Detection of SARS-CoV-2 by Canine Olfaction: A Pilot Study

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Background. As the number of coronavirus disease 2019 (COVID-19) cases continue to surge worldwide and new variants emerge, additional accurate, rapid, and noninvasive screening methods to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are needed. The number of COVID-19 cases reported globally is >455 million, and deaths have surpassed 6 million. Current diagnostic methods are expensive, invasive, and produce delayed results. While COVID-19 vaccinations are proven to help slow the spread of infection and prevent serious illness, they are not equitably available worldwide. Almost 40% of the world’s population remains unvaccinated. Evidence suggests that SARS-CoV-2 virus–associated volatile organic compounds found in the breath, urine, and sweat of infected individuals can be detected by canine olfaction. Medical detection dogs may be a feasible, accurate, and affordable SARS-CoV-2 screening method.

Methods. In this double-blinded, case–control, validation study, we obtained sweat samples from inpatients and outpatients tested for SARS-CoV-2 by a polymerase chain reaction test. Medical detection dogs were trained to distinguish SARS-CoV-2-positive samples from SARS-CoV-2-negative samples using reward-based reinforcement.

Results. Samples were obtained from 584 individuals (6–97 years of age; 24% positive SARS-CoV-2 samples and 76% negative SARS-CoV-2 samples). In the testing phase, all dogs performed with high accuracy in detecting SARS-CoV-2. The overall diagnostic sensitivity was 98%, and specificity was 92%. In a follow-up phase, 1 dog screened 153 patients for SARS-CoV-2 in a hospital setting with 96% diagnostic sensitivity and 100% specificity.

Conclusions. Canine olfaction is an accurate and feasible method for diagnosis of SARS-CoV-2, including asymptomatic and presymptomatic infected individuals.

Keywords. medical detection dogs; SARS-CoV-2; volatile organic compounds; virus detection; canine olfaction.

The World Health Organization declared coronavirus disease (COVID-19) a global pandemic on March 11, 2020. Currently, >455 million individuals globally have been infected with severe acute respiratory syndrome coronavirus (SARS-CoV-2), and COVID-19 deaths have surpassed 6 million [1].

The pandemic continues to surge worldwide as more virulent and contagious variants emerge. COVID-19 vaccinations have been proven to slow the spread of infection and help prevent serious illness and death [2]. According to researchers at the Our World in Data Project, >60% of the world’s population had received at least 1 dose of a COVID-19 vaccination as of January 2022; however, vaccine doses remain relatively scarce in low-income countries, where only 9.6% of people have received at least 1 dose of a vaccine. At present, there are limited outpatient treatment options specific to SARS-CoV-2 infection with proven efficacy in randomized controlled trials [3].

Early diagnosis and quarantine remain key strategies to reduce transmission of the virus. However, the effectiveness of these strategies is dependent upon timely testing and screening methods that identify individuals infected with the virus, especially before symptom onset [4].

Thermal screening is utilized as a method for detecting COVID-19, but this screening method alone is an ineffective marker of viral infection, as it has low sensitivity rates and can miss more than half of infected individuals [5]. Real-time reverse transcriptase polymerase chain reaction (RT-PCR) tests are considered the gold standard for COVID-19 diagnoses; however, testing is invasive, expensive, and produces delayed results [6].

Additional effective, affordable, and noninvasive screening and testing methods are needed to provide real-time diagnosis of SARS-CoV-2 in the fight against COVID-19. Early diagnosis of COVID-19 in infected individuals, especially those who are asymptomatic or presymptomatic, is the key to minimizing the spread of infection as well as ensuring early treatment to prevent serious illness or death [4].
A promising approach to rapid screening is through identifying the volatile organic compound (VOC) patterns of SARS-CoV-2 infection. Studies have shown that viral and bacterial cultures have pathogen-related VOCs [7] and that SARS-CoV-2 and influenza A infections have different emitted VOCs in breath samples [8]. Research also indicates that SARS-CoV-2 produces distinct VOCs emitted through the urine, saliva, and sweat of individuals infected with the virus and that body fluids are similarly suited for reliable detection of SARS-CoV-2 in infected individuals by canine scent detection [9].

Electronic sensor technologies, “electronic noses,” have demonstrated promise in detecting various diseases including prostate and other cancers [10,11], as well as bacteria in blood samples, but currently there is no device for use in clinical practice [12]. Dogs were shown to outperform current technology in a recent prostate cancer detection study [13]. In another study, dogs indicated a detection limit of <0.001 parts per billion (ppb; 1 \times 10^{-12}) [14], while “electronic noses” had a detection threshold of 100 to 400 (ppb; 1 \times 10^{-2}) [15].

Canine scent detection is gaining attention as an effective and reliable method for identifying infections, viruses, and diseases [16,17]. Dogs’ olfactory acuity is >100 000 times stronger than humans’, with the ability to detect odors in parts per trillion [14,18]. There is evidence that dogs can learn and detect the smell of virus-associated VOCs with sensitivities of up to 96% and specificities of up to 98% [19]. Medical detection dogs are being utilized in olfactory research of diseases such as cancer [20–22], diabetes [23], and malaria [24]. The supplementary material associated with Maurer et al. [25] and other research [26] have shown that dogs can detect bacterial and viral infectious with a high rate of precision.

Medical detection dogs can distinguish pathogen-specific body odors in the breath, saliva, and skin of individuals infected with SARS-CoV-2 with a high degree of accuracy, and also distinguish infected individuals from those not infected with the virus [27–30]. Research indicates that dogs can discriminate between SARS-CoV-2 and other viral respiratory infections [31] and may be superior to RT-PCR tests in screening for SARS-CoV-2 [32]. A recent study also suggests that dogs can generalize the odor of COVID-19 and have the same accuracy rate when identifying new SARS-CoV-2 variants they have not previously been conditioned to [33].

These findings suggest that trained medical detection dogs may present a novel method for screening and detecting SARS-CoV-2-infected individuals, including those who are asymptomatic or presymptomatic, as well as those infected with different variants. However, samples have primarily consisted of blood, bronchial secretions, etc., which are more challenging to collect and less applicable in real-world SARS-CoV-2 screening scenarios. Sweat is a bodily odor that can be immediately and noninvasively screened by medical detection dogs.

Our primary objective for this study was to determine the ability of medical detection dogs to distinguish SARS-CoV-2-positive (case) sweat samples from SARS-CoV-2-negative (control) sweat samples using an efficient methodology that can be implemented in real-world settings. This research could help lead to the development of an accurate, noninvasive, and rapid-result mobile diagnostic tool for screening people with SARS-CoV-2 infections. There is also potential for medical detection dogs to be deployed in regions where other screening and testing methods are not readily available.

METHODS

Collection of Samples From Human Subjects

Study Participants
Eligible subjects were males or females who underwent testing for SARS-CoV-2 by a real-time reverse transcriptase polymerase chain reaction (RT-PCR) test within 72 hours of sample collection. RT-PCR was performed for a variety of reasons: as part of usual clinical care for subjects with symptoms suggestive of COVID-19 and for asymptomatic subjects who may have been exposed to COVID-19 or were required to test for work, travel, etc. At the time of sample collection, subjects were asked if they had symptoms of COVID-19. We included subjects who were symptomatic and asymptomatic and those who were hospitalized and nonhospitalized. We excluded those who had a prior positive COVID-19 RT-PCR test within the prior 90 days and those hospitalized with severe COVID-19 (eg, requiring mechanical ventilation). There were no other restrictions. We defined “cases” as those who were COVID-19 RT-PCR positive and “controls” as those who were COVID-19 RT-PCR negative.

Patient Consent
We received institutional review board (IRB) approval from The Queen’s Medical Center Institutional Review Committee. The study involved no more than minimal risk to subjects, and written consent was obtained for study participation.

Sample Collection
Positive and negative samples were obtained from individuals at various sites to ensure the dogs were not conditioned to the environments where the samples were collected. Samples from hospitalized patients were collected from The Queen’s Medical Center. Samples from nonhospitalized patients were collected at outpatient COVID-19 testing sites and at the homes of participating individuals. All samples were collected by trained nursing or research staff, who wore standard personal protective equipment and utilized methods to avoid sample contamination. Hypoallergenic cotton pads were wiped for 15 seconds on the side of the neck in a back-and-forth motion under the angle of the jawbone from below the ear to below the
Samples were placed into 4-ounce specimen cups, labeled, and placed into individually sealed plastic bags. Anonymized samples were stored and transported in coolers and kept under continuous refrigeration until used.

A total of 584 samples were collected, including 141 from individuals who had tested positive for SARS-CoV-2 by RT-PCR and 443 from individuals who had tested negative for SARS-CoV-2 by RT-PCR using nasal swab specimens. COVID-19 is more transmissible through aerosol transmission than contact transmission of the virus [34]. Therefore, not only is sweat collection more efficient than collecting blood or other bodily fluid samples; it also decreases the risk of transmission of SARS-CoV-2 compared with other collection methods.

**Teaching Dogs to Detect SARS-CoV-2**

**Institutional Animal Care and Use Committee**

A US Public Health Service–compliant Institutional Animal Care and Use Committee (IACUC) was established and consisted of 5 members: 1 veterinarian chairperson, 1 institutional member, and 3 lay members representing general community interests in the proper care and treatment of animals [35]. The IACUC approved the training facility and study protocol.

**Training Site and Equipment**

Canine scent detection training took place at the Assistance Dogs of Hawaii campus (Makawao, Maui, HI, USA). The training room was 9.14 square meters, temperature controlled (21°C–26°C), and cleaned at the end of each day using an unscented, nontoxic cleaner. The laboratory room (3.04 m × 3.66 m) was adjacent to the training room, with a 1-way privacy window that allowed researchers to observe double-blinded runs. Samples were stored in a refrigerator (1.8°C–3.3°C). Samples were removed from the refrigerator before the run in which they were used and discarded immediately after each session. Sample handlers wore standard personal protective equipment.

The hypoallergenic cotton pads containing the skin odor samples collected from study participants were transferred to sterile 4-ounce specimen cups (Thomas Scientific) and then placed in individual plastic scent detection boxes. The boxes were 17.5-cm square with a 4.0-cm circular opening at the top and a snug-fitting, removable lid. The size of the specimen jar within the boxes allowed for sufficient air circulation and spacing so the samples could not be reached by the dogs’ noses or mouths. Boxes were lined up on the floor at 56.0 cm apart.

**Personnel**

Research staff included dog handlers, sample handlers, and data recorders. Dog handlers worked with the dogs 1 at a time. A sample handler prepared the samples at the start of each run and disposed of samples immediately after use. The sample handler, stationed in a separate laboratory room, observed runs through a 1-way privacy window and was the only person who was unblinded to the SARS-CoV-2 status of the test samples during the testing phase of the study. During the testing phase, a data recorder was positioned behind a solid curtain in the far corner of the training room and observed and recorded results on paper and video. Information was entered at the end of each day in a spreadsheet, which was sent to the researcher for analysis.

**Dog Selection and Training**

Four dogs were trained using reward-based methods. The dogs included 3 Labradors and 1 Golden Retriever ranging in age from 1 to 5 years old (Figure 1). All dogs had prior assistance.

![Medical detection dogs Yuki, Tess, Sadie, and Samson.](image-url)
dog training but no prior scent detection training. Training included introducing an adequate number of positive and negative samples to ensure that both generalization and discrimination took place by the dogs. Generalization was necessary to ensure that the dogs were not memorizing the individual training samples, which would impact their ability to identify new samples as positive or negative. Discrimination ensured that the dogs learned to recognize the specific disease they were conditioned to and were able to distinguish it from similar odors, such as other respiratory diseases.

The dogs were presented with a lineup of 5 scent detection boxes. Dogs demonstrated a recognizable alerting behavior for SARS-CoV-2-positive samples and were rewarded for correctly alerting to the case samples. Handheld clickers and food rewards were used as positive reinforcement if the dog correctly alerted to SARS-CoV-2-positive case samples. Training lasted 6 weeks and took place 3 days per week, 1–2 hours per day. The duration of the training phase was dictated by the number of samples received. The total training time for each dog averaged 20 hours.

**Training and Testing Phases**

**Training Phase: (6 Weeks).** During the training phase, 73 samples from individuals who tested positive and 82 samples from individuals who tested negative for SARS-CoV-2 were utilized. The dogs were first taught to identify the positive (case) samples and then to distinguish the positive samples from the negative (control) samples. Initially, the dogs found 1 positive sample in a lineup of 5 containers, including 4 empty containers, then gradually transitioned to a lineup of 5 containers with 1 target sample and 4 different control samples. Dogs worked off leash and were encouraged to sniff the study samples with a verbal cue of “go find.” The dogs learned to demonstrate a recognizable alerting behavior to a positive sample by pawing or sitting directly in front of the container. Dogs were taught to sniff the negative samples and to move onto the next container (Figure 2). The dogs’ responses were compared with the laboratory results to evaluate accuracy. At the end of the training phase, it was determined that 3 of the 4 dogs were ready to participate in the testing phase. The 3 dogs were Labradors and included Sadie (5 years old), Tess (2 years old), and Yuki (1 year old).

**Validation Testing Phase: (3 Weeks).** During the validation testing phase, we utilized 52 SARS-CoV-2-positive samples and 208 SARS-CoV-2-negative samples. During this phase, the dog, dog handler, and data collector were blinded to the SARS-CoV-2 status of the sweat sample. The sample handler was unblinded. Only new positive and negative samples that had not been utilized during the training phase were used in the validation testing phase.

A random number table was utilized to determine the placement of case and control samples in each row. Each testing run included a line-up of 5 boxes, with 1 box containing a case sample and the remaining boxes containing control samples. Testing runs included age-matched control samples within 5 years. The dogs worked off-leash and were allowed to sniff the boxes more than once. To ensure that the dogs were not learning to alert to a particular box or at a particular station, the placement of the boxes containing the case samples was determined using a random number table. At each of the 5 stations, sample handlers randomly rotated among the 37 different scent detection boxes, so that each location would have equal probability of holding a case sample without regard to prior sessions.

Correct responses by the dogs included (a) sniffing and then pawing or sitting at case samples (a true positive in sensitivity calculations) and (b) sniffing but not sitting or pawing at control samples (a true negative in specificity calculations). Incorrect responses included (i) sniffing and then sitting or pawing at a control sample (false positive) and (ii) sniffing but not sitting or pawing at a case sample (false negative). When a dog correctly alerted to a case sample, the sample handler, located behind a 1-way mirror in the adjoining room, activated the clicker, and the dog handler dispensed a food reward. At the end of each run, the dog handler called out the results: “sniffed at [which stations]” and “alerted at [which stations]” (Table 1). The data recorder entered the results of each testing run on paper forms, and at the end of each day the data were entered into an electronic spreadsheet for analysis. All testing runs were video-recorded and audited daily.

**Analysis**

Diagnostic accuracy of the double-blinded testing phase was calculated as sensitivity and specificity, with dogs’ indication of samples for the presence or absence of SARS-CoV-2 compared with the gold standard of SARS-CoV-2 RT-PCR test result (positive or negative). Sensitivity was calculated as the frequency with which the dogs correctly alerted to SARS-CoV-2-positive (case) samples. Specificity was calculated...
as the frequency with which the dogs correctly ignored SARS-CoV-2-negative (control) samples [36]. Sensitivity and specificity, with exact binomial confidence limits, were calculated using R statistical software (https://www.r-project.org/).

## RESULTS

### Subjects

Samples were obtained from 584 individuals at a variety of testing sites, including indoor and outdoor locations. Ages ranged from 6 to 97 years with a mean age of 40 years and a standard deviation of 18.16. Samples included both inpatients and outpatients, of whom 46.4% were female and 53.6% were male. One hundred forty-one samples (24%) were from subjects who had tested positive for SARS-CoV-2 (cases), and 443 (76%) of samples were from subjects who tested negative for SARS-CoV-2 (controls). A total of 64 subjects were hospitalized, 520 were ambulatory, 423 patients were asymptomatic, and 161 were symptomatic.

### Diagnostic Accuracy

Overall, the 3 dogs detected positive SARS-CoV-2 samples with a sensitivity of 0.98 (95% CI, 0.94 to 0.99) and specificity of 0.92 (95% CI, 0.90 to 0.94) and positive and negative predictive values of 0.80 (95% CI, 0.74 to 0.85) and 0.99 (95% CI, −0.98 to 1.00), respectively (Table 2). Individually, the 3 dogs all had excellent sensitivity, varying slightly from 0.96 to 1.00. Specificity was also excellent and varied slightly more (0.87 to 0.99) (Table 2). Positive and negative predictive values were calculated from the 2×2 table using the standard equations $PPV = TP/(TP + FP)$ and $NPV = TN/(TN + FN)$.

### Implementation Phase

Immediately following the testing phase, we conducted a pilot project to test applicability in a hospital setting. Tess provided additional screening for patients of The Queen’s Medical Center who were scheduled for surgery (Figure 3). Patients received a PCR test before surgery, and at the same time laboratory technicians collected sweat samples on a cotton pad. These were refrigerated and transported to a designated room at the hospital, where they were screened by Tess and results were recorded.

Tess worked with a handler and screened 153 new patient samples, while PCR test results were pending. In addition, 16 positive (case) samples that had not previously been utilized were included in this phase to keep Tess motivated to search for the target scent. Samples were placed in scent detection boxes, and Tess screened a lineup of 5–10 boxes at a time. Tess’s responses were recorded and compared with the patient’s PCR test results when they became available. All samples were collected and stored using the same process as the testing phase. Tess was encouraged with a verbal cue of “go find” to continue working after alerting to positive samples. Each lineup included the unknown patient samples that were pending PCR results, plus 0, 1, or 2 case samples. During this pilot project, Tess performed with 96.4% diagnostic sensitivity and 100% diagnostic specificity.

### DISCUSSION

Our research demonstrates a safe, accurate, and noninvasive method to screen individuals for COVID-19. The results
confirm that canines can be taught to discriminate between sweat samples from SARS-CoV-2-positive and SARS-CoV-2-negative individuals. The results also suggest that dogs can detect SARS-CoV-2 in asymptomatic or presymptomatic individuals infected with the virus. The dogs’ high accuracy rates confirmed the findings of earlier studies that used various bodily fluids in testing dogs’ ability to detect SARS-CoV-2. Sensitivity and specificity were equally high in an implementation phase that took place in a hospital, demonstrating the potential for medical detection dogs to provide screening for COVID-19 in public places such as hospitals, schools, and businesses.

**Limitations**

A limitation of our study was using a 1:4 case to control ratio during the testing phase. However, during the implementation phase, trials with 0 and multiple case samples were included, and similar accuracy was achieved. Another limitation was the 72-hour window for the RT-PCR test and sweat sample collection, which could lead to change in the participant’s status. Excluding subjects who had been infected within 90 days limits the results to this subgroup of patients. The vaccination status of the subjects was unknown, presenting another limitation of this research, though there are indications that vaccination status does not affect the dogs’ ability to detect SARS-CoV-2. Additionally, because our study population included subjects who were symptomatic or exposed to COVID-19, our estimated positive and negative predictive values would likely differ if performed in a general population with lower COVID-19 prevalence.

**Future Directions**

Our research demonstrates that dogs can detect a signature odor for COVID-19 based on volatile organic compounds found in sweat. Medical detection dogs could be utilized as an additional screening tool in various settings, with individuals they identify as positive receiving rapid PCR tests to confirm their status. With recent technological advances, electronic noses may be developed and mass-produced to help screen and provide early detection of SARS-CoV-2 and other diseases. The dogs’ high accuracy rate at detecting asymptomatic individuals suggests that they may be able to identify those who are presymptomatic. Currently, we are screening students for COVID-19 at schools and investigating how early dogs can detect the presence of SARS-CoV-2 compared with PCR tests. We are also researching their ability to generalize their training to new variants. The results so far are very promising. Going forward, medical detection dogs may prove to be a valuable ally by providing rapid screening of emerging diseases and helping control the spread of future pandemics.
CONCLUSIONS

Dogs’ ability to detect SARS-CoV-2 suggests the possibility of using canine scent detection as an efficient and inexpensive mobile diagnostic tool for screening people with SARS-CoV-2 infections. Medical detection dogs could potentially be deployed at hospitals, schools, and other public places to detect SARS-CoV-2 and help prevent the spread of infection.

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