicable to all types of detection dogs, not only COVID-19 detection dogs.

Except for the fact that the filmed material was the basis for this study, it was also of great use for the dog trainers as they could go back and analyze what improvements could be done. As the filmed material allows repeated viewing of the searches, it makes it possible to observe details that could easily be missed by only observing once.

5.1.1. Limitations of the study

As this is a retrospective study, there are some aspects that were suboptimal. First, the dogs were trained in one track after another. As they constantly learned and evolved it is likely that the dogs had developed their searching skills during the training period, which is to say that they had different prerequisites for the different tracks. Other prerequisites that differed between the tracks were what counted as a correct marking, as well as what type of scent samples were used.

Another limitation of the study is that only two dogs were observed. The results can still give an indication, but they are very tied to the individuals rather than detection dogs as a group. Even though these two dogs had similar results in the tracks, further studies are needed for validation of what would reflect detection dogs as a group.

5.1.2. Suggested improvements

To obtain a more comparable study result between the tracks, everything except the shape of the track should be as similar as possible. As we are not interested in the individuals' performances, the study could be done with several groups of dogs, each group trained in a particular track design. The requirements for a marking to count as correct would be the same throughout the study, and the samples used in the study would be of the same kind in all tracks.

5.2. Scent sample storing

As the dogs did not detect the Kong scent in any of the searches, the result suggests that the Falcon tubes contain scent well, at least for one week stored in minus 18°C. Since both empty tubes and tubes with Kong were used in the test, two tests were actually performed. If the dogs had been able to detect the Kong scent from the sealed tubes with Kong inside, that would suggest that the material leaks detectable amounts at least after one week. The second test would then be if the empty tubes would absorb the leaked smell, and thereby get contaminated. In this study the dogs did not detect the pieces of Kong inside the sealed Falcon tubes. This indicates that the Falcon tubes are suitable for scent sample storing. This result is valuable as this has not been described before and could potentially lead to more space-efficient storing of scent samples.

It can be assumed that scent contamination occurs in the same way regardless of the source of the scent. Irrespective of what scent samples are being stored, it is always relevant to keep the scents separated and it is important that scent contamination does not occur. As long as the scent is not stronger than that of Kong, it is reasonable to assume that scent contamination would not occur in other scent samples stored in Falcon tubes either.

As Goss (2019) describes, plastic containers absorb scent to a higher degree compared to glass or metal, which makes it hard to reuse the containers. For this reason, a glass container with a metal lid, or an all-metal container could be even more suitable.

5.2.1. Limitations of the study

There were certain limitations of the study on scent contamination between scent samples stored in Falcon tubes. For example, a low number of Falcon tubes with

Kong inside were used, as there were only three. The number of dogs who performed the searches were also only three. Another limitation of the study was the lack of positive control to ensure that the dogs would have reacted if they felt the scent of Kong. Moreover, the test has only been made with Kong as the source of smell, which means that anything with a stronger scent could potentially leak through the plastic more easily.

5.2.2. Suggested improvements

A more optimal study would include a larger number of detection dogs performing the searches as well as a larger number of Falcon tubes included in the test. The dogs were trained to detect Kong, but a positive control could have been added to increase the reliability of the test. To be certain that other scent sample types do not leak through the plastic of the Falcon tube, the test should be done with a source of scent that has a strong scent and that the dogs are trained to detect.

6. Conclusions

According to the statistical analysis, a straight track shape seems most beneficial for the detection dogs' learning. However, the results may have been influenced by prerequisites that differed between the tracks, which makes the results difficult to interpret.

The results from the study on Falcon tubes indicate that the scent of Kong does not escape the sealed container, at least within a week when stored in minus 18°C. Falcon tubes are therefore a promising alternative for storing scent samples in a space-efficient way, and they would be valuable to study further.

References

Aberle, D.R., Berg, C.D., Black, W.C., Church, T.R., Fagerstrom, R.M., Galen, B., Gareen, I.F., Gatsonis, C., Goldin, J., Gohagan, J.K., Hillman, B., Jaffe, C., Kramer, B.S., Lynch, D., Marcus, P.M., Schnall, M., Sullivan, D.C., Sullivan, D. & Zylak, C.J. (2011). The National Lung Screening Trial: overview and study design. *Radiology*, 258 (1), 243–253. https://doi.org/10.1148/radiol.10091808

Aksenov, A.A., Sandrock, C.E., Zhao, W., Sankaran, S., Schivo, M., Harper, R., Cardona, C.J., Xing, Z. & Davis, C.E. (2014). Cellular scent of influenza virus infection. *Chembiochem : a European Journal of Chemical Biology*, 15 (7), 1040–1048. https://doi.org/10.1002/cbic.201300695

Angle, C.T., Wakshlag, J.J., Gillette, R.L., Steury, T., Haney, P., Barrett, J. & Fisher, T. (2014). The effects of exercise and diet on olfactory capability in detection dogs. *Journal of Nutritional Science (Cambridge)*, 3, e44–e44. https://doi.org/10.1017/jns.2014.35

Angle, T.C., Passler, T., Waggoner, P.L., Fischer, T.D., Rogers, B., Galik, P.K. & Maxwell, H.S. (2015). Real-time detection of a virus using detection dogs. *Frontiers in Veterinary Science*, 2, 79–79. https://doi.org/10.3389/fvets.2015.00079

Buck, L. & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, 65 (1), 175–187. https://doi.org/10.1016/0962-8924(91)90063-F

Buljubasic, F. & Buchbauer, G. (2015). The scent of human diseases: a review on specific volatile organic compounds as diagnostic biomarkers. *Flavour and Fragrance Journal*, 30 (1), 5–25. https://doi.org/10.1002/ffj.3219

Catala, A., Grandgeorge, M., Schaff, J.-L., Cousillas, H., Hausberger, M. & Cattet, J. (2019). Dogs demonstrate the existence of an epileptic seizure odour in humans. *Scientific Reports*, 9 (1), 4103–4103. https://doi.org/10.1038/s41598-019-40721-4

Central Engineering Design Ltd. (u.å.). *Scent Detection Training Devices*. *Scent Detection*. https://www.scentdetection.co.uk/ [2022-01-01]

Corning Incorporated (2013). *Falcon Tubes and Pipets*. [Brochure]. Tewksbury: Corning. https://www.corning.com/catalog/cls/documents/brochures/CLS-DL-CC-012.pdf [2022-08-07]

Ehmann, R., Boedeker, E., Friedrich, U., Sagert, J., Dippon, J., Friedel, G. & Walles, T. (2012). Canine scent detection in the diagnosis of lung cancer: revisiting a puzzling phenomenon. *The European Respiratory Journal*, 39 (3), 669–676. https://doi.org/10.1183/09031936.00051711

Else, H. (2020). Can dogs smell COVID? Here’s what the science says. *Nature*, 587 (7835), 530–531. https://doi.org/10.1038/d41586-020-03149-9

Eskandari, E., Ahmadi Marzaleh, M., Roudgari, H., Hamidi Farahani, R., Nezami-Asl, A., Laripour, R., Aliyazdi, H., Dabbagh Moghaddam, A., Zibaseresht, R., Akbarialiabad, H., Yousefi Zoshk, M., Shiri, H. & Shiri, M. (2021). Sniffer dogs as a screening/diagnostic tool for COVID-19: a proof of concept study. *BMC infectious Diseases*, 21 (1), 243–8. https://doi.org/10.1186/s12879-021-05939-6

Essler, J.L., Kane, S.A., Nolan, P., Akaho, E.H., Berna, A.Z., DeAngelo, A., Berk, R.A., Kaynaroglu, P., Plymouth, V.L., Frank, I.D., Weiss, S.R., Odom John, A.R. & Otto, C.M. (2021). Discrimination of SARS-CoV-2 infected patient samples by detection dogs: A proof of concept study. *PloS One*, 16 (4), e0250158–e0250158. https://doi.org/10.1371/journal.pone.0250158

Fjellanger, R., Andersen, E.K. & McLean, I. (2002). A training program for filter-search mine detection dogs. *International Journal of Comparative Psychology*, 15 (4), 278–287

Fredholm, L. (2004). Luktvärlden avslöjad. *Forskning & Framsteg*, 2004 (8). https://fof.se/tidning/2004/8/artikel/medicin-luktvarlden-avslojad [2021-10-21]

Furton, K.G. & Myers, L.J. (2001). The scientific foundation and efficacy of the use of canines as chemical detectors for explosives. *Talanta*, 54 (3), 487–500. https://doi.org/10.1016/S0039-9140(00)00546-4

Gialamas, D.M. (1996). Enhancement of fire scene investigations using Accelerant Detection Canines. *Science & Justice*, 36 (1), 51–54. https://doi.org/10.1016/S1355-0306(96)72555-8

Glusman, G., Yanai, I., Rubin, I. & Lancet, D. (2001). The complete human olfactory subgenome. *Genome Research*, 11 (5), 685–702. https://doi.org/10.1101/gr.171001

Gorbalenya, A.E., Baker, S.C., Baric, R.S., de Groot, R.J., Drosten, C., Gulyaeva, A.A., Haagmans, B.L., Lauber, C., Leontovich, A.M., Neuman, B.W., Penzar, D., Perlman, S., Poon, L.L.M., Samborskiy, D.V., Sidorov, I.A., Sola, I. & Ziebuhr, J. (2020). The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nature Microbiology*, 5 (4), 536–544. https://doi.org/10.1038/s41564-020-0695-z

Goss, K.-U. (2019). The physical chemistry of odors - Consequences for the work with detection dogs. *Forensic Science International*, 296, 110–114. https://doi.org/10.1016/j.forsciint.2019.01.023

Grandjean, D., Al Marzooqi, D.H., Lecoq-Julien, C., Al Hammadi, H.K., Alvergnat, G., Al Blooshi, K.M., Al Mazrooei, S.K., Alhmoudi, M.S., Al Ahbabi, F.M. & Mohammed, Y.S. (2021). Use of canine olfactory detection for COVID-19 testing study on UAE trained detection dog sensitivity. *Journal of Veterinary Science & Research*, 6 (2). https://doi.org/10.23880/oajvsr-16000210

Grandjean, D., Sarkis, R., Lecoq-Julien, C., Benard, A., Roger, V., Levesque, E., Bernes-Luciani, E., Maestracci, B., Morvan, P., Gully, E., Berceau-Falancourt, D., Haufstater, P., Herin, G., Cabrera, J., Muzzin, Q., Gallet, C., Bacque, H., Broc, J.-M., Thomas, L., Lichaa, A., Moujaes, G., Saliba, M., Kuhn, A., Galey, M., Berthail, B., Lapeyre, L., Capelli, A., Renault, S., Bachir, K., Kovinger, A., Comas, E., Stainmesse, A., Etienne, E., Voeltzel, S., Mansouri, S., Berceau-Falancourt, M., Dami, A., Charlet, L., Ruau, E., Issa, M., Grenet, C., Billy, C., Tourtier, J.-P. & Desquilbet, L. (2020). Can the detection dog alert on COVID-19 positive persons by sniffing axillary sweat samples? A proof-of-concept study. *PloS One*, 15 (12), e0243122–e0243122. https://doi.org/10.1371/journal.pone.0243122

Guest, C., Dewhirst, S.Y., Allen, D.J., Aziz, S., Baerenbold, O., Bradley, J., Chabildas, U., Chen-Hussey, V., Clifford, S., Cottis, L., Dennehy, J., Foley, E., Gezan, S.A., Gibson, T., Greaves, C.K., Kleinschmidt, I., Lambert, S., Last, A., Lindsay, S.W., Morant, S., Parker, J.E.A., Pickett, J., Quilty, B.J., Rooney, A., Shah, M., Somerville, M., Squires, C., Walker, M. & Logan, J.G. (2022). Using trained dogs and organic semi-conducting sensors to identify asymptomatic and mild SARS-CoV-2 infections. *Journal of Travel Medicine*. 29 (3), taac043. https://doi.org/10.1093/jtm/taac043

Jendrny, P., Schulz, C., Twele, F., Meller, S., von Koeckritz-Blickwede, M., Osterhaus, A.D.M.E., Ebbers, J., Pilchova, V., Pink, I., Welte, T., Manns, M.P., Fathi, A., Ernst, C., Addo, M.M., Schalke, E. & Volk, H.A. (2020). Scent dog identification of samples from COVID-19 patients - a pilot study. *BMC Infectious Diseases*, 20 (1), 536–536. https://doi.org/10.1186/s12879-020-05281-3

Jendrny, P., Twele, F., Meller, S., Osterhaus, A.D.M.E., Schalke, E. & Volk, H.A. (2021a). Canine olfactory detection and its relevance to medical detection. *BMC Infectious Diseases*, 21 (1), 838–838. https://doi.org/10.1186/s12879-021-06523-8

Jendrny, P., Twele, F., Meller, S., Schulz, C., von Köckritz-Blickwede, M., Osterhaus, A.D.M.E., Ebbers, H., Ebbers, J., Pilchová, V., Pink, I., Welte, T., Manns, M.P., Fathi, A., Addo, M.M., Ernst, C., Schäfer, W., Engels, M., Petrov, A., Marquart, K., Schotte, U., Schalke, E. & Volk, H.A. (2021b). Scent dog identification of SARS-CoV-2 infections in different body fluids. *BMC Infectious Diseases*, 21 (1), 707–707. https://doi.org/10.1186/s12879-021-06411-1

Jenkins, E.K., DeChant, M.T. & Perry, E.B. (2018). When the nose doesn't know: Canine olfactory function associated with health, management, and potential links to microbiota. *Frontiers in Veterinary Science*, 5, 56–56. https://doi.org/10.3389/fvets.2018.00056

Jezierski, T., Adamkiewicz, E., Walczak, M., Sobczyńska, M., Górecka-Bruzda, A., Ensminger, J. & Papet, E. (2014). Efficacy of drug detection by fully-trained police dogs varies by breed, training level, type of drug and search environment. *Forensic Science International*, 237, 112–118. https://doi.org/10.1016/j.forsciint.2014.01.013

Johnen, D., Heuwieser, W. & Fischer-Tenhagen, C. (2017). An approach to identify bias in scent detection dog testing. *Applied Animal Behaviour Science*, 189, 1–12. https://doi.org/10.1016/j.applanim.2017.01.001

Kishida, T., Thewissen, J., Hayakawa, T., Imai, H. & Agata, K. (2015). Aquatic adaptation and the evolution of smell and taste in whales. *Zoological Letters*, 1 (1), 9–9. https://doi.org/10.1186/s40851-014-0002-z

Kitiyakara, T., Redmond, S., Unwanatham, N., Rattanasiri, S., Thakkinstian, A., Tangtawee, P., Mingphruedhi, S., Sobhonslidsuk, A., Intaraprasong, P. & Kositchaiwat, C. (2017). The detection of hepatocellular carcinoma (HCC) from patients' breath using canine scent detection: a proof-of-concept study. *Journal of Breath Research*, 11 (4), 046002

KONG Company (2014). *KONG® Classic*. https://www.kongcompany.com/kong-classic [2021-12-08]

Kynoscience (u.å.). Detection dog training system. *Kynoscience*. https://www.kynoscience.de/en/detection-dog-training-system/ [2022-01-01]

de Lahunta, A. & Glass, E. (2009). *Veterinary Neuroanatomy and Clinical Neurology*. 3rd edition. St. Louis, Mo: Saunders Elsevier.

Maa, E., Arnold, J., Ninedorf, K. & Olsen, H. (2021). Canine detection of volatile organic compounds unique to human epileptic seizure. *Epilepsy & Behavior*, 115, 107690-. https://doi.org/10.1016/j.yebeh.2020.107690

Malmberg, A. (2021). *Hunden som hjälpmedel vid diagnostik av covid-19*. Uppsala universitet - nyhet. https://www.uu.se/nyheter/artikel/?id=16494&typ=artikel [2021-09-03]

McCulloch, M., Jezierski, T., Broffman, M., Hubbard, A., Turner, K. & Janecki, T. (2006). Diagnostic accuracy of canine scent detection in early- and late-stage lung and breast cancers. *Integrative Cancer Therapies*, 5 (1), 30–39. https://doi.org/10.1177/1534735405285096

McCulloch, M., Turner, K. & Broffman, M. (2012). Lung cancer detection by canine scent: will there be a lab in the lab? *The European Respiratory Journal*, 39 (3), 511–512. https://doi.org/10.1183/09031936.00215511

Mellgren, F. (2021). Nu ska Sveriges första coronahundar utbildas. *Svenska Dagbladet*. https://www.svd.se/nu-ska-sveriges-forsta-coronahundar-utbildas [2021-11-02]

Mombaerts, P. (2004). Love at first smell — The 2004 Nobel Prize in physiology or medicine. *The New England Journal of Medicine*, 351 (25), 2579–2580. https://doi.org/10.1056/NEJMp048325

Moser, E. & McCulloch, M. (2010). Canine scent detection of human cancers: A review of methods and accuracy. *Journal of Veterinary Behavior*, 5 (3), 145–152. https://doi.org/10.1016/j.jveb.2010.01.002

Niimura, Y. & Nei, M. (2007). Extensive gains and losses of olfactory receptor genes in mammalian evolution. *PloS One*, 2 (8), e708–e708. https://doi.org/10.1371/journal.pone.0000708

Olender, T., Fuchs, T., Linhart, C., Shamir, R., Adams, M., Kalush, F., Khen, M. & Lancet, D. (2004). The canine olfactory subgenome. *Genomics (San Diego, Calif.)*, 83 (3), 361–372. https://doi.org/10.1016/j.ygeno.2003.08.009

Olender, T., Waszak, S.M., Viavant, M., Khen, M., Ben-Asher, E., Reyes, A., Nativ, N., Wysocki, C.J., Ge, D. & Lancet, D. (2012). Personal receptor repertoires: olfaction as a model. *BMC Genomics*, 13 (1), 414–414. https://doi.org/10.1186/1471-2164-13-414

Quignon, P., Kirkness, E., Cadieu, E., Touleimat, N., Guyon, R., Renier, C., Hitte, C., Ane, C., Fraser, C. & Galibert, F. (2003). Comparison of the canine and human olfactory receptor gene repertoires. *Genome Biology*, 4 (12), R80–R80. https://doi.org/10.1186/gb-2003-4-12-r80

Quignon, P., Rimbault, M., Robin, S. & Galibert, F. (2012). Genetics of canine olfaction and receptor diversity. *Mammalian Genome*, 23 (1), 132–143. https://doi.org/10.1007/s00335-011-9371-1

Rochford, K., Chen, F., Waguespack, Y., Figliozzi, R.W., Kharel, M.K., Zhang, Q., Martin-Caraballo, M. & Hsia, S.V. (2016). Volatile organic compound gamma-butyrolactone released upon herpes simplex virus type -1 acute infection modulated membrane potential and repressed viral infection in human neuron-like cells. *PloS One*, 11 (8), e0161119–e0161119. https://doi.org/10.1371/journal.pone.0161119

Rooney, N.J., Guest, C.M., Swanson, L.C.M. & Morant, S.V. (2019). How effective are trained dogs at alerting their owners to changes in blood glycaemic levels?: Variations in performance of glycaemia alert dogs. *PloS One*, 14 (1), e0210092–e0210092. https://doi.org/10.1371/journal.pone.0210092

Sarkis, R., Lichaa, A., Mjaess, G., Saliba, M., Selman, C., Lecoq-Julien, C., Grandjean, D. & Jabbour, N.M. (2021). New method of screening for COVID-19 disease using sniffer dogs and scents from axillary sweat samples. *Journal of Public Health*, fdab215, 1–6. https://doi.org/10.1093/pubmed/fdab215

Shirasu, M. & Touhara, K. (2011). The scent of disease: volatile organic compounds of the human body related to disease and disorder. *Journal of Biochemistry (Tokyo)*, 150 (3), 257–266. https://doi.org/10.1093/jb/mvr090

Sjaastad, Ø.V., Hove, K. & Sand, O. (2016). *Physiology of Domestic Animals*. 3rd edition. Oslo: Scandinavian Veterinary Press.

Sonoda, H., Kohnoe, S., Yamazato, T., Satoh, Y., Morizono, G., Shikata, K., Morita, M., Watanabe, A., Morita, M. & Kakeji, Y. (2011). Colorectal cancer screening with odour material by canine scent detection. *Gut*, 60 (6), 814–819

Stone, R.L. & Waugh, D. (2014). Canine scent-specific detection of serous ovarian cancer. *Gynecologic Oncology*, 133, 103–104. https://doi.org/10.1016/j.ygyno.2014.03.275

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A. & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, 71 (3), 209–249. https://doi.org/10.3322/caac.21660

Taverna, G., Tidu, L., Grizzi, F., Torri, V., Mandressi, A., Sardella, P., La Torre, G., Cocciolone, G., Seveso, M. & Giusti, G. (2015). Olfactory system of highly trained dogs detects prostate cancer in urine samples. *The Journal of Urology*, 193 (4), 1382–1387

The Nobel Assembly at Karolinska Institutet (2004). *The 2004 Nobel Prize in Physiology or Medicine to Richard Axel and Linda B. Buck*. [Press release]. https://www.nobelprize.org/prizes/medicine/2004/press-release/ [2021-10-21]

Trimmer, C., Keller, A., Murphy, N.R., Snyder, L.L., Willer, J.R., Nagai, M.H., Katsanis, N., Vosshall, L.B., Matsunami, H. & Mainland, J.D. (2019). Genetic variation across the human olfactory receptor repertoire alters odor perception. *Proceedings of the National Academy of Sciences - PNAS*, 116 (19), 9475–9480. https://doi.org/10.1073/pnas.1804106115

Vesga, O., Valencia, A.F., Mira, A., Ossa, F., Ocampo, E., Agudelo, M., Čiuoderis, K., Perez, L., Cardona, A. & Aguilar, Y. (2020). Dog savior: immediate scent-detection of SARS-COV-2 by trained dogs. *BioRxiv*,. https://doi.org/10.1101/2020.06.17.158105

Willis, C.M., Britton, L.E., Harris, R., Wallace, J. & Guest, C.M. (2011). Volatile organic compounds as biomarkers of bladder cancer: Sensitivity and specificity using trained sniffer dogs. *Cancer Biomarkers*, 8 (3), 145–153

World Health Organization (2020a). *IHR Emergency Committee on Novel Coronavirus (2019-nCoV).* World Health Organization. https://www.who.int/director-general/speeches/detail/who-director-general-s-statement-on-ihr-emergency-committee-on-novel-coronavirus-(2019-ncov) [2021-11-02]

World Health Organization (2020b). *Novel Coronavirus(2019-nCoV)*. (Situation Report, 22). World Health Organization. https://apps.who.int/iris/handle/10665/330991 [2021-11-02]

World Health Organization (2020c). *Pneumonia of Unknown Cause – China*. World Health Organization. https://www.who.int/emergencies/disease-outbreak-news/item/2020-DON229 [2021-11-02]

World Health Organization (2020d). *WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020*. World Health Organization. https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020 [2021-11-02]

World Health Organization (2021a). *Consultation on the Use of Trained Dogs for Screening COVID-19 cases.* World Health Organization. https://cdn.who.int/media/docs/default-source/blue-print/who-consultation-screening-dogs--8th-march-2021.pdf?sfvrsn=a0d8cbda_1&download=true [2021-11-09]

World Health Organization (2021b). *Timeline: WHO's COVID-19 response*. World Health Organization. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/interactive-timeline [2021-10-11]

Young, J.M., Friedman, C., Williams, E.M., Ross, J.A., Tonnes-Priddy, L. & Trask, B.J. (2002). Different evolutionary processes shaped the mouse and human olfactory receptor gene families. *Human Molecular Genetics*, 11 (5), 535–546. https://doi.org/10.1093/hmg/11.5.535

Zozulya, S., Echeverri, F. & Nguyen, T. (2001). The human olfactory receptor repertoire. *Genome Biology*, 2 (6), 1–12. http://genomebiology.com/2001/2/6/research/0018 [2021-10-24]

Popular Science Summary

Dogs have a very well-developed sense of smell, and they have therefore been used by humans as a detection tool in many different areas. A detection dog can be trained to detect anything with a specific smell, and they are a common sight in airports where they search for drugs, explosives, and firearms. Dogs can even detect different types of diseases as these change a person's body odor. A person's smell can be collected with gauze wipes that absorb sweat. Sweat samples can then be used when training detection dogs to detect a specific illness.

Detection dogs are used in many areas and in some cases used instead of a diagnostic test. It is therefore of high importance that the dogs are reliable and trained in a structured manner. This study evaluated the performance of two detection dogs, when training to detect COVID-19 infected individuals through sweat samples. No previous research has been published on what method to use when training dogs for scent detection. Therefore, this study focused on comparing three different track designs used in the training. All tracks consisted of six consoles. In the first track the consoles were positioned in a straight line. This design caused the dogs to be curious which made them skip the first consoles in many searches. The consoles were therefore positioned in a circle so the dogs had to go around the track to search every console. The dogs skipped fewer consoles with this design, but the circular design made it possible for the dogs to pick up on visual cues the handlers were unaware of. As this was considered a risk factor that could potentially lead to false results, the consoles were then placed in a slightly curved line.

According to the statistical analysis of the dogs' performance in the different tracks, both dogs had the highest accuracy in the first track, and the lowest accuracy in the third track. However, the results may have been influenced by things that differed between the tracks, which makes the results difficult to interpret.

This study also examined whether a particular type of centrifuge tube is suitable as a storage container for scent samples. In this study, no dog could detect the scent of natural rubber that was kept in the sealed tube. This indicates that the tubes are a promising alternative for storing scent samples since scent contamination between samples would not be possible if the containers do not leak the scent. Further studies would be valuable as this could potentially facilitate storing scent samples in a space-efficient way.