**Controlling the Spread of SARS-CoV-2 via Sniffer Dogs**

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**Abstract**

COVID-19 outbreak leads to morbidity and mortality. It is undeniable fact that both controlling infection and treatment are important for preventing the transmission of COVID-19. So, diagnosis of early stage of the disease and rapid identification of people who are infected with SARS-CoV-2 are vital. Various methods are currently used to diagnose of this disease, however there are limitations for current diagnostic methods such as cost, test results, time consuming, require specialized equipment and expertise. It is specified that a large number of human diseases have a specific smell. Probably each pathogen in individual can influence the released Volatile Organic Compounds (VOC) signals/profiles. Tracing changes in VOCs associated with certain disease can be used as unique fingerprints; also it can provide clues for early disease detection in order to prevent further outbreaks of infectious diseases. Dogs are be trained for smelling/ identifying any scented materials. Innumerable scientific articles indicate the importance of sniffer dogs in a spectrum range of applications. In this study, we scrutinize the properties of trained dogs for detection infected people with COVID-19.

**Keywords:**SARS-CoV-2, COVID-19, Trained dogs, Sniffer dogs, Olfactory system,Volatile Organic Compounds, VOC

**Introduction**

Corona Virus Disease 2019 (COVID-19) is still expanding worldwide. One of the most important issues in dealing with this pandemic is the rapid identification of asymptomatic carriers of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus. Various methods are currently used to diagnose of this disease, however there are limitations for current diagnostic methods such as cost, test results, time consuming, require specialized equipment and expertise (1).

Delaying appropriate COVID-19 infection control measures such as transferring patients to a single room can result in spread of SARS-CoV-2 virus. Early detection of individuals with COVID-19 is of importance for a community to subside the epidemic. Although, routine screening of all asymptomatic and pre-symptomatic patients in the community theoretically seems to prevent COVID-19 delayed diagnosis, it is not cost benefit and practical. Timely and accurate diagnosis of early stage of the disease and rapid identification of infections can defeat prevalent diagnostic delays (delays in sampling, laboratory procedures and lack of clinical suspicion) and result in prompting hygienic and treatment of individuals infected with SARS-CoV-2. It is therefore desirable to develop a sufficiently reliable, non-invasive, faster, versatile screening tool and inexpensive method of diagnosing COVID-19, which would usefully supplement the diagnostic techniques currently in common use (2).

It is specified that a large number of human diseases have a specific smell (3). Research is conducted to develop electronic noses technology that these sensors can recognize smells and tastes (4, 5). Electronic nose can be useful as affordable diagnostic tools and potential non-invasive in order to identifying volatile organic compounds (VOCs) in association with cancers. (5, 6). Despite important developments in this field, utilization of electronic sensing technologies has limitations, for instance, fasting of patients before process of sample breathing and not smoking are limitations of their applications (7, 8). Other challenges we will face in using electronic noses are an optimized sample collection is necessary and need long durations for sample analysis (9).

Practically, dogs are be trained for smelling/ identifying any scented materials. In dairy research, dogs are used to detect odors of estrus-specific in biofluids (10, 11). In comparison with electronic diagnostic sensors, even in the presence of unpleasant odors is trustworthy (12). Poor concentration of substances with different shape and size, parts in trillion, can be detected by the highly sensitivity of olfactory sensory system in dogs (13). Sniffer dogs olfactory sensory system is three times more sensitive than available tools which detect low concentration of substances, parts per million or billion (14). It has been more than a century that diagnosis of canine olfactive due to well-developed sense of smell is used as operational instruments. The efficacy of sniffer dogs are proven in various conditions such as police forces, finding people under the rubble and avalanches, discovering drugs and also identification of human illnesses (12, 15, 16). In 1978, magazine editor Gillian stated that her dog was paying a lot of attention to small brown spots on her leg. When her dog smelled the areas of freckles on Gillian's skin, Gillian's attention was drawn to those areas. Then, she went a physician and found out that it was malignant tumor (3). It is believed that the diagnosis of cancer by dogs are associated to VOCs produced by cells that become diseased. Studies showed that protein changes in malignant cells during tumor growth cause peroxidation of cell membrane components, which leads to VOCs production(17). Studies have shown that extraordinary sensitivity of the dog's nose in terms of accuracy for detection people who are infected in malaria or bacterial, and viral infections is wonderful (2, 18, 19). Definite patterns of VOCs may be the cause of identification of odors associated with particular pathogens by dogs.(2). Virus-infected cells have been reported to have unique VOC properties (20, 21). In contrast to bacteria, viruses do not enjoy a metabolism. Therefore, cellular infections that produced VOCs are caused by the metabolism of host processes (22). In this study, we intend to discuss the preventive effect of sniffer dogs on SARS-CoV-2 distribution; also, attention will be paid the role of these animals in the rapid diagnosis of people with COVID-19.

**Volatile Organic Compounds (VOCs)**

Organic compounds are established in all living things, all organic compounds contain carbon (23). Volatile organic compounds (VOCs) are defined as small (less than 400 Da molecular mass) organic molecules. They are volatile at ambient temperatures (24, 25). Conventionally, VOCs have always been categorized as either a pleasant smell or an “off” smell (26).

Odour fingerprints emitted from a body are linked directly to metabolic activity in the body. Particular patterns of these VOCs transfer metabolic information, pathological processes, or individual’s mindset. (7, 8). Some organs, in particular the liver can contribute to the VOCs conversion present in the bloodstream, thereby increasing the concentration of VOCs in the bloodstream (27).

Around 400 before Christ (BC), Hippocrates identified the usefulness of detecting smell of body. He talked about various illnesses have a particular smell emanated from urine or sputum (28). Different studies announced that specific pathogen or an infection with a specific pathogen shows a distinctive VOC pattern. (19, 29, 30). But based upon our guesses chemical composition are the origin smell of diseases. Both in cancer diseases and noncancerous diseases some compounds are produced in the body during metabolism. In cancer diseased releasing of acetone, hexanal, heptanal formaldehyde, alkanes, and benzene derivatives can be signs of having cancer, as well as highest concentration of acetone can be a potential marker of having diabetes (31-34).

**Canine olfactory system**

Functional olfaction in dogs develops between 8 and 13 days after birth (35). Olfactory epithelium, nasal cavity receptor cells, vomeronasal organ (VNO), which is an olfactory chamber rich in receptors and olfactory bulb, are major components of dog’s nose. (17, 36). The VNO gives this capability to dogs to conceive pheromones produced sexual signals and analyze other animals’ physiological condition (36). The ability of exploring olfactory stimuli are associated with the surface area of the olfactory epithelium and olfactory cells. A dog has 44 times more olfactory neurons than human. Dogs have multiple odorant recognition ability. This higher capability of dogs in comparison with humans has originated from the fact that number of olfactory receptors in dogs exceed 220 million per 170 cm2 but humans have only 5 million on the area about 5 cm2 olfactory receptors. (37, 38). This diagnosis ability in dogs has been reported 10,000-1,000,000 times more than a human (17).

Caniformia could easily learn 10 odors in a search task and subsequently identify it (39). Dogs have improved the accuracy of air sampling and odor collection, regardless of the dog's body size, through active inhalation, which generates short and sharp breaths at 4-7 Hz (40). These animals have a lower threshold for diagnosis of poor concentrations than other mammals and some odor detectors, because the anatomy of the dog's nose causes the odor to accumulate. Subethmoidal shelf makes a pocket-like cavity, which traps odor during exhalation. This phenomenon promotes odor identification by concentrating weak odors (36). By this way dogs can detect low amount of odors in present. The lower limit of the detectable dog for volatile organic compounds is one part per trillion (ppt) (13).

The initial process of odor and the identification and discrimination of millions of odors in vertebrates starts in the superfamily of G-protein-coupled olfactory receptor (OR) proteins located in the nasal cavity (41, 42). The thin layer covering the mucous membrane, dissolves Odorants and distributed gaseous substances in the air. These compounds stimulated olfactory receptors (ORs) on the cell surface of an olfactory neuron that starts transmitting more signals to the brain (38, 43, 44). It is believed that clusters of the ORs are the largest gene family in the mammalian genome which each cluster of the ORs possesses up to 100 intronless coding regions (∼1000 bp long). There are almost 1300 OR genes in the canine that are belong to the superfamiliy of protein–coupled receptor (GPCR) (16, 42, 45, 46). GPCR receptors which illustrate polymorphism contain 7 transmembrane (TM) domains, intercellular (IC) and extracellular (EC) loops (16). Polymorphism of OR genes may also impact olfactory capability and accuracy of odor discrimination in breeds and individuals (17). The percentage of pseudogenes (nonfunctioning genes) and the frequency of specific gene polymorphism varied in several species, including dogs, humans, and mice (47), identified nonfunctioning pseudogenes are in human 67%, pseudogenes represent 20% of olfactory receptor genes in mice and only 12% in dogs (36).

**Patiens identification by dogs**

Attention to the exploitation of VOCs for diagnostic intentions is in progress (31, 48). Diagnosis of VOC can be done using sophisticated biochemical methods or animals that in a letter Williams and Pembroke first suggested to the Lancet in 1989 that canine can diagnose malignant tumor based upon the odor (49). According to this concept, in 2017, a research team led by Carola Fischer-Tenhagen stated, dogs were able in selecting a prevalent odor among mixturing of odors. They showed chamomile as the targeting odor with an average sensitivity of 72% and a specificity of 84% detected by trained dogs.(11). In the field of oncology, sniffer dogs have the phenomenal ability to identify a specific "odor signature" (VOCs produced by tumors) that are added in the air by urines, sweat, breath and even blood (11, 33, 50) . In view of this,  study by McCulloch et al. in order to accuracy of canine scent to distinguish cancer patients from controls revealed high sensitivities and specificities (51). Also, R. Ehmann et al. studied on 60 lung cancer patients and 110 healthy controls. In this study, detecting lung cancer by sniffer dogs with sensitivity of 90% and specificity of 72% were reported (52). Study by Joanna Rudnicka et al. demonstrated that among lung cancer patients, overall sensitivity and specificity of trained dogs detection was rthat detection of ovarian cancer by sniffer dogs with the sensitivity rate was 100% and the specificity rate was 98% (53). Jean-Nicolas Cornu and et al. prostate carcinoma release scent signature to urine. They proved that identification of smelling urine for identifying prostate cancer is possible by sniffer dog; As they stated in their report dog can correctly detected the samples of cancer in 30 of 33 cases (33).

In 2016, study performed by T. Craig Angle and et al. assessed potentiality of two sniffer dogs to recognize bovine viral diarrhea virus (BVDV) infected cell cultures and to discriminate cultured cells infected BVDV from uninfected cell cultures and from bovine herpes virus 1 (BHV 1) and bovine parainfluenza virus 3 (BPIV 3) infected cell cultures. They demonstrated that sniffer dogs could make different cell between cultures infected with BPIV3 and BVDV, BHV (14). Furthermore, researchers performed an examination on the VOCs release from cultured several microorganisms such as influenza A, influenza B, adenovirus, respiratory syncitial virus, and parainfluenza 1 virus, and four following bacteria: Moraxella catarrhalis, Haemophilus influenza, Legionella pneumophila, and Mycoplasma pneumoniae. They found out that the growth of viral and bacterial lead to unique VOC profiles. They concluded that microorganisms produce species-specific VOC by distinguished metabolisms (21). Also, in a cell culture model, researchers determined VOC produced by primary human tracheobronchial cells following infection with human rhinovirus different from uninfected cells (54). In this regard, sniffer dogs assist with African physicians to identify cancer and probably epidemic infectious diseases such as AIDS and TB (37). In another study, researchers examined the capability of sniffer dogs to identify *Clostridium difficile*. They reported that sniffer dogs had ability to identify *Clostridium difficile* in fecal samples and patients infected with *Clostridium difficile* with 100% estimation, not only in sensitivity, but also in specificity (55).

**Sniffer dogs and COVID-19 identification**

COVID-19 surges throughout the world, the most important step to prevent the spread of the SARS-CoV-2 is to identify infected people. Unpleasant happenings like these make it necessary for the performance of public health measures including contact tracing, quarantine, social distancing, and isolation, in order to reduce the burden of the disease (56). Rapid identification of individuals that described as asymptomatic carriers of SARS-CoV-2 is the diagnostic gold standard, these individuals are identified as super spreaders (57). Identify these asymptomatic individuals are important strategies to against the prevalent of the SARS-CoV-2. Swift and early laboratory detection of SARS-CoV-2 and screening of huge portions of the society should get attention certainly during the SARS-CoV-2 epidemic.

The significant instrument for diagnosis of pathogenic viruses is based on culture, nevertheless of being long, therefore, this method is not appropriate to the rapid laboratory diagnosis of viruses in correlation with acute infectious diseases (58). Probing viral nucleic acid with RT-PCR are recommended methods for detection SARS-CoV-2 in upper and lower respiratory tract. Molecular diagnostic methods based on reverse transcription (RT) can diagnose of virus RNA among patients who are infected to SRAS-CoV-2. Not only this technique has been demonstrated to be time-consuming, but also different agents such as great amounts of genetic mutation in RNA viruses, as well as mismatches of target sequences, primers and probes might lead to reduce the sensitivity and accuracy of this method and increase false -negative results (56, 59, 60). Detecting SARS-CoV-2 antibodies via serological tests are widely accessible (59). Chest computed tomography scans are another way to diagnose COVID-19 patients (58). Nevertheless, these methods have disadvantages, for example, its devices are not portable and also are needed specialists and medical centers to use this method, therefore is not possible to use it in public places.

Using a method to be rapid, precise, transportable, affordable and non-specific device can be effective for preventing the spread of COVID-19 disease by quickly diagnosis and detection asymptomatic SARS-CoV-2 infected individuals. In comparison with current diagnostic methods, the dog detection system has outstanding features such as phenomenal sensitivity, mobility inherently and trace an odor from target source and ability to move towards of it. Current diagnostic system no longer meet such criteria (14, 61). Table 1 summarized the benefits and limitations of using trained detection dogs.

Animal training is generally divided into two categories: associative or non-associative learning. Classical (i.e. Pavlovian) conditioning or operant conditioning are subsets of associative learning (62). The training method of operant conditioning for the sniffer dog is usually based upon positive reinforcement. This method is occurred via providing reward to the dogs with correct response (e.g. sitting) as soon as prosperous discovery of the target. However, in the absence of the detection of the target, withholding the reward is often used as a form of negative punishment (63, 64).

 Strong scientific evidence supports that dogs can play an effective role in controlling the prevalence of SARS-CoV-2 alongside medical staff. According to this concept, Paula Jendrny and et al. studied utilization of dogs for making differences in both COVID-19 positive samples and non-infected controls. They collected samples from saliva and tracheobronchial secretion of hospitalized COVID-19 patients. To prevent dogs and their handlers from becoming infected with the SARS-CoV-2 during training, they inactivated all samples by beta propiolactone (BPL). In this study they provided the samples to the dogs using a device called the Detection Dog Training System (DDTS; Kynoscience UG, Germany), which can present samples in a randomised automated manner without trainer interference. The device design prevents the physical contact of dogs with the samples and to exclude any visual signs that may have enabled further detection abilities. For each experiment, only one of the holes SARS-CoV-2 positive specimen was presented and negative samples were presented in the other holes. When the dog detects a positive specimen, a reward is given to the animal automatically. After eating the reward, the position of the specimens for the next trial run changes by the software of the device randomly and naturally, and as a result positive odor specimen is only available in one hole. Moreover, team members used to stand behind the dogs during the trial to minimize distraction in the experiment. This study reported the sensitivity and specificity of the sniffer dogs ability to distinguish respiratory secretions of SARS-CoV-2 infected patients from healthy individuals 82.63% and 96.35% respectively (2).

The presence of the virus (SARS-CoV-2) in the sweat secretions of patients with COVID-19 has been documented by a group of researchers. Sweat samples collected from 25 patients with COVID-19 were tested for SARS-CoV-2 RNA by Real-time Polymerase Chain Reaction (RT-PCR) method. They found that sweat samples taken from the foreheads of infected people do not contain any SARS-CoV-2 RNA. According to this concept, they concluded that transmission of SARS-CoV-2 via sweat of infected people with COVID- 19 is not possible unless be contaminated with other body secretions (65). Scientists at the National Veterinary School of Alfort and French-Lebanese University Saint Joseph evaluated the capability of trained dogs in discrimination between sweat specimens from COVID-19 positive symptomatic individuals (SARS-CoV-2 PCR positive) and those from COVID-19 asymptomatic negative people. This research comprised of overall 18 dogs and 198 sweats of armpits samples were gained from various hospitals. Personnel were trained to collect Sweat samples from the underarm area in a way to prevent samples from getting contaminated with their own odors. Essential precautions should be obeyed during collecting samples by personnel such as wearing two new pairs of gloves and full COVID-19 safety protection. Two various materials either gauze or polymer tubes were used as a sampling material due to the uncertainty about the most effective sampling substance. For 20 minutes the sampling substance was in contact with the skin. Hospital anti-UV sterile containers were used to store marked samples (collected from left or right underarm) , then kept into another plastic bag. Dogs were trained to sit in front of a cone containing the COVID-19 positive sample. In this study, 4-step procedures for training dogs are utilized: 1- Learning line-up work: all cones in the line-up are empty. When the dog smells empty cones, he receives a toy as a prize. 2- Remembering the smell of an infected sample with SARS-CoV-2: The dog will be rewarded when he can detect the odor of infected samples with SARS-CoV-2, whereas the rest of the cones in the line-up are empty. 3- Replacing empty cones with mocks: The dog will be rewarded when he can detect the odor of the infected samples with SARS-CoV-2, whereas the rest of the cones are mocks. 4- In the line-up, cones are included one infected samples with SARS-CoV-2, one or two mock(s), and COVID-19 negative sample(s). The dog training sessions lasted between one and three weeks. Training of the dogs took about one to three weeks. After the end of dog training sessions, the dog must be able to detect the positive COVID-19 specimen, which is located behind one of three or four olfactory cones; at least one cone contained a COVID-19 negative sample and the other cones contained mocks. The success rate per dog ranged from 76% to 100%. They concluded that there is numerous evidence that the scent of underarm sweat of individuals with COVID-19 is different, by which dogs can diagnose infectious people with the virus of SARS-CoV-2. Although, they stated that the study was limitations, including using some COVID-19 samples more than once and potentially confounding biases. As a result, these results should be authenticated in validation studies (12). A team of researchers from Florida International University (FIU) to presentation of the samples to the sniffer dogs, used face coverings worn by individuals who tested positive and negative for the virus, as well as utilization of ultraviolet C light in order to inactivate the virus. The result of their studies indicated that the accuracy of detection of the dogs can be more than 90 percent (66). A group of researchers with the use of four COVID-19 sniffer dogs monitored passengers in terms of infected to SARS-CoV-2 at Finland’s Helsinki airport. The passengers wipe their skin with a wipe according to the instructions, then drops the sample into the container provided for it. The dog and its trainer are behind a wall, where the dog sniffs the given sample. When the dog detects the COVID-19 positive samples, starts lying down or pawing. The results of this study showed that in 83% of cases, sweat samples infected with SARS-CoV-2 were correctly identified by dogs. Some dogs had 100% accuracy (67). Researchers Saint Joseph University, screened 1,680 passengers at airport in Lebanon by medical detection dogs. Dogs diagnosed 158 COVID-19 cases that were confirmed by PCR tests. According to unpublished results, the findings of this study proved that sniffer dogs can identify negative results with 100% accuracy, and in 92% of cases, dogs were able to identify positive samples (68). All these results lead us to believe that if we can train canine to diagnose odors related with COVID-19, the dog can be used in frontline operational environments and completed the tracing tool progress for diagnosis of SARS-CoV-2.

**Conclusion**

COVID-19 continues to lead illness and fatality. Timely and quick detection of the infectious of COVID-19 is important issue to for control infection and treatment among populations for preventing transmission (69). Current detection techniques are utilized to control infection dependent in the complex of diagnostic specimen from people or polluted environment, transferring of specimen to a lab, and subsequent testing in laboratory, leading to considerable postponement in times of response. It has been determined that communities where mastering their outbreak and had ability to preserve the small number of infected individuals, require to carry out fewer tests to control the outbreak accurately, than those communities where the virus has become more prevalent. For the same reason, swift action of patient identification process, diagnosing and treating disease will be more effective way to reduce the prevalence if carried out earlier, when coronavirus has fewer infectious (70).

Utilizing of an extraordinary sensitive and easily mobile technique that can be deployed in areas of strategic interest for SARS-CoV-2 transmission such as borders, crowded areas including sport stadiums, airports would be suitable to help prevent more expanding of the virus or more prevalence.

Probably each pathogen in individual can influence the released VOC signals/profiles. Changes in VOCs associated with each disease can be used as diagnostic olfactory biomarkers. Tracing changes in VOCs associated with certain disease can be used as unique fingerprints; also it can provide clues for early disease detection in order to prevent further outbreaks of infectious diseases (25, 71). Innumerable scientific articles indicate the importance of sniffer dogs in a spectrum range of applications (72). Forasmuch as canine can be trained fast, they can be used as a potential new non-invasive, early warning measure to diagnose SARS-CoV-2 in the future. dogs can differentiate between specimens of persons with COVID-19 and non-infected controls via training. They could be employed as an alternative or addition laboratory testing to helping to prevent further spreading of the virus or be deployed at strategic locations to prevent entry and transmission of disease (2, 14).

Although the olfactory test appears to be a promising tool for the detection of COVID-19, we will face various challenges in using this animal. One of the most important issues to manage the recent epidemic to distinguish whether the dog can efficiently differentiate between SARS-CoV-2 infected patients who are hospitalized, and pre-symptomatic COVID-19 patients, or asymptomatic carriers (73). Therefore, future study have to concentrate on capability of canine for diagnosis of dilution of specimen, various disease phases including pre-symptomat, asymptomatic, chronic and acute clinical cases, and differentiation to other infection diseases or pathogens such as influenza, respiratory syncitial virus, adenovirus, rhinovirus. Since one way to prevent and control the spread of the virus is to avoid contact with infected specimens, another challenge of using this method is the possibility of dogs becoming infected with SARS-CoV-2, recently new studies have reported that dogs are infected to SARS-CoV-2 (74). So should be more studies on the risk of dogs becoming infected with this virus and transmission to humans. This problem could be decreased via utilizing measures of prevention organizational including avoiding surroundings environment of patients and physical contact with them (14). On the other hand the olfactory test depends on various factors including environmental conditions, such as relative humidity and also intercurrent infections or allergies, given that the dog is a living creature may be influenced by these condition (17, 75).

In conclusion, the concept of the olfactory test is a promising way to detect COVID-19 at an early stage. Future research should cause increases our deeper knowledge regarding the metabolic origins of secreted volatile metabolites from affected organs and further levels of information about analysis of VOCs, to the extent that the olfactory test can be utilized as a clinical screening instrument for COVID-19.

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 Table 1.The Advantages, disadvantages and limitations of using trained detection dogs.

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| --- | --- |
| Advantages of use of trained detection dogs | Disadvantages and limitations of use of trained detection dogs |
| Mass detection of infected people (2). | Extensive studies are needed to identify pre-symptomatic COVID-19 patients and asymptomatic carriers (2). |
| Dogs can examine thousands of samples or scan large surface areas (14). | Dogs could be infected with SARS-CoV-2 and could potentially play a role in viral spread (2, 55). |
| Detection dogs have an ability to search for unique odor patterns in field conditions amidst substantial “odor noise” (i.e., varied and/or strong odors) (14, 22).  | Disease-related VOC are largely unknown (76, 77).  |
| No need for trained personnel and laboratory instrumentation (14). | The need to study dogs ’attitude towards differentiating COVID-19 from other types of respiratory diseases (12). |
| Dogs are mobile and widely available (14). | Certain foods, drinks, or drugs may interfere with the odor of disease related compounds (31, 75).  |
| No need for surveillance methods processes rely on the collection of diagnostic samples from individuals, transportation of samples to a laboratory, and laboratory testing (14) | The qualification of the dog trainer, training time and number of different training samples (11). |
| Dogs are adapted to difficult work environments (14). | the choice of the dog breed, due to the polymorphism of the scent receptors (16, 78). |
| Non-invasive diagnostic method (5). | The dog is a living creature that gets hurt and may be influenced by intercurrent infections or allergies, which may reduce the quality of the olfactorial test conditions (75).  |
| Immediate identification of infected people. | Environmental conditions, such as relative humidity and barometric pressure can have direct impacts on olfaction, in addition to the impacts those factors have on the generation and movement of odor itself (17). |
| Affordable method of diagnosing COVID-19 (79). |  |