ACTA VET. BRNO 2023, 92: 303–307; https://doi.org/10.2754/avb202392030303

# The effect of urine sample temperature on the efficacy of olfactory detection of prostate cancer in men by a specially trained dog

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*Received October 31, 2022*
*Accepted July 11, 2023*

**Abstract**

The objective of the study was to determine whether the temperature of urine samples from patients with prostate cancer or the temperature of urine samples from men not suffering from this condition affects the efficacy of prostate cancer detection performed by a specially trained dog using its olfactory abilities. A total of 218 urine samples, divided into two sets differing in temperature, were tested for male prostate cancer by a dog sniffing urine samples. In group 1 (urine temperature 15–23 °C), 120 urine samples were examined by a sniffer dog, of which 57 samples were positive (from the group of men with established prostate cancer) and 63 samples were negative (from men not affected by prostate cancer). In group 2 (urine temperature 2–14 °C), 98 urine samples were examined by a sniffer dog, of which 51 were positive (from the group of men with established prostate cancer) and 47 were negative (from men not affected by prostate cancer). The results of our study demonstrated no link between the efficacy of male prostate cancer detection performed by a specially trained dog using its olfactory abilities from a urine sample and the temperature range from 2–23 °C ($P > 0.05$).

*Urology, tumour, prostate gland, auxiliary examination methods*

Early diagnosis of prostate cancer in men is one of the key factors determining the effectiveness of treatment for this serious disease (Sonoda et al. 2011). Therefore, auxiliary minimally invasive diagnostic methods are constantly being designed and developed. The ability of dogs to detect the odour of cancer in humans was first described by Williams and Pembroke in 1989 (Pirrone and Albertini 2017). More than ten years later, a study by Church and Williams was published (Pirrone and Albertini 2017). These two studies focused additional attention on further investigation of the olfactory abilities of dogs to detect the chemical markers of cancer. At our department, we focused on the diagnosis of prostate cancer in men using canine olfactory abilities and we showed that the diagnosis of prostate cancer in men using the olfactory abilities of a specially trained dog can be considered a reliable, non-invasive, and relatively inexpensive method of diagnosing a prostate carcinoma (Urbanova et al. 2015). Following the results of our previous study (Urbanova et al. 2015), we decided to continue looking at some factors that could potentially affect the reliability of prostate cancer detection in men from a urine sample using canine olfactory abilities.

Therefore, the objective of this study was to determine whether the temperature of urine samples from men with prostate cancer ("positive urine samples") and urine samples from men without prostate cancer ("negative urine samples") presented to a specially trained dog would affect the accuracy of its ability to discriminate between urine samples from men with prostate cancer and urine samples from men without prostate cancer.

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### Materials and Methods

In the period from 7 January 2016 to 1 March 2016, 218 urine samples were examined by a dog trained to detect prostate cancer from male urine (female German Shepherd named Agáta Jankari) (Urbanova et al. 2015). The samples were divided into two groups differing in urine temperature. A total of 120 urine samples with a temperature in the range of 15–23 °C included in group 1 were tested by the dog's olfaction, of which 57 were positive (from the group of men with established prostate cancer) and 63 were negative (from men without prostate cancer). A total of 98 urine samples with a temperature in the range of 2–14 °C included in group 2 were tested by the dog's olfaction, of which 51 were positive (from the group of men with established prostate cancer) and 47 were negative (from men without prostate cancer).

The samples presented to the dog for olfactory testing were contained in plastic cups with a perforated lid and were placed on the floor in a room designated for that purpose, with its floor covered by marmoleum which was washed by a disinfectant solution every day. The cups were fixed to the floor with an adhesive to eliminate the risk of them being knocked over and the urine contaminating the floor. During each run, the dog was commanded to examine 3 urine samples by sniffing, of which 2 were negative (from men without prostate cancer) and 1 was positive (from a patient with prostate cancer). Detection was considered correct if the dog lay down in front of the positive urine sample with its nose pointing towards it (Plate XV, Fig. 1).

Where the dog gave a positive urine sample (from a patient with prostate cancer) only a short sniff but immediately moved on to another sample to examine it (olfactory test), such detection was considered incorrect. A negative urine sample (from a man without prostate cancer) was correctly identified when the dog sniffed at it and moved on to test the next sample without lying down, whereas lying down in front of the sample was considered an incorrect detection.

During one olfactory detection run, each of the three sets of tested samples within each group (1 or 2) had the same temperature. Three different hypotheses were subjected to statistical analysis and these were subsequently tested (test 1, test 2, and test 3) by Chi-squared test using a contingency table verifying the independence and homogeneity of the tested data.

Test 1 – relation between the success/failure detection rate and sample temperature
Zero hypothesis H0: Frequency distribution is dependent on the sample temperature.
Alternative hypothesis HA: Frequency distribution is independent of the sample temperature.

Test 2 – relation between correct detection and sample temperature
Zero hypothesis H0: Correct detection is affected by sample temperature.
Alternative hypothesis HA: Correct detection is not affected by sample temperature.

Test 3 – relation between incorrect detection and sample temperature
Zero hypothesis H0: Incorrect detection is affected by sample temperature.
Alternative hypothesis HA: Incorrect detection is not affected by sample temperature.

## Results

The frequency rate of positive and negative urine sample detection from group 1 (urine at 15–23 °C) correctly or incorrectly detected by the dog using its olfactory abilities is shown in Table 1.

The frequency rate of positive and negative urine sample detection from group 2 (samples at 2–14 °C) correctly or incorrectly detected by the dog based on the dog's olfactory test is shown in Table 2.

None of the three tests performed showed a link between the efficacy of detection of the relevant urine sample and its temperature within 2–23 °C ($P > 0.05$) by a specialist trained dog using its olfactory abilities.

Table 1. Results of urine sample detection using dog's olfactory abilities (urine temperature 15–23 °C).

| Urine sample | Number of urine samples | Results of olfactory tests performed by the dog | Number of urine samples |
|---|---|---|---|
| Positive | 57 | Detected correctly | 47 |
| | | Detected incorrectly | 10 |
| Negative | 63 | Detected correctly | 46 |
| | | Detected incorrectly | 17 |
| Total | 120 | | |

Table 2. Results of detection of urine samples using the dog's olfactory abilities (urine temperature 2–14 °C).

| Urine sample | Number of urine samples | Results of olfactory tests performed by the dog | Number of urine samples |
|---|---|---|---|
| Positive | 51 | Detected correctly | 40 |
| | | Detected incorrectly | 11 |
| Negative | 47 | Detected correctly | 26 |
| | | Detected incorrectly | 21 |
| Total | 98 | | |

## Discussion

The sense of smell is the primary sense for dogs and is thousands of times more sensitive than the sense of smell in humans (Flanders 2011). Dogs are able to smell around half a million odour compounds at concentrations that are imperceptible to the human nose (Buszewski et al. 2012).

Dogs that can use their olfactory abilities are an indispensable aid to humans, for example, in helping to locate drugs, explosives, currency, and missing or dead people (DeGreeff et al. 2012). The odour profile consists of many volatile organic compounds (VOCs) in the gaseous state (Lorenzo et al. 2003). Many studies have addressed the issue of canine olfactory abilities (Lorenzo et al. 2003; Statheropoulos et al. 2005; Vass et al. 2008; Hoffman et al. 2009; Cablk et al. 2012; DeGreeff et al. 2012; Stadler et al. 2012; Forbes et al. 2014) to detect the chemical composition of the odour of decomposing tissue and its key components used by dogs. In recent years, studies have been published describing the dogs' ability to detect cancer patients by scent (Yoel et al. 2015). This ability is based on the sniffing of volatile organic elements that are secreted by malignant cells, or reacting to them (Yoel et al. 2015).

The sensitivity of dogs to odours is attributed to their highly developed olfactory system and a high number of olfactory receptors on their nasal cells, approximately 20–60 times higher than in humans (Craven et al. 2010). During the process of sniffing, VOCs bond to odour receptors in neurons, sending an electrical signal which is processed by the olfactory bulb of the brain (Rebman et al. 2000). Chemical properties such as the molecule size, shape, stereochemistry, solubility, volatility, and polarity will determine the pattern of neuron firing, producing different electrical signals in the brain and creating a recognizable identifier for a particular odour (Rebman et al. 2000).

The first work by Williams and Pembroke from 1989 dealing with the ability of dogs to detect the presence of carcinoma in humans and a study published more than a decade later by Church and Williams (Pirrone and Albertini 2017) led to further investigations into the dog olfactory abilities to detect chemical markers of carcinomas, demonstrating that with appropriate training, dogs are able to distinguish the breath, urine, faeces, and tissue samples (lung, mammary gland, prostate, skin, ovary) of cancer patients from those of healthy individuals (Pirrone and Albertini 2017). However, biological samples consist of hundreds of VOCs with low concentrations, making it challenging to determine exactly which VOCs are the markers that dogs perceive and respond to (Pirrone and Albertini 2017). A number of factors can influence the correct determination of a sample. Among others, the welfare of sniffer dogs, involving physiological or behavioural signs of stress which can affect the dog's performance both in terms of motivation and work precision (Pirrone and Albertini 2017). However, in contrast to analytical instruments, dogs are subject to boredom, fatigue, hunger, and external distractions (Hackner and Pleil 2017). The analytical instrument approach has limitations in that they can only detect that for which they are programmed (Hackner and Pleil 2017). Even instruments designed to mimic olfaction such as the "electronic nose" are relegated to sensor arrays of 16, 32, or 64, whereas the dog has about 200 million olfactory cells (Kateb et al. 2009; Buszewski et al. 2012; Bikov et al. 2015). Other factors affecting the results of a urine sample examination in patients with prostate cancer using the dog's olfactory abilities may be associated with the patient's illnesses or the food consumed (Cornu et al. 2011).

The authors of one of the studies (Willis et al. 2004) presented a group of dogs with dried urine samples and compared the detection rate of that group (positive urine samples from bladder cancer patients) to a group of dogs presented with liquid urine samples. The group of dogs sniffing the liquid samples performed significantly better in terms of sample detection than the group of dogs sniffing the dried urine samples (Willis et al. 2004). However, small sample sizes, along with other potential variables of the two groups of dogs (such as breed, age, and environmental conditions during testing, etc.), limit the reliability of this finding (Willis et al. 2004).

Storage conditions and presenting of samples to the dog for an odour test may also affect the outcome of the dog's sample detection. In the study by Cornu et al. (2011), the dog was presented with thawed samples heated to 37 °C for examination.

With regard to the potential influence of some physical factors (state, urine sample temperature) on the reliability of detection of urine samples from patients with prostate cancer by a dog using its olfactory abilities, we studied the effect of the temperature of the tested urine sample on the detection rate of prostate cancer in men by the sense of smell of a specially trained dog.

Based on the results of odour tests performed by the sniffer dog using its olfactory abilities, involving indication of urine samples from men with prostate cancer ("positive urine samples") and urine samples from men not suffering from prostate cancer ("negative urine samples") of different temperatures, we can conclude that no relation between detection efficacy of the relevant urine sample and the sample temperature was demonstrated ($P > 0.05$).

Considering that a number of physical properties of the test urine samples as well as other factors may potentially affect the accuracy and reliability of prostate cancer detection in humans and the utilisation of the demonstrated olfactory abilities of dogs has not been thoroughly studied, this area of research focusing on early, non-invasive, and relatively inexpensive urological diagnostics definitely deserves further attention.

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Fig. 1. The sniffer dog indicating a positive urine sample.