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“Dogs as diagnostic tools”

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Abstract

For many years, dogs, often referred to as man's closest companions, served as the primary scent-detection tool in both civilian and military applications. Recently, numerous studies have shed light on the remarkable olfactory abilities of dogs, particularly in the realm of medical diagnostics, where they excel in identifying various infectious, metabolic, and neoplastic conditions. This newfound recognition is especially significant given that cancer ranks among the leading causes of death, and during pandemics like Covid-19, when human resources may be insufficient. It prompts us to reconsider the invaluable role our beloved canine friends can play. Dogs possess an exceptional sense of smell, allowing them to detect diseases and metabolic changes, often long before they become detectable by advanced technological machines and complex laboratory methods.

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1 Introduction

Next to cats, dogs are the most popular pets worldwide, they are very sensitive, intelligent and have a unique olfactory organ, which makes them the most popular companions of humans, dogs have been accompanying humans on their way for around 15.000 years and even today in the 21st century they are still indispensable in many areas.

Nowadays dogs are used due to their extraordinary nose in many different fields:

- they work together with the police to find drugs, explosives, counterfeit money, or criminals,
- they help mountain rescuers to find and rescue avalanche victims,
- they accompany blind people safely through their everyday lives,
- they are indispensable for patients with diabetes mellitus because they can recognize metabolic changes early than any machine.

In times of the Covid-19 pandemic, dogs were trained with a high success rate to recognize Covid-19 [1] and recently dogs are also to be used in preventive medicine for infection diseases and especially for cancer prevention.

Dogs have extremely fine noses: they have around 220 million olfactory receptors, while humans have only around 5 million, and even their mucosal membrane of the olfactory organ is about 10 times larger than that of the human nose which makes it very easy for them to recognize odours from which we humans do not even know that they exist [2].

But while police and rescue dogs have become indispensable, human medicine is still reluctant to work with four-legged colleagues, even though they work very accurately and precisely and could cover such an important area, especially in preventive medicine and in cancer screening.

1.1 Objective

In this literature review I tried to summarize the advantage to use the qualification of dogs' sense of smell in human medicine. As we can see in the results of different studies canine diagnose can achieve very high success rates, of course a dog diagnose cannot replace are CT or MRI scan, but they are very efficient and able to diagnose cancer in a very early stage and they have at least the same success rate as a covid rapid antigen test.

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1.2 History

Throughout ancient medical history, the human sense of smell has served as a diagnostic tool. Dating back to the era of Hippocrates, a prominent figure in medicine, practitioners used their sense of smell to identify illnesses. Notable examples from that time, still relevant in modern medicine, include detecting advanced liver disease through the distinctive musty breath odour known as fetor hepaticus, and identifying diabetic ketoacidosis through the sweet scent of ketones in exhaled breath. Even before the advent of proper diagnostic tools, infectious diseases were associated with specific odours. For example, the foul odour of gas gangrene, caused by Clostridium perfringens bacteria, was recognized during medieval times. In the context of the First and Second World Wars, physicians heavily relied on their senses, particularly smell, due to the absence of other diagnostic resources. They could diagnose Pseudomonas aeruginosa infection based on a fruity odour emanating from wound infections [3].

Numerous anecdotal accounts have been documented regarding instances where dogs exhibited spontaneous curiosity towards specific body parts of their owner.

Perhaps one of the most renowned instances of a dog serving as a diagnostic instrument is the anecdote documented by Williams and Pembroke in 1989. They recounted a situation in which a patient's dog consistently sniffed at a mole on her leg while ignoring other moles. This unusual behaviour aroused the owner's concern, prompting them to consult a dermatologist. The dermatologist's evaluation ultimately confirmed the presence of melanoma. This incident spurred numerous research endeavours to explore the potential of dogs' keen sense of smell in the detection and diagnosis of various medical ailments [4].

Church and Williams (2001) shared a case involving a man whose dog continually sniffed a patch of eczema on his leg. Following the removal of the lesion, it was revealed to be a basal cell carcinoma. In another report by Campbell et al. (2013), a man's dog persistently licked a lesion behind his right ear, which was later confirmed as malignant melanoma [5].

Since than science focused on the dogs for screening diagnosis for cancer, diabetes, Parkinson, schizophrenia etc. and in the year 2020 when Covid-19 pandemic ravaged Europe and the rest of the world, dogs were also trained to detect people affected with the SARS-CoV-2 virus [4].

2 The olfactory system

2.1 Anatomy of olfaction

Humans have made practical use of the exceptional sense of smell possessed by dogs for the purpose of identifying and distinguishing various odours. Dogs possess an incredibly acute sense of smell, which is estimated to be significantly more sensitive, approximately 10,000 to100,000 times, than that of the typical human. Furthermore, dogs have the remarkable ability to detect volatile organic compounds at extremely low concentrations, sometimes as minuscule as one part per trillion. This heightened olfactory capacity enables them to perceive and identify a diverse array of chemical substances [6],[7].

In order to properly care for and maintain the health of detection dogs, it is crucial to possess knowledge about the anatomy and physiology related to their sense of smell. Key elements of the olfactory system in canines encompass the nasal passage, the olfactory epithelium along with its receptors, the vomeronasal organ (VNO), and the olfactory bulb (Figure 1) [8].

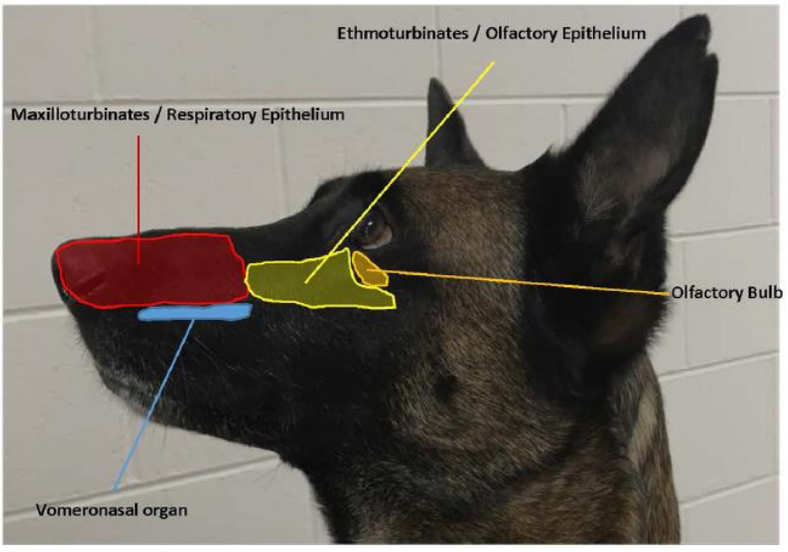

Figure 1. Demonstration of the canine olfactory compartments [2]

The nasal passage in dogs consists of two sections that are divided by a nasal septum. These sections have a rich blood supply from the sphenopalatine artery and are equipped with three different types of turbinates (nasal, maxillary, and ethmoturbinate), which increase the surface area of the nasal lining [8]. The size and shape of a dog's muzzle can impact the overall mucosal surface area. Inside the nasal chambers, there are structures called turbinates made up of vein networks. Just above the turbinates lies the olfactory cleft, where a portion

of the inhaled air is directed, and numerous cranial nerves end in this area. The sense of smell in dogs relies entirely on the olfactory epithelium and olfactory nerves [9].

The olfactory epithelium, found in the nasal lining, is made up of various components, including the cribriform plate, dorsal septum, dorsal and middle turbinates, and pseudostratified columnar epithelium. Within this structure, there are millions of olfactory receptor cells (ORNs), also called olfactory receptor neurons, along with support cells known as sustentacular cells. These sustentacular cells have multiple functions, including regulating the composition of nasal mucus, protecting the epithelium from harmful inhaled substances, and helping to maintain proper humidity levels in the nasal passages. Bowman's glands embedded in the olfactory epithelium contribute to the production of the nasal mucus layer, which aids in trapping odour molecules [2] [9]. Olfactory receptor cells directly transmit signals to the olfactory bulb for the processing and transmission of odour information. [10].

In dogs, olfaction is a highly specialized sense, with olfactory receptor cells (ORCs) possessing numerous cilia that are responsible for surface odour detection. Compared to humans, dogs have a greater number of cilia per ORC, allowing them to detect even extremely low concentrations of odorants. The canine nasal cavity contains over 220 million olfactory receptors, each with a single type of odour receptor [11]. Activation of these receptors triggers a signal amplification process involving G-proteins and cAMP, resulting in the generation of action potentials transmitted through the olfactory bulb [2].

The olfactory bulb functions as a hub that receives signals from olfactory receptor cells, enhances them via connections to mitral cells, and subsequently relays these signals This intricate olfactory system gives dogs an extraordinary ability to detect and discriminate between various odours, making their sense of smell highly sophisticated and critical for various aspects of their behaviour and survival [8].

2.2 Physiology of olfaction

During sniffing, dogs draw air from the environment towards their nostrils, capturing odour molecules in the process. The airflow patterns created during sniffing enhance their olfactory acuity by directing the air to specific areas within the nasal cavity. Dogs have a distinctive quality where the olfactory recess remains isolated from airflow during exhalation, ensuring a constant exposure of incoming air to their olfactory receptors. These adaptations enable

dogs to maintain olfactory stimulation throughout the respiratory cycle, enhancing their ability to detect and track scents [12],[2].

Environmental factors, such as humidity and barometric pressure, can directly affect a dog's sense of smell. Higher humidity levels improve nasal humidity and odorant trapping, resulting in better performance for search and rescue dogs. Similarly, increased humidity intensifies odorant detection and tracking tasks. Dogs rely on sniffing, which produces unidirectional laminar airflow, enhancing their sensitivity to odours and facilitating the processing of odours in the olfactory cortex [13],[14].

Dogs exhibit specialized brain processing for emotions, sounds, and odours, with their olfactory pathways connected to specific brain hemispheres. The right nostril typically processes threatening stimuli, while the left nostril is responsible for familiar scents and non-threatening cues, enhancing emotional communication between dogs and humans. In the case of detection dogs, it's likely that they predominantly process target odours through their left nostril [13].

Olfaction and memory have a strong connection in dogs. The pathways responsible for smell are linked to the limbic system, enabling the integration of smell and memory. Dogs initiate the process of learning and remembering scents even before birth, influenced by their mother's diet and the content of amniotic fluid. However, it seems that the memory of these learned scents fades by the time they reach 10 weeks of age. While both their sense of smell and cognitive abilities decline as they age, there isn't a specific age at which dogs stop learning. Dogs can retain and distinguish between numerous scents, and their memory of trained scents can persist for several months [2].

3 Sampling

3.1 Cancer samples

The positive samples came from patients with histologically confirmed cancer (Table 1), while negative samples came from patients with a negative histological reports [15].
For the diagnosis samples from different body excretes are taken, depending on which cancer type they are looking for.

Table 1.: Samples from patient with different types of cancer

Tumour	**Material**
Lung cancer[4]	Exhaled air, urine
Breast cancer	Exhaled air, urine
Colon cancer	Exhaled air, faeces
Ovarian cancer[2]	Tumour tissue, blood plasma
Prostate cancer[1]	Urine
Bladder cancer[3]	Urine
[15][1] [16][2] [17] [18][3][19][4]	

3.2 Covid samples

As sample for the detection of Covid-19 different studies used different secretions/objects, in some studies they used sweat and saliva [1], in others of pharyngeal secretion samples, face masks and cloths [4] or even urine. Prior to collecting the samples, the RT-PCR was used to verify the positive or negative status of each patient [4].

3.3 Sampling

In the case of urine sample for the detection of prostate cancer all patients underwent a prostate massage before the urine collection. The urine is collected in collection urine cups. During the transport the sample must be cooled, then the urine is centrifuged [20].

In case of the detection of lung cancer breath and urine samples were taken. The urine sample were collected by the patient itself in specialized sterile urine cups, the urine sample should be taken spontaneously in the morning and from the midstream. Whereas the breath samples were collected in the in the hospital under aseptic conditions and all patients were assisted by the same person to achieve same condition and to minimize the contamination of other odours [19].

3.4 Sample storage

Urine, was stored in test tubes and frozen at a temperature between -20°C [15]. For the tissue samples, the tumour was divided in small pieces and then stored at -80°C until needed. Before presenting the samples to the dog, they were thawed and brought to room temperature [16]. Breath sample were stored in test tubes of 120x22cm, filled with a hydrophobic and one hydrophilic siliconized 2.5x60cm piece of propylene cotton [19]. During the training the samples are placed in line-up cones (Figure 2).

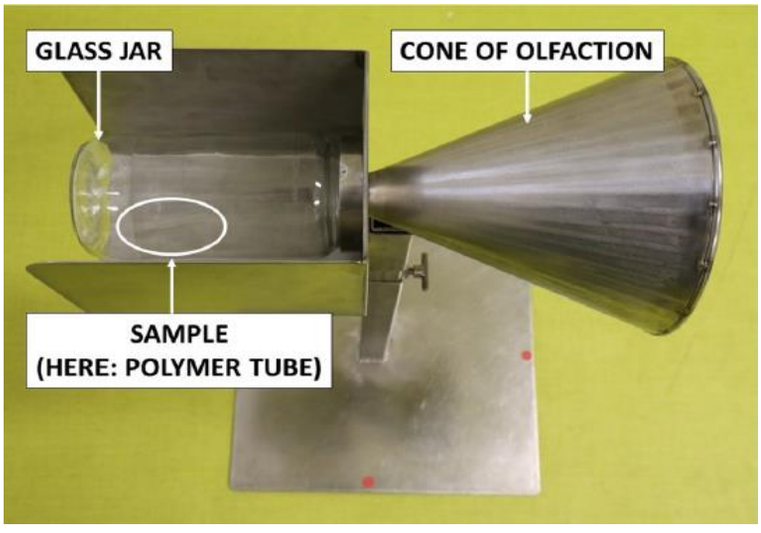

Figure 2. Demonstration of a line-up cone [23]

4 Training of cancer sniffing and Covid-19 detecting dogs

Dogs are trained to detect disease markers by recognizing specific signs and responding with actions like barking. This training, called "operant discriminant training", reinforces positive responses to disease indicators and avoids rewards for negative ones. This method relies on two main skills: generalization and discrimination. Generalization means dogs can respond to similar targets not seen in training, like detecting various viruses in a family. Discrimination is their ability to tell different things apart, enhancing accuracy in finding slight differences between related viruses or explosives. Balancing generalization and discrimination are crucial to reduce errors in disease detection, affecting accuracy and precision. Dogs are used to detect diseases in humans, including cancer and infections. Applying dogs' disease-detection skills practically involves gradually giving fewer rewards during training. This readies dogs for real tasks by transitioning from consistent to occasional rewards, solving issues with delayed testing in labs due to sample transportation [3].

4.1 Breeds

Almost every dog breed can be trained as a Biomedical detecting dog, but nowadays mainly Labradors retrievers, Belgian Malinois or German shepherds are used for those jobs, because they have an advantage due to their long nose, where more receptors are placed and even because they like to work with humans, are good trainable and easy to motivate [1].

4.2 Different training techniques

In most of the studies the concept of the training is very similar, but they vary in detail. Two examples for cancer diagnostic training (see more in the studies) are discussed below, the diagnostic training with Prostate cancer samples and with Ovarian cancer samples.

In the Prostate cancer study a German Shepherd was used, she underwent specialized training starting at seven months of age. The dog had undergone prior training in fundamental obedience and scent work, with a specific emphasis on harnessing its olfactory skills for the purpose of detecting prostate cancer in males. This training employed positive reinforcement methods involving the use of a clicker.

The dog was rewarded with treats or games when it exhibited desired behaviours, such as approaching and sniffing the positive urine sample [15].

The Ovarian cancer training began by exposing the dog to rags attached to strings, with one of them containing an ovarian cancer sample. the dog's interest in the target was reinforced by quickly removing it. Initially, high-grade and advanced-stage seropapillary carcinomas were used, and as the dog successfully identified them, mucinous and endometroid carcinomas and carcinosarcomas were introduced. Different tumour grades and stages were also used to train the dog to detect various forms of the disease [16].

4.3 Timing

Over a period of 11 months, the dog underwent training sessions 4-5 times per week. Each session involved dispatching the dog to evaluate one to three samples (one positive and two negative). During different sessions, the training centred on enhancing the dog's physical fitness and honing its olfactory skills by practicing scent trails [15].

4.4 Training process

The process of training the dog to detect cancer entailed introducing it to the scent of a positive urine sample, breath sample, and so on. The dog was progressively taught to sit or lie down near the positive sample and place its nose on the container (Figure 3). As the training progressed, additional containers were introduced, including those with an empty test tube, water, and negative urine samples. The criteria for rewarding the dog's behaviour were adjusted accordingly. The training gradually transitioned from using test tubes to using beakers to prevent contamination [15].

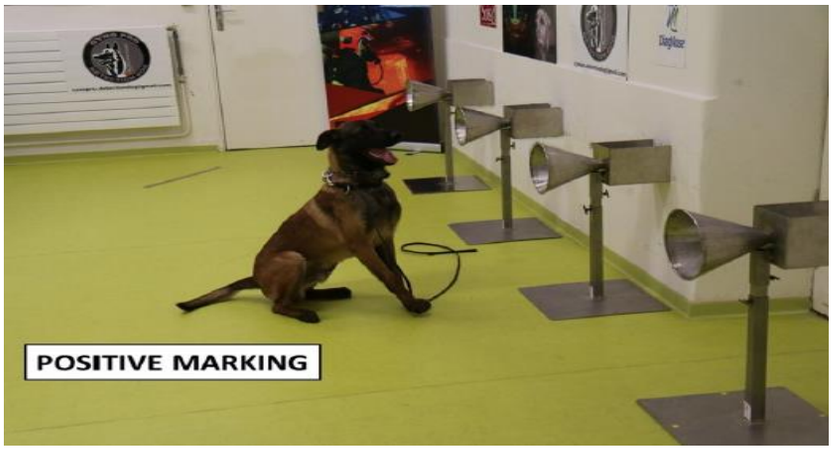

Figure 3. Dog during the training [23]

During the training, the samples were positioned in various ways to challenge the dog's ability to identify the positive sample.

The dog was directed from various angles to identify the correct sample upon receiving the "OK" command. As the training advanced, a different individual positioned the samples to remove any potential cues from the dog's handler [15].

5 Development of the specific odours

5.1 Body odours

Body odours are generated by the intermingling of numerous odorous volatile organic compounds (VOCs) emitted through metabolic processes by different cells in the body. The primary sources of these VOCs consist of exhaled air, sweat, skin, urine, faeces, and vaginal secretions. Additionally, blood plays a significant role as a source of body odour, as certain VOCs produced metabolically are expelled into the bloodstream and eventually released into the external environment through breathing and/or sweating [17].

Advancements in analytical chemistry have made it possible to quantify and compare VOCs (volatile organic compounds) that come from cells. VOCs are low molecular weight compounds that can produce odours and can be detected in real-time. This progress has enhanced our understanding of what dogs can identify as biological targets. All odours are VOCs, and these developments have opened up new opportunities in the field. Every organ in the body generates cellular VOCs as by-products of metabolism, and they are simultaneously released by millions of cells, making them detectable. Once VOCs enter the bloodstream, they are released into the air through various bodily excretions like breath, saliva, urine, faeces, skin emanations, and blood. This collection of VOCs surrounding a person is referred to as the "volatilome," which reflects the organism's unique metabolic state. VOCs are emitted at different concentrations, ranging from parts per billion (ppb) to parts per trillion (ppt) in human breath, and parts per million (ppm) to ppb in human blood and urine. Dogs have the ability to detect VOCs even at extremely low concentrations, such as in the ppt range. Specific HLA (Human leukosis antigen) sequences produce distinct VOC patterns or "odour prints," which can be reliably identified by well-trained dogs. Pathogen-specific odours consist of particular VOC patterns and non-volatile molecules, like exhaled breath condensate and aerosols. These VOCs are released by infected cells as a result of metabolic processes in the host. Dogs' exceptional sense of smell is especially valuable for detecting pathogens in various scenarios, including large animal herds, crowds of people, objects like ships or airplanes, buildings, or different areas of land [3].

5.2 Disease-specific odours

The different microbial species release a diverse range of volatile compounds inside the host's body, which are then emitted through body excretions, like: breath, urine, faeces, and sweat. Researchers have utilized Gas Chromatography (GC) or Gas Chromatography-Mass

Spectrometry (GC-MS) analysis of the gaseous environment around these cultures to demonstrate that microorganisms generate various odorous substances like alcohols, aliphatic acids, and terpenes. These compounds proportions vary depending on the specific infectious microorganism. Clinicians have long recognized distinct odours associated with different diseases caused by infections, and the profiles of these volatile compounds have the potential to serve as biomarkers for diseases [17].

6 Detection of different cancer types (Studies)

6.1 Methods

6.1.1 Lung Cancer Study

- 432 patients, 2 phases, 2 groups,
- Patient: histology,
- Control: no detectable tumour, physiological heart, and lung sounds.

Feil et al. (2021) conducted a comprehensive study on lung cancer involving a total of 432 patients, which was carried out in two distinct phases and divided into two groups. The primary focus was to evaluate the acoustic characteristics of lung sounds and their correlation with different medical conditions. The study participants were categorized into two groups: patients with confirmed histology of lung cancer and controls. For the control group, individuals were selected who exhibited no detectable tumours and displayed normal physiological heart and lung sounds. This distinction allowed for a comparative analysis between the lung sounds of patients and healthy individuals [19].

6.1.2 Ovarian Cancer Study

- 31 different histopathological samples with various grades, and stages including borderline tumours,
- Control: abdominal fat, muscle and small bowl samples were used.

Horvath and colleagues (2008) carried out an in-depth exploration into ovarian cancer, covering a broad spectrum of 31 histopathological types characterized by diverse grades and stages, which also included borderline tumours. The primary focus of their study revolved around assessing the distinctive acoustic attributes linked to various classifications of ovarian cancer. For comparison purposes against ovarian cancer samples, control samples were sourced from entities such as abdominal fat, muscle tissues, and small bowel samples. The meticulous selection of these control samples aimed to provide a foundational reference

point. To ensure the dependability of the samples, meticulous cytological evaluations were performed on both tumour and control specimens. Tumour samples were classified as malignant if at least half of their cells exhibited malignancy, while control samples were included only when they demonstrated an absolute absence of malignant cells [16].

6.1.3 Prostate Cancer Study

- 108 urine samples 59 cancers and 49 controls,
- Frozen samples,
- Two phases:
    - Training phase: 26 cancers samples and 16 controls,
    - Testing phase: 33 cancers samples and 33 controls.

Cornu et al. (2011) conducted a thorough examination of prostate cancer, concentrating on individuals who exhibited elevated levels of prostate-specific antigen (PSA) or showed abnormal results during a digital rectal examination. During their initial appointments, urine samples were obtained from these individuals, followed by prostate biopsies performed on all participants. Following the pathological examination of the biopsy specimens, patients were then classified into two groups: cases (indicating the presence of prostate cancer) and controls (indicating the absence of cancer). Patients with a history of urothelial carcinoma or other malignancies were excluded from the study to ensure the specificity of the results. The study comprised a total of 108 urine samples, consisting of 59 cancer samples and 49 control samples. The research was executed in two distinct phases: a training phase involving 26 cancer samples and 16 controls, and a testing phase featuring 33 cancer samples and 33 controls. Notably, factors such as alcohol consumption, drug use, dietary habits, and tobacco use were not considered as exclusion criteria for patient selection [21].

6.1.4 Bladder Cancer Study

In total 108 participants healthy and diseases, both diseased and healthy controls

- 54 men, age range 18-85, mean age 45,
- 54 women, age range 18-85, mean age 40.

In a comprehensive study conducted by Willis et al. (2004), the focus was on bladder cancer, encompassing a diverse participant pool of 108 individuals, including both healthy subjects and those afflicted by the disease. The study encompassed both diseased and healthy control groups for a comprehensive analysis.

The participants were divided into distinct groups, with 54 men and 54 women included. The age range of the participants spanned from 18 to 85, with an average age of 45 for men and 40 for women. The selection criteria for this study involved participants who had recently undergone procedures like cystoscopy with no observable bladder malignancy or negative prostate histology results for men over 50 years of age. By examining both healthy and diseased participants, the study aimed to decipher the characteristic acoustic profiles associated with bladder cancer and evaluate its diagnostic potential [18].

6.2 Canine participants

In a series of cancer studies involving canine participants, each dog played a unique role. The lung cancer study featured a 7-year-old golden retriever, a cherished member of a household, intimately intertwined with the daily lives of its owners. Similarly, the ovarian cancer study involved a 4-year-old black Riesen schnauzer, enjoying a life of familial care, including fresh water, regular meals, and leisurely walks. In the prostate cancer research project, a Belgian Malinois shepherd, which played a crucial role in the French army's veterinary department, was chosen from a pool of dogs originally intended for explosive detection training [19], [16], [21], [18].

In contrast, the bladder cancer study employed a diverse approach, enlisting the involvement of six dogs representing various breeds and spanning different age groups. These dogs collectively contributed to the research, offering a wide range of perspectives in the study's exploration of bladder-related concerns. Each canine participant, in their own way, played a significant part in advancing our understanding of cancer in these distinct areas [19], [16], [21], [18].

6.3 Training methods

In each of the canine studies focusing on various medical conditions, specific training protocols were implemented to harness the dogs' exceptional olfactory abilities and enhance their capacity to distinguish distinct scents.

In the lung study, the canine participant underwent a year-long training regimen led by a professional dog trainer affiliated with Teamcanin, with sessions occurring once or twice per week. The training method employed was centred around the clicker technique, a widely recognized approach for conditioning dogs [19].

Similarly, in the ovarian study, the canine participant underwent an extensive two-year training program, with training sessions held twice a week to progressively develop the dog's olfactory acumen [16].

For the prostate study, a rigorous training routine was established, utilizing the clicker training method five days a week for a duration of 16 months. This comprehensive and intensive training aimed to sharpen the dog's ability to discern specific scents associated with prostate-related conditions [21].

In preparation for the scent discrimination training, which lasted seven months in the bladder study, the dogs were initially acquainted with basic obedience commands. This initial phase laid the foundation for their subsequent scent-oriented training. The primary aim was to instruct the dogs in distinguishing between urine samples obtained from individuals with bladder cancer and samples from individuals with different medical conditions. Clicker training, a highly regarded positive reinforcement technique, was used to shape their behaviour [18].

Throughout the training, diverse urine samples were employed:

- Urine from bladder cancer patients as a key marker.
- Diluted urine from healthy individuals for contrast.
- Urine from completely healthy people as a baseline.
- Urine containing menstrual blood, introducing unique scents.
- Urine from non-malignant urological conditions for comprehensive exposure.

To ensure versatility, samples were presented in fresh, defrosted, and dried states. This thorough approach honed the dogs' scent detection capabilities, enabling them to effectively identify specific bladder health conditions [18].

6.4 Study Design

In each of the distinct studies, meticulous study designs and testing protocols were employed to evaluate the efficacy of the dogs' scent detection abilities.

In the lung study, a rigorous double-blind testing methodology was employed, encompassing 41 cancer samples and 205 control samples. Importantly, only samples that had not been used during the training phase were included to prevent the dog from recognizing scents encountered before [19].

For the ovarian study, a combination of single-blind and double-blind testing was implemented. The single-blind test included 20 cancer samples and 80 control samples, followed by a double-blind test with the same sample composition. These tests were carefully structured to assess the dog's ability to distinguish between ovarian cancer-related scents and those from healthy individuals [16].

In the prostate study, a double-blind testing approach was utilized, consisting of 33 runs, with each run presenting the dog with six samples: five control samples and one cancer sample, each lasting 30 seconds, facilitating efficient assessment of the dog's performance [21].

In the bladder study, a blind testing approach was adopted, featuring nine runs with varying testing durations based on the dogs' pace. The primary focus was on evaluating the dogs' ability to detect scents associated with bladder conditions [18].

6.5 Results

6.5.1 Lung Cancer Study

The study showed that urine samples had a sensitivity of at least 85.4%, which is higher than the reference value of 78%. When looking at breath samples alone, their sensitivity could be a bit lower. Both breath and urine samples had better specificity than the reference value of 71.5%. Combining breath and urine samples is likely to improve sensitivity, detecting cancer in 40 out of 41 cases.

Interestingly, the study found that dogs could detect lung cancer from urine and breath samples better than bronchoscopy. Bronchoscopy only had a sensitivity of 56.1%, while using smell for urine and breath samples achieved sensitivities of at least 84.5% and 73.7%, respectively. Although CT scanning is the most accurate (100% sensitivity), it's limited due to radiation and false positives. Analysing both urine and breath samples together in the study identified 97.6% of cases (40 out of 41), offering a promising way to improve lung cancer detection [19].

6.5.2 Ovarian Cancer Study

In the latter stages of the training period, two single-blind tests were conducted to evaluate the training's effectiveness and address specific concerns. The main goal of these tests was to differentiate between ovarian carcinomas and healthy tissues. Tumour samples previously used in training were employed, with ten sets created from four samples each from five patients' tumours. These sets were tested on separate days, with 80 other sample boxes filled randomly with fat and intra-abdominal muscle tumour (myoma) samples from different patients, along with healthy ovary samples as controls. During these tests, the dog accurately identified all cancer samples and didn't signal any control samples, resulting in a perfect sensitivity and specificity of 100%. The likelihood of the dog achieving this by chance alone was extremely low. These results highlight the dog's exceptional ability to distinguish between ovarian carcinomas and healthy tissues [16].

In the subsequent double-blind test, 20 different individuals' ovarian carcinoma samples were used as targets, not previously encountered in training or single-blind tests.

To distinguish between ovarian carcinomas and healthy tissues, in the study 20 different individuals as targets ovarian carcinoma samples were used. These tumours were not previously utilized in the training or single-blind tests. Control samples consisted of small bowel, muscle, fat, and two pieces of healthy postmenopausal ovary. In a total of 10 series, involving 100 examined boxes, the dog successfully identified all 20 ovarian carcinomas without difficulty. However, in one series, the dog did indicate one fat sample and one muscle sample as targets. The dog achieved a sensitivity of 100% in correctly identifying all cancer samples. Additionally, the dog exhibited a specificity of 97.5% (78 out of 80 samples) since there were two instances of positive indications for control samples [16].

6.5.3 Prostate Cancer Study

During the double-blind testing, there were a total of 33 rounds, each lasting around 30 seconds (Table 2). In 30 rounds, the dog correctly identified the cancer sample by sitting in front of it. However, in three rounds, the dog mistakenly sat in front of a control sample. These three incorrect identifications were considered as false positives, and the cancer samples corresponding to them were considered as false negatives. Overall, out of the 66 samples tested in this phase, the dog correctly classified 60 of them. After each mistake, they repeated the test using the same cancer sample but different control samples. The dog consistently detected cancer samples as true positives, but there were a few cases where it identified control samples incorrectly as positives [21].

Table 2. The double-blind testing involving the dogs identification of cancer and control samples

Parameter	Value	Percentage
Total Rounds	33	
Correct Identification by Dogs	60	60/66 x 100 = 90,91 %
Incorrect identification by Dogs	3	3/66 x 100 = 4,55 %
False positive (control sample)	3	
False negative (cancer sample)	3	
True positive (cancer sample)	60	
True negative (control sample)	Not specified	

6.5.4 Bladder Cancer Study

When looking at all attempts combined, the dogs correctly identified positive bladder cancer urine in 22 out of 54 instances. This yielded an average success rate of 41% (with a confidence range of 23% to 58% using normality assumptions, and 26% to 52% using bootstrap methods). In contrast, if it was just by random chance, the expected success rate would be 14%. The correlation between the dogs' selections and the presence of cancer appeared to be somewhat more pronounced when analysing the multivariate model, which factors in variables such as the presence of blood and ketones, in contrast to the simpler univariate model. This implies that the dogs' ability to detect bladder cancer urine was not significantly influenced by other measured factors in the urine analysis [18].

Moreover, the four dogs trained with wet urine samples performed better, achieving a 50% success rate, compared to the two dogs trained with dried samples, who achieved a 22% success rate. However, the researchers acknowledge that due to the limited number of dogs used, more data would be needed to have stronger confidence in this observation [18].

7 Covid-19 studies

7.1 Methods

Study A

The study's inclusion criteria involved individuals who displayed clinical symptoms of Covid-19 and had confirmed positive results for Covid-19 through RT-PCR or PCR testing for the SARS-CoV-2 virus. Exclusions were made for those who had undergone medical treatment exceeding 36 hours before the PCR test to avoid any influence on sweat VOCs or prolonged treatments. Furthermore, in order to minimize potential interference from the "hospital odour," for every Covid-19 positive participant enlisted, a Covid-19 negative participant was also recruited from the same hospital. These negative participants exhibited no Covid-19 symptoms and tested negative for SARS-CoV-2 in PCR tests [22].

Study B

Sweat and saliva specimens were collected from individuals who tested both positive and negative for Covid-19, with the intention of training and field-testing dogs for detection purposes. Prior to sample collection, patients were requested to complete a questionnaire aimed at gathering epidemiological information, which encompassed their full name, age, gender, chronic health conditions, substance usage, symptoms, medical history, and contact with confirmed Covid-19-positive individuals. All positive samples were sourced from individuals experiencing symptoms, either mildly or with more pronounced symptoms like fever, headache, diarrhoea, or cold-like symptoms. Specifically, patients with symptoms lasting up to 9 days, particularly those in the early stages of infection exhibiting mild symptoms for 1-3 days, were preferred. Inclusion criteria for Covid-19-positive patients encompassed an age range of 18 to 60 years, symptoms persisting up to 9 days, and confirmation of SARS-CoV-2 infection through either RT-PCR or the Panbio Covid-19 Ag Rapid Test Device antigen test [1].

The majority of negative samples were collected from individuals experiencing mild symptoms such as diarrhoea, headache, fever, or symptoms resembling a common cold, but not the distinct Covid-19 symptoms such as loss of smell or taste, or respiratory problems. Additionally, negative samples were sourced from asymptomatic individuals, all of whom tested negative for both RT-PCR [1].

Study C

This study encompassed not only hospitalized Covid-19 patients but also non-hospitalized asymptomatic individuals and those with mild clinical symptoms. The criteria for inclusion involved either a positive diagnosis of SARS-CoV-2 infection via RT-PCR of nasopharyngeal swabs (for positive samples), a negative result on the SARS-CoV-2 RT-PCR test and being in a healthy state (for negative control samples), or a negative result on the SARS-CoV-2 RT-PCR test along with symptoms indicative of another respiratory ailment [23].

7.2 Samples

Study A

In the Study A they used sweat samples, gathered from the underarm area (Figure 4) by trained medical staff. The choice of collecting sweat from the underarm region was motivated by its potential as a reliable substrate for canine detection, especially for search and rescue or tracking dogs. Additionally, this area is easily accessible and less likely to be contaminated by a Covid-19 positive patient's saliva [22].

The collection material was left in contact with the skin for a duration of 20 minutes,

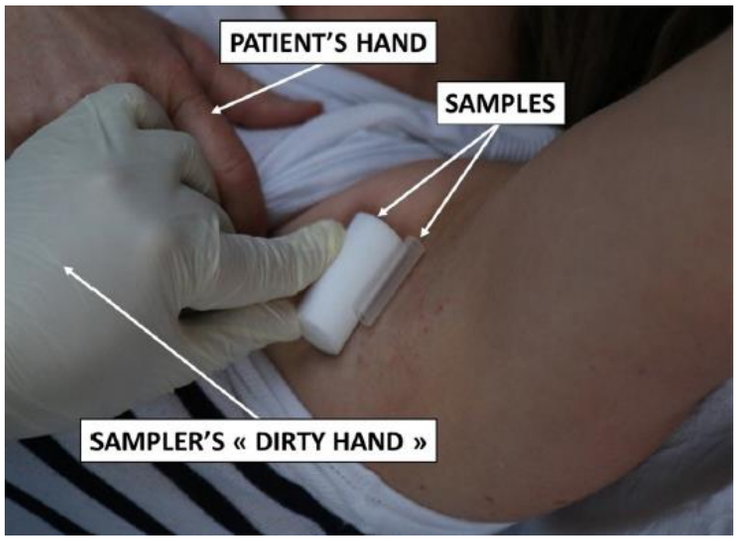

Figure 4. Demonstration of a sweat sampling [23]

resulting in an average sweat volume of approximately 75 mg for both gauze swabs and cellulosic filters. After collection, the samples were stored in sterile containers with anti-UV properties [22].

Study B

In study B samples for the RT-PCR and antigen tests were collected from the throat and nasopharynx of every patient using established and standard protocols [1].

For the collection of sweat samples, each patient was provided with a set of clean nitrile gloves and a resealable Ziploc bag containing two sterilized translucent glass flasks equipped with metal caps, along with sterile, odourless gauze and dental swabs. Patients were instructed to gently rub their neck, face, and forearms for approximately 1 minute using the gauze on one side of their head and then repeat the process on the other side. Additionally, they were asked to place dental swabs in their mouth and beneath each armpit for 1 minute. Subsequently, patients were guided to place the gauze pieces and swabs containing the collected sweat samples into the glass flask, seal it securely, and return it to the resealable bag. This procedure was brief, requiring less than 3 minutes per patient. No additional substances were introduced to the saliva samples during the dog training process. The gathered samples were then transported in coolers to the laboratory and stored at a temperature of 18°C until they were utilized [1].

For saliva samples, patients were provided with nitrile gloves and a resealable Ziploc bag containing sterilized glass flasks with metal caps and dental swabs. Patients were instructed to place dental swabs in their mouth for 1 minute, after which they were placed in the glass flask, sealed, and returned to the resealable bag. This process also took less than 3 minutes per patient [1].

Study C

To obtain saliva samples, individuals were instructed to produce approximately 1 to 3 millilitres of saliva by using a straw to collect it in tubes. In the training period, saliva samples were obtained from twelve individuals who had received a diagnosis of SARS-CoV-2 infection, with a spectrum of symptoms that ranged from having no noticeable symptoms to experiencing severe Covid-19 symptoms. These samples were treated with beta propiolactone (BPL) and inactivated following a specified protocol to ensure the safety of both the dogs and their handlers during training. Sweat samples were produced by instructing participants to use a cotton pad to wipe the inner part of their elbow. Urine samples were obtained from individuals by having them urinate into a cup and subsequently transferring 5 millilitres of urine into a sample tube. Subsequently, all collected samples were subjected to deep-freezing at -80 degrees Celsius within the laboratory until they were ready for use. The

study utilized samples from a total of ninety-three participants, consisting of 32 males and 61 females [23].

7.3 Canine participants

Study A

The dogs utilized (Table 3) in this study included explosives detection dogs, search and rescue dogs, and colon cancer detection dogs. Explosives detection dogs are trained to identify numerous types of explosives, working through a line of samples that they individually sniff. These dogs would only need to learn and generalize any specific odour associated with Covid-19 positive samples. Drug detection dogs were not chosen due to the possibility that both Covid-19 positive and negative individuals might have used prohibited substances, which could result in the detection of their metabolites in sweat [22].

Table 3. Dogs used in the study [23]

	Name	Gender	Breed	Age	Organisation involved	Specialty
Paris	Guess	F	Belgian Malinois	8 years	Cynopro detection Dog	Explosives
	Maika	F	Belgian Malinois	3 years	Fire and Emerg. Dept. 77	Search and rescue
	Gun	M	Belgian Malinois	16 months	Cynopro detection Dog	Explosives
	Oslo	M	Belgian Malinois	18 months	DiagNose	Explosives
Beirut	Bella	F	Belgian Malinois	6 years	Mario-K9	Colon Cancer
	Jacky	M	Jack Russell terrier	3 years	Mario-K9	Colon Cancer

Study B

Originally, nine dogs were chosen for training. These dogs, known as "green dogs," had not received previous training but possessed natural instincts and potential to pass the various training phases. However, the training was successfully completed by only six out of the nine dogs. These six dogs consisted of a 1-year-old dog named Leia, initially being trained for epilepsy detection, two 2-year-old male German Shepherds named Mike and Sam, who had no prior training experience, a 1-year-old Belgian Malinois named Krilling, also without previous training, and two 2-year-old Belgian Malinois named Harry and Spaidy, who were new to detection work, originating from narcotic and sport lines. The remaining three dogs did not complete their training due to various reasons, such as illness or an inability to meet the requirements for detection work [1].

Study C

All ten dogs in this study were previously employed by the German armed forces and had specific backgrounds in protection work, explosives detection, or had no prior training except for basic obedience. These dogs encompassed various breeds such as Belgian Malinois, Labrador Retriever, German Shepherd, and a Dutch Shepherd crossbreed, with ages spanning from one to nine years, and a median age of 3.7 years. The group consisted of six female and four male dogs [23].

7.4 Training method

Study A

The dogs were trained to identify a Covid-19 positive sample among a line-up of cones. The training employed positive reinforcement, with dogs receiving a toy as a reward for correct identification. The training proceeded in four steps:

1. Learning to work with the line-up of empty cones.
2. Memorizing the odour of the Covid-19 sample.
3. Transitioning to cones with sample material (mocks).
4. Introducing Covid-19 negative samples alongside the positive one.

Dogs took less than a day to memorize the Covid-19 positive odour, typically sniffing four to ten samples. The handler determined the readiness for testing based on the dog's behaviour [22].

After completing training, testing sessions began using new Covid-19 positive and negative samples that weren't part of the training. Each test took place in a designated room with cones at the back. A data recorder placed a Covid-19 positive sample behind one cone, along with Covid-19 negative samples and possibly mock samples behind the other cones. Sample types were consistent (gauze or polymer tubes) within a session. The number of cones in a line-up remained constant for each dog. The Covid-19 positive and negative samples' positions were randomized using a dedicated website, and the data recorder knew their placement but had no visual contact with the dog or handler during testing [22].

The dog, accompanied by its handler, entered the room, and sniffed each cone to identify the positive sample. The dog was trained to mark only one Covid-19 positive cone. After marking, the trial ended, and the handler rewarded the dog if correct. Successful trials involved accurate marking of the positive cone; otherwise, it was considered a failure. After each trial, new samples were placed in the line-up. The testing spanned 21 days to

accommodate non-daily work schedules. Environmental conditions adhered to canine olfactory standards (temperature and humidity). Dogs showed no health issues during training and testing [22].

Study B

The training program for the Covid-19 detection dogs spanned approximately 12 weeks, consisting of two daily sessions six days a week. These dogs, part of the inaugural cohort trained for Covid-19 detection, underwent training exclusively using human sweat samples [1].

The training process consisted of three primary phases: target odour association, discrimination, and training evaluation. During the initial target odour association phase, which spanned three weeks, the dogs were trained to concentrate on a designated scent and were rewarded with a toy as a recognition when they correctly identified it. They also became skilled at locating this toy inside a stainless-steel container. During this phase, the dogs were gradually introduced to sweat samples from Covid-19-positive individuals. These samples were placed alongside the familiar toy odour to help the dogs associate the Covid-19-positive sweat scent with a reward. The dogs were then evaluated on their ability to correctly identify the scent of the sweat alone [1].

Moving on to the discrimination phase, which took several weeks, the dogs worked with increasingly concentrated positive samples before being tasked with distinguishing between negative and positive samples. This phase involved a setup with two salt shakers containing sweat swabs from RT-PCR-positive and RT-PCR-negative patients, placed randomly in a line of four holes. The dogs learned to follow a specific search sequence and differentiate between positive and negative samples, even when the positive sample's location varied [1].

In the 2-week evaluation phase, conducted under double-blind conditions, the dogs had to identify positive samples among negative ones. Their objective was to consistently mark five positive samples in a row, marking the completion of this phase [1].

Following their training, the dogs transitioned to fieldwork in the second generation of training. Saliva samples from SARS-CoV-2-positive patients with loss of taste and smell were exclusively used. These samples were obtained from Covid-19 wards in hospitals. In this phase, food was initially used as a reward to associate the dogs with the Covid-19-positive saliva sample's odour. Food was provided when the dogs were near a table

containing the sample. Later, a second empty vial was introduced, and food was given only when the dogs placed their noses inside the salt shaker with the positive sample. After three weeks, two of the dogs returned to using the Kong toy as a reward, as they had been originally trained with it and performed better with it. Training with saliva samples reduced the overall training time to 10 weeks, enabling the dogs to detect the Covid-19 odour more rapidly due to exposure to symptomatic patients [1].

Study C

The training method relied solely on positive reinforcement. The dogs underwent a 6-day familiarization period with a substitute odour, followed by an 8-day specialized training phase to acclimate them to a distinct SARS-CoV-2 specific scent. This particular scent was created using twelve inactivated positive saliva samples and negative control samples obtained from healthy individuals. The concluding phase of the research lasted for four days and included non-inactivated saliva samples, along with urine and sweat samples. It is crucial to note that all the samples introduced during this final phase were entirely new to the dogs and had not been encountered by them previously [23].

7.5 Study design

Study A

In this proof-of-concept investigation, the primary objective was to showcase the dogs' ability to distinguish a solitary Covid-19 positive sample from a selection of cones, encompassing both mock samples and samples that were Covid-19 negative. Due to the simultaneous nature of the lineup, metrics like sensitivity, specificity, and false alert rates couldn't be computed, as suggested by Johnen et al. (2013). Initially, the dogs were trained to differentiate between sweat samples and imitation samples before proceeding to distinguish between Covid-19 positive and negative samples, which was part of the third training step. To calculate the random choice proportion, the number of imitation samples was subtracted from the total number of cones in the line-up, and then 1 was divided by the number of Covid-19 positive or negative sweat samples. For example, in a 4-cone line-up with one imitation sample, the random choice proportion would be 33% To address the potential influence of repeated exposure to the same Covid-19 positive sample, success rates were categorized based on whether it was the first, second, or third presentation of that particular sample [22].

Study B

Samples used in the trials, both positive and negative, were exclusively sourced from the Anticipa Health Center, a two-story facility where patient samples were collected on the ground floor and transported to the upper floor for organization into testing line-ups. In these experiments, two identical odour lines were established, with each line having four holes. However, due to a scarcity of available samples, only one salt shaker containing a positive sample and another with a negative sample were employed in each trial. The remaining two holes in the setup were left empty and were not factored into the statistical analysis. The assessment of an individual's Covid-19 status depended on the Ag test outcome in 52% of instances, whereas for the remaining 48%, Covid-19 status was ascertained through the presence of associated symptoms and subsequently confirmed by RT-PCR testing conducted two weeks later [1].

The testing procedure included guiding each dog through all the samples arranged in a row on two occasions: initially for identification and subsequently for a conclusive detection. This approach, inspired by previous research, aimed to improve the dogs' sample discrimination capabilities by introducing additional memory demands. In this adapted approach, inspired by the work of Grandjean and colleagues, the dogs began by sniffing both salt shakers in every trial. During the second phase, the dog handler directed the dog to pinpoint the positive sample, and the salt shaker marked as such was regarded as the definitive identification for that particular trial [1].

In contrast to earlier training phases, the samples used in these experiments consisted of two pieces of sterile gauze exposed to axillary sweat for one minute and one piece exposed to corporal sweat from a patient for the same duration. These gauze pieces were then placed in a plastic container, sealed for 1 to 5 minutes to allow the odours to permeate, and subsequently transferred into sanitized stainless-steel salt shakers measuring 10 cm in height and 7 cm in diameter. Negative samples for each trial were gathered from individuals at the health centre and underwent a similar processing procedure as the positive samples. For each trial, fresh positive and negative samples were employed, and previous samples were not reused. The allocation of positive and negative samples was randomized by the data recorder, ensuring that neither the dog handler nor the dog had any knowledge of the locations of the positive samples. This procedure adhered to a double-blind approach, with the recorder signaling when the lineup was ready, at which point both the dog handler and the dog faced the lineup. When the dog marked one of the salt shakers, typically by sitting or lying on it,

the dog handler indicated the completion of the trial with a closed fist raised upright. The data recorder verbally confirmed the accuracy of the mark, and if it was correct, the dog handler rewarded the dog with a Kong toy [1].The process for exposing the dogs to saliva samples closely mirrored that of sweat samples, except for the use of dental swabs. These swabs, either positive or negative for SARS-CoV-2, were placed and opened within sterilized salt shakers. This testing phase spanned 12 weeks, during which none of the dogs displayed any signs of illness [1].

Study C

Every sample was meticulously managed by a single person who donned personal protective gear to ensure that there was no chance of odour contamination that could impact the dogs' performance.

The study comprised multiple sessions, starting with the use of non-inactivated saliva samples to determine if the dogs could effectively apply their trained scent detection skills to non-inactivated samples. Subsequent sessions evaluated the dogs' detection abilities with non-inactivated sweat, urine, and once again, saliva samples [23].

The dogs had four possible responses when presented with odours:

1. True positive (TP): The dog correctly identified a SARS-CoV-2 positive sample.
2. False positive (FP): The dog incorrectly identified a negative control or distractor.
3. True negative (TN): The dog briefly sniffed at a negative sample but correctly did not indicate it.
4. False negative (FN): The dog briefly sniffed at a positive sample but did not indicate it.

For a detection trial to be considered successful, the dog had to keep its snout in the target scent-presenting hole of the DDTS for at least 2 seconds, triggering the automatic release of a reward from the device and the commencement of the next randomized trial. The software-controlled DDTS ensured that the dogs were rewarded automatically when they correctly identified a positive sample, all while maintaining the study's double-blind status. In every trial, the placement of the target odour among seven distinct positions was determined at random, and neither the dog nor its handler had any prior knowledge of which hole contained the positive odour. The outcomes were electronically documented for subsequent examination and corroborated by a manual review of timestamped video recordings [23].

Throughout the study, the dog training laboratory was maintained at a constant temperature of 24 ± 1°C. While the samples were introduced to the dogs in sealed specimen containers, experiments involving potentially infectious materials were carried out within a biosafety level 2 laboratory to completely mitigate any infection risk [23].

7.6 Results

Study A

In Study A, a total of 95 individuals who tested positive for Covid-19 and exhibited symptoms, along with 82 individuals who tested negative for Covid-19 and were asymptomatic, were enrolled, resulting in 177 sweat samples. At the Paris site, there were 27 participants who were symptomatic and tested positive for Covid-19, as well as 34 individuals who were asymptomatic and tested negative for Covid-19. The gender distribution was similar in both groups, but the Covid-19 positive group at this site was notably older, with an average age of 70 compared to 42 in the negative group. Meanwhile, the Beirut site included 68 individuals who were symptomatic and tested positive for Covid-19, as well as 48 individuals who were asymptomatic and tested negative for Covid-19. The gender and age characteristics were comparable between both groups at this site [22].

Of the 14 trained dogs, six participated in testing after 1 to 3 weeks of training. They were primarily Belgian Malinois, comprising explosive detection, colon cancer detection, and search and rescue dogs. Testing involved line-ups of cones containing Covid-19 positive and negative samples, with or without mocks. Success rates varied from 76% to 100%, often surpassing random choice proportions. Success rates were also analysed based on whether Covid-19 positive samples were presented for the first, second, or third time to the dogs, revealing varied patterns of performance [22].

Study B

A total of 138 individuals, provided both sweat and saliva samples for this study. Among these samples, 69 of the sweat samples tested positive for SARS-CoV-2, while 69 were negative. In the case of saliva samples, 128 were collected, with 54 testing positive for the virus and 74 samples testing negative. The gender distribution in the entire sample was 59% women and 41% men [1].

The age range of the entire sample spanned from 18 to 60 years, with women having an average age of 37 ± 10 years and men 38 ± 12 years. Additionally, there were no significant variations in the average age observed between the groups with Covid-19-positive and Covid-19-negative individuals [1].

Table 4 presents the sensitivity and specificity measurements for four dogs exposed to sweat samples, and these results are compared with the outcomes of RT-PCR and antigen tests. Among these dogs, Sam and Leia exhibited marginal performance, with their 95% confidence intervals overlapping the randomness region (50%) [1].

Table 4. Results of sensitivity and specificity values of sweat samples [1]

Name	Number of samples	Sensitivity (%)	Specificity (%)
Sam	132	58	69
Leila	132	60	64
Mike	124	76	75
Harry	95	80	88

Conversely, Mike and Harry exhibited elevated sensitivity rates of 76% and 80%, respectively, and their 95% confidence intervals were distinctly separate from the range expected due to chance. Regarding specificity, Sam and Leia had results that fell within the expected range for randomness, while Mike and Harry demonstrated specificity rates of 75% and 88%, respectively, and their 95% confidence intervals did not overlap with the range expected by chance [1].

Table 5 presents the sensitivity and specificity data for three dogs exposed to saliva samples, and these results are compared with the outcomes of RT-PCR and antigen tests. Among these dogs, their sensitivity ranged from 70% to 78%. Notably, Spaidy and Krilling had 95% confidence intervals that were distinct from the range expected by chance, indicating more reliable sensitivity. In contrast, Leia's 95% confidence interval overlapped with the range expected due to chance. The specificity trends for these three dogs followed a similar pattern, with Spaidy and Krilling having 95% confidence intervals that did not overlap with the expected range for randomness, while Leia's did [1].

Table 5. Results of sensitivity and specificity values of sweat samples [1]

Name	Number of samples	Sensitivity (%)	Specificity (%)
Spaidy	138	70	69
Krilling	138	78	69
Leila	32	73	53

In essence, this data sheds light on the dogs' performance in detecting Covid-19 using sweat and saliva samples, with variations in sensitivity and specificity observed among individual dogs [1].

Study C

Following training using deactivated saliva samples, the dogs demonstrated their ability to distinguish between samples from individuals who were infected with SARS-CoV-2), those who were not infected (RT-PCR negative), and samples from individuals with respiratory symptoms but tested negative for SARS-CoV-2. Their diagnostic sensitivity was found to be 84% and their specificity was 95% [23].

In subsequent detection sessions, where the device used non-inactivated samples of the same bodily fluids (saliva, sweat, or urine), the dogs continued to perform well. For saliva samples, their diagnostic sensitivity and specificity were 82% and 96% respectively. In the case of sweat samples, the values were 91% for sensitivity and 94%for specificity. Finally, for urine samples, the dogs achieved a sensitivity of 95% and a specificity of 98% These results are summarized in (Table 6). The average disease prevalence observed was approximately 18% [23].

Throughout 5,308 randomized sample presentations, the dogs exhibited an overall success rate of 92%, with 723 correct indications of positive samples, 4,140 correct rejections of negative or distractor samples, and 214 incorrect indications of negative samples along with incorrect rejections of 231 positive sample presentations (List 1) [23].

Out of the total 93 subjects, 46 tested positive for SARS-CoV-2, while 47 tested negative via RT-PCR of nasopharyngeal swabs. Interestingly, RT-PCR results of sample materials (saliva, sweat, urine) from individuals with confirmed SARS-CoV-2 infection via nasopharyngeal swabs were only positive in twelve cases. Notably, there was a time gap of 2 to 5 months between the RT-PCR tests of nasopharyngeal swabs and the sample materials, which were stored and frozen at -80°C. Additionally, nasopharyngeal swabs from each dog and the exterior of the membranes used after each day of testing all yielded negative results [23].

Table 6. Sensitivity and specificity of the samples [23]

Test type	Diagnostic sensitivity	Diagnostic specificity
Saliva	82 %	96 %
Sweat	91 %	94 %
Urine	95 %	98 %

List 1. Summary of the results [25]

- Overall success rate during 5,308 sample presentations was 92%.
- Correct indications of positive samples: 723.
- Correct rejections of negative or distractor samples: 4,140.
- Incorrect indications of negative samples: 214.
- Incorrect rejections of positive sample presentations: 231.

8 E-Nose

The e-nose is a device that mimics mammalian olfaction using an array of gas sensors and pattern recognition. It finds already applications in various fields like industry, food, cosmetics, environmental monitoring, military, pharmaceuticals, and microbiological analyses [24].

The artificial nose is trained to detect VOCs produced by the body due to metabolic changes, similar as the dog's nose, the e-nose should become a diagnostic tool for the diagnosis of cancer. The idea behind this technology is not only to create a tool for diagnostic workup in the hospitals, but furthermore to design a simple tool, that can be used at home, similar to the already existing and widely used breathalyser to detect the alcohol concentration in human breath [25].

8.1 Technique

There are many different technologies regarding the e-nose, but most of them are using the air sampling method, there are differences in air storing as well, but almost every system needs a closed environment system, during the process of sampling [26].

The e-nose instead of having olfactory receptors (ORs) it contains a sensory array (Figure 5) including gas sensors that detect odorant molecules when they come into contact with the sensor's material. While in the real nose the signals are transmitted via nerves, here measurable electronic signals are produced and become converted from analog to digital signals, then they are processed by various algorithms and finally as alternative to the brain they are recognized and analysed a pattern recognition algorithm [27].

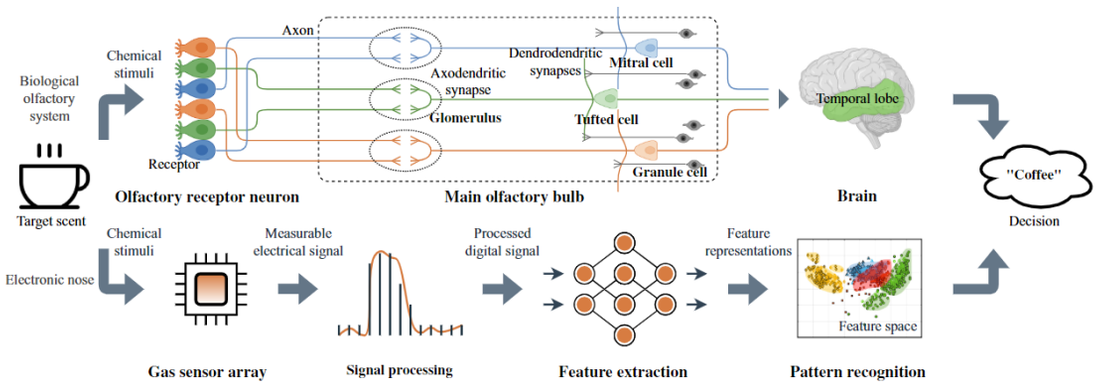

Figure 5. Demonstration of the biological olfactory system and the electronic olfactory system [29].

8.2 E-Nose Studies

In these studies, they detect: prostate cancer (PCa) in urine samples [24], and lung cancer in breath samples [28] with the help of the e-nose.

8.2.1 Methods

Prostate cancer

The study enrolled a total of 174 participants, comprising 88 cancer patients and 86 control individuals. The participants were categorized into two distinct groups: the PCa group and the Control group. The PCa group consisted of patients with a confirmed history of PCa, who had undergone procedures such as prostate biopsy, radical prostatectomy, or transurethral resection of the prostate (TURP). On the other hand, the Control group was composed of young female volunteers with non-neoplastic or neoplastic diseases and healthy young men (aged 18-25), along with adult men (aged > 45) who exhibited negative family history of PCa, negative digital rectal examination (DRE), and prostate-specific antigen (PSA) levels < 2.5 ng/mL that remained stable over time [24].

The study did not employ any exclusion criteria based on lifestyle factors such as smoking, alcohol consumption, drugs, or diet.

Sample Collection and Storage: For each participant, a spontaneous 30cc urine sample was collected in two separate sterile containers. These samples were obtained either during hospital administration or prior to surgery. To maintain sample integrity, the urine specimens were promptly frozen at a temperature of -20°C. Following collection, the samples were transported under controlled temperature conditions to ensure preservation and prevent degradation. Upon arrival at the laboratory, the samples were stored at -20°C until they were ready for analysis [24].

Lung cancer

The study included a total of 167 patients, comprising two distinct groups: 107 patients with benign lung diseases and 60 patients with histologically proven lung carcinoma. To ensure a reliable evaluation, the study employed strict exclusion criteria, which included age under 18 years and any history of cancer or ongoing tumour treatment [24].

8.2.2 Results

Prostate cancer

The study's results revealed the promising potential of the e-nose in detecting prostate cancer. With a sensitivity rate of 85.2%, the e-nose successfully identified the majority of patients with prostate cancer. Out of the 88 cases of prostate cancer, there were only 13 false negatives. Moreover, the e-nose demonstrated a specificity rate of 79.1%, indicating its ability to accurately distinguish healthy controls from cancer patients. The occurrence of 18 false positives out of the 86 control cases was observed [24].

Lung cancer

The findings of the study highlighted the diagnostic accuracy of the method across training and validation sets. In the training set, the method demonstrated a sensitivity of 83% and a specificity of 84%, resulting in an overall accuracy rate of 83%. These results indicate the method's ability to accurately identify true positives and true negatives within this subset of patients. Furthermore, the validation set results depicted an even higher performance level. The method exhibited a sensitivity of 88% and a specificity of 86%, leading to an overall accuracy rate of 86%. This increase in sensitivity and specificity in the validation set suggests that the method's diagnostic capabilities hold consistent promise across different patient populations [24].

8.3 E-nose advantage and disadvantage compared to dog nose

In general, both dog nose and e-nose have two big advantages compared to all other diagnostic methods for cancer that they aren't invasive or harmful for the patient.

Advantage

E-nose should become a tool which can be easily handled at home, or even if the diagnosis is done in a clinic, in most of the cases no biopsy or other invasive procedures must be expect, it is enough to give a urine, or breath sample or in the worst a few drops of blood [25].

Dogs can thrive in a home environment with the right care and attention. While they do require proper training and regular exercise, they come with some incredible advantages. One notable advantage is their innate ability to detect various scents without needing to learn the specifics of each odour. Instead, they simply need to master the art of focusing on particular scents and effectively conveying their findings to their handlers [3]

Disadvantage

Electronic noses are sophisticated devices used for odour detection and identification, but they come with several notable limitations.

1. Loss of Sensitivity in Certain Conditions

   E-noses may experience a loss of sensitivity when exposed to specific environmental factors, such as high humidity or the presence of high concentrations of a single component like alcohol. These conditions can interfere with the accuracy and reliability of scent detection, limiting their effectiveness in certain settings [29].

2. Sensor drift and lack of absolute calibration

   One common challenge with e-noses is sensor drift, where the sensors' response to odours may change over time or due to environmental factors. This can lead to inconsistent results and the need for frequent recalibration. Additionally, e-noses typically provide relative measurements rather than absolute values, making it difficult to obtain precise quantitative data [29].

3. Limited sensor lifespan

   Some sensors used in e-noses have a relatively short lifespan. Over time, sensor performance can degrade, necessitating sensor replacement or recalibration. This maintenance requirement can add to the cost and complexity of using e-noses in long-term applications [26].

4. Method development for each application

   E-noses require considerable method development work for each specific application. Tailoring the device to effectively detect and differentiate between specific odours or compounds can be time-consuming and resource intensive. This limitation can hinder the rapid deployment of e-noses in new or specialized areas [26].

5. Inability to provide quantitative data for Odour differences

   While e-noses are adept at detecting and comparing different odours, they often struggle to provide precise quantitative data for aroma differences. This limitation can make it challenging to quantify subtle variations in scent profiles [29].

The biggest disadvantages with dogs probably are that they are mortal and living beings, they need to rest and can´t work 24/7, not every dog is suitable for this job and of course every single dog needs to undergo a long training period [30], [1].

9 Summary

The utilization of dogs as diagnostic tools represents both a novel concept and one with historical roots dating back to ancient times. Historically, dogs have served as hunting companions and protectors against various threats, and in contemporary contexts, they continue to be indispensable across a wide spectrum of applications. Their remarkable olfactory capabilities, which exceed human abilities by a factor of a million, afford them the capacity to detect hazards well in advance of human perception. Nevertheless, the training required for these dogs is resource-intensive, and within critical domains such as cancer or COVID diagnosis, professionals in the scientific and medical communities exhibit a degree of reluctance to place significant reliance on canine assessments. Consequently, research in this domain remains limited. However, it is noteworthy that the studies conducted thus far show considerable promise, yielding remarkable outcomes.

There are series of studies that investigate the potential of canine scent detection in diagnosing various types of cancer and Covid-19. These studies involve rigorous methodologies, special canine participants, and training techniques tailored to each investigation. For cancer detection, the studies cover lung, ovarian, prostate, and bladder cancers, showcasing the remarkable abilities of trained dogs in detecting these diseases. In Covid-19 studies, specific eligibility criteria were applied to participants, and various sample collection methods were utilized. The training of dogs in Covid-19 detection involved systematic approaches with an emphasis on positive reinforcement. The results from both cancer and Covid-19 studies demonstrate the promising potential of canine scent detection as a valuable addition to existing diagnostic methods.

In the realm of technology, the potential of odours in diagnostics has also been unveiled, and researchers are diligently developing a tool that could potentially replace the canine nose. Some initial studies have already yielded promising results. But both the dogs and the e-nose, of course, come with their own set of advantages and disadvantages, and further research is essential in both cases, before they can be adopted as diagnostic standards.

10 Összefoglalás

A kutyák diagnosztikai eszközként való felhasználása újszerű koncepciót jelent, amelynek gyökerei azonban egészen korai időkig nyúlnak vissza. Történelmileg a kutyák vadászati kísérőként és különböző veszélyekkel szembeni őrző-védőkként szolgáltak, és alkalmazásuk napjainkban továbbra is nélkülözhetetlen több területen. Figyelemre méltó szaglóképességük, amely milliószorosan meghaladja az emberi képességeket, lehetővé teszi számukra, hogy jóval az emberi érzékelés előtt észleljék a veszélyeket. Mindazonáltal a kutyák képzése erőforrás-igényes, és az olyan kritikus területeken, mint a daganatos betegségek vagy a COVID diagnosztizálása, a társadalom, a tudományos testületek és az orvosi közösségek szakemberei bizonyos fokú vonakodást tanúsítanak abban, hogy a kutyák szaglására és így a kutyák döntésére hagyatkozzanak. Következésképpen az e területen végzett kutatások továbbra is korlátozottak. Figyelemre méltó azonban, hogy az eddig elvégzett tanulmányok ígéretesek, és számos pozitív eredményt hoztak.

Megjelentek tanulmánysorozatok, amelyek a kutyás szagérzékelés lehetőségeit vizsgálják a különböző ráktípusok és a Covid-19 diagnosztizálásában. Ezek a tanulmányok szigorú módszertant, speciális kutya résztvevőket és a vizsgálatokhoz igazított képzési technikákat foglalnak magukban. A rákfelismerés tekintetében a vizsgálatok a tüdő-, a petefészek-, a prosztata- és a húgyhólyagrákra terjednek ki, bemutatva a kiképzett kutyák figyelemre méltó képességeit e betegségek felismerésében. A Covid-19 vizsgálatokban résztvevőkre speciális alkalmassági kritériumokat alkalmaztak, és különböző mintavételi módszereket alkalmaztak. A kutyák Covid-19 kimutatására való kiképzése szisztematikus megközelítéseket alkalmazott és a pozitív megerősítésre helyezve a hangsúlyt. Mind a rákkal kapcsolatos, mind a Covid-19 vizsgálatok eredményei azt mutatják, hogy a kutyákkal végzett szagdetektálás a meglévő diagnosztikai módszerek értékes kiegészítőjeként ígéretes lehetőségeket rejt magában. Ezek az eredmények aláhúzzák a további kutatások szükségességét az orvosi diagnosztikai munka ezen innovatív területén.

A technológia fejlődésével a szagok diagnosztikában rejlő lehetőségeit is felfedezték, és a kutatók szorgalmasan fejlesztenek egy olyan eszközt, amely potenciálisan helyettesítheti a kutya orrát. Néhány kezdeti tanulmány már ígéretes eredményeket hozott. De természetesen mind a kutyáknak, mind az e-orrnak megvannak a maga előnyei és hátrányai, és mindkét esetben további kutatások szükségesek, mielőtt diagnosztikai szabványként alkalmazhatjuk őket.

11 Conclusion

Dogs are utilized in various fields today, not only in the service of the police but also for assisting the visually impaired and many diabetics to prevent hypoglycaemic shocks. It is proven that dogs possess an incredibly keen sense of smell, allowing them to detect things imperceptible to us humans. Moreover, dogs thrive on challenges, and both physical and mental tasks are crucial for their well-being.

As most studies indicate, dogs excel at identifying metabolic changes and, with the appropriate training, can communicate these findings. Their success rates often surpass those of conventional methods. I do not believe that dogs should or can replace conventional methods, but in the realm of prevention, where we currently lack alternatives, and for conducting regular check-ups without long wait times or costly procedures, especially in crisis or emergency situations, we can harness the tremendous potential of a dog's olfactory senses.

Certainly, electronic noses are also an intriguing option, yielding excellent results. However, they are not yet fully developed, and we cannot predict when they will be. Additionally, their development is labour-intensive and expensive. Given that three million people in Europe are diagnosed with cancer annually, with 1.2 million succumbing to the disease, often due to late detection, I believe we should act as swiftly as possible and train dogs for these purposes.

Dogs with a basic education in this field can also be quickly trained or retrained in emergency situations, such as during the outbreak of a virus like Covid-19, to detect the relevant pathogens or metabolic changes. I believe that new diagnostic tools for cancer, emerging viral or bacterial diseases will play a significant role in human medicine in the near future, and we should prepare ourselves as effectively as possible.

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Supervisor's consent form

I hereby confirm that I am familiar with the content of the thesis entitled „ **Dogs as diagnostic tools**" written by Anna Gräfin von Pfeil, which I deem suitable for submission and defence.

Date: 10th of November 2023

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Dr. Mándoki, Míra

Pathology Department

Thesis progress report for veterinary students

Name of student: **Gräfin von Pfeil, Anna**

Neptun code of the student: **AB0Z3S**

Name and title of the supervisor: **dr. Mándoki Míra, professor**

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Thesis title: **„Dogs as diagnostic tools"**

Consultation – 1st semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	03	20	Consultation about the thesis guide, requirements	
2.	2022	04	24	Introduction, title of the thesis	
3.	2022	05	22	Consultation about current literature 1	
4.	2022	06	05	Consultation about current literature 2	
5.	2023	06	12	Consultation about the timing	

Grade achieved at the end of the first semester: …..excellent (5)……..

Consultation – 2nd semester

Timing				Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	09	29	Correction of Introduction	
2.	2023	10	12	Correction of literature review part 1	
3.	2023	10	16	Discussion on the study descriptions, interpretation	
4.	2023	10	25	Correction of literature review part 2	
5.	2023	11	02	Finalizing of the thesis	

Grade achieved at the end of the second semester: …..excellent (5)……..

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing. I accept the thesis and find suitable to defence.

……
signature of the supervisor

Signature of the student: ……

Signature of the secretary of the department: ……

Date of handing the thesis in……