Collection and disinfection of forensic biological specimens in five cases concerning COVID-19 in Guangzhou, China

Xingyi Yang a,1, Quyi Xu a,1, Hong Liu a, Jichao Xu a, Dian Yang a, Cheng xiao a, Huiying Hu a, Yunyun Liu b,2, Chao Liu a,2,*

a Guangzhou Forensic Science Institute, Guangzhou, China
b Department of Neurology, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China

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ABSTRACT

There have been many cases of pneumonia caused by novel coronavirus infections in China and around the world. This will inevitably lead to a rise in the number of patients. At the present time, clinical and forensic autopsies have given guidance and explanations in relation to the problem of COVID-19 transmission and defense. However, less attention is paid to the handling of COVID-19 biological samples in forensic practice. Particularly, COVID-19 can survive on some surfaces for days. Since there were many cases involving COVID-19 during the epidemic, this article shares the methods and strategies for handling such inspection materials and the biological samples related specifically to COVID-19 cases.

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1. Introduction

Presently, the novel Corona Virus Disease 2019 (COVID-19) has overwhelmed the globe. With the onset of the epidemic caused by COVID-19, as of June 9, 2020 the number of confirmed COVID-19 cases in China had reached 83,046, including 4634 deaths. The cumulative number of international infections was at this time 7,039,918 of which 404,396 of these were deaths related to the COVID-19 infection, but also, to estimate whether biological samples can still extract high-quality DNA and obtain effective STR typing. Most DNA extraction laboratories do not currently meet the protective conditions of clinical laboratories and autopsy laboratories. The issue of how to deal with biological samples to achieve the protection from the transmission of COVID-19 has been barely addressed. The research presented here is based on the current understanding of COVID-19 in June 2020 and is subject to change as more information is released.

In forensic biology, we often have to use different biological samples, including human saliva, blood, tears, vaginal secretions, organs and exfoliated cells. Studies have shown that COVID-19 can be detected in pharyngeal swabs, perianal swabs, blood, visceral slices, masks used by patients and elevator buttons [3,5]. COVID-19 was detected in the saliva samples of 91.7% (11/12) of patients [6]. Especially during the epidemic, medical personnel cannot avoid being exposed to biological samples from patients with confirmed or suspected COVID-19 infection. In addition to this, a major problem in the extraction of biological samples from COVID-19 related cases is not only to help prevent the forensic staff from the COVID-19 infection, but also, to estimate whether biological samples can still extract high-quality DNA and obtain effective STR typing. Most DNA extraction laboratories do not currently meet the protective conditions of clinical laboratories and autopsy laboratories. The issue of how to deal with biological samples to achieve the protection from the transmission of COVID-19 has been barely addressed. The research presented here is based on the current understanding of COVID-19 in June 2020 along with case numbers from March. Cases concerning infected specimen collection and the
processing methods are summarized below.

2. Methods

2.1. Cases

The collection process of these biological specimens requires close contact between staff and patients, which causes a high risk of transmission of the virus to us. Therefore, before biological sample collection, body temperatures and background of contact in the epidemic area must be well known. Unless the case was urgent, we would wait for a few days before we handled biological samples relating to COVID-19 suspected infected patients that had a fever. If the body temperature is lower than 37.3 °C, we would choose the level of protection according to whether the patient was within the epidemic area (Fig. 1).

Case 1 concerns the COVID-19 patient who died in a hospital, that was initially thought to be suicide. To rule out homicide, her bloodstains from the initial point of the fall, exfoliated epithelial cell from the initial falling point, and vaginal swab samples were collected.

Case 2 concerns the COVID-19 old man in his house, who was assumed to commit suicide by hanging. There were some suspicious bruises found on the arm that was brought to our attention. To rule out homicide, his blood sample and exfoliated epithelial cell from rope used to hang him were collected.

Case 3 concerns the bottle in the Infectious Diseases section of a hospital where the owner was accused of stealing three mobile phones and one laptop.

Case 4 concerns an intentional injury, in which a mentally disturbed young man with COVID-19 bit a nurse’s face and caused her to bleed, during his treatment in an Infectious Disease Hospital. To collect the evidence in this intentional injury case, the buccal swab of this manic man and exfoliated epithelial cells left the suspect from the nurse’s bite wound were collected.

Case 5 concerns the deceased that is suspected of having COVID-19 whose organ was found in Pear River, China. To verify his identity, his viscera was collected.

2.2. Sample collection and preparation protocols

Before collecting biological samples on-site, 75% alcohol was sprayed on-site for disinfection, and the specimens were subjected to ultraviolet radiation for 30 min. The biological samples were then packaged in multi-layer bags.

The biological samples sent to the inspection laboratory were placed in a fume hood first and then the thermostat was set to 56 °C for 30–40 min (Fig. 2). After this process of disinfecting of the biological evidence, it was labeled as “sterilized”.

The operator wore a scope, N-95 mask and ULTITEC 3000T protective clothing and prepares tweezers and scissors. Upon entry of the ventilation room, 5% hypochlorous acid was used to wipe the table. Every time a layer of a packaged bag was opened, 75% alcohol was sprayed. The cotton swabs used were sprayed with 75% alcohol spray before wiping the inspection materials.

2.3. Preprocessing

The biological samples were transferred to an Eppendorf (EP) tube and sample pre-processing was performed using the Promega Casework Extraction Kit. The extraction mix was prepared by adding 430 μl of Lysis Buffer, 10 μl of 18 mg/ml Proteinase K and 10 μl 2 M dithiothreitol (DTT) for a final volume of 450 μl per sample for tissue samples.

2.4. DNA extraction of samples

This process involved the isolation of the DNA from the cell nucleus. In the process of DNA Extraction, each sample was incubated at 56 °C for 30 min and centrifuged at the maximum speed for 2 min at room temperature. The CW Spin Baskets were then discarded, and the centrifugal Lysis Buffer was added to each tube containing extract. Following thorough mixing, the samples were added to the Maxwell® FSC Cartridge. DNA was extracted from these samples using the DNA IQ™ System, (Promega, Madison, WI, USA), and the instrument protocol was initiated according to manufacturer instructions. The lysate was purified using reagents and components in the Maxwell® FSC DNA IQ™ Casework Kit. All samples were eluted to a final volume of 50 μl.

2.5. DNA quantitation

The quantity of DNA isolated with this approach is based on the number and capacity of the magnetic particles used. The extracted DNA was quantified using the Quantifier Human DNA Quantification Kit and the 7500 real-time PCR system (Life Technologies).
2.6. PCR

STR analysis was carried out using a 25 µl reaction volume with the PowerPlex 21 kit (Promega, Madison, WI, USA) [7]. Amplification reactions generally contained 0.5 ng DNA in 25 µl and used 28–30 cycles as described in the PowerPlex 21 System technical manual.

2.7. STR analysis

DNA fragments were denatured and size-fractionated using capillary electrophoresis on an ABI 3500xl Genetic Analyzer. Injection time set at 5s and the injection voltage was set at 1.2kVolts, the run time was 1500s. Fragment nomenclature was processed and analyzed using the Sequence Detection System version 1.4 software package (Thermo Fisher Scientific). Peak amplitude thresholds were limited to above 50rfu. Cut-off value of allelic ladder spike was 0.2. SQ weighting was fix to 0.5.

After sample processing, the laboratory surfaces were wiped with 5% hypo-chloric acid and 75% alcohol were sterilized further by ultraviolet light. All of the consumables were discarded in a bucket marked with the COVID-19 logo for appropriate disposal. During the entire process of the experiment, the lid of the EP tube was not opened except for the addition of the lysis reagent, Maxwell automated extraction. To produce aerosols carrying viruses, only these two links can be produced.

3. Results and discussion

All the high template DNA (above 0.1ng/ul) biological samples such as blood samples, buccal swabs, and vaginal swabs in cases 1–5, have obtained full profile STR-typing. The DNA profile from the bite mark (outside of nurse’s mask) in case 4 shows a single profile of the suspect because of the strict adherence to wearing personal protection by the nurse. Biological components from the outside of nurse’s mask shared 17 STR profiles with the suspect and the LR ratio is 8.39 × 10^{27} (Fig. 3). However, it is not ideal for low template DNA (LT-DNA), where the quantity is lower than 0.005 ng/µl, such as epithelial cells collected at the jumping point in Case 1 and epithelial cells on the rope in Case 2 both in which most of the loci were lost. Even so, the STR typing of low template DNA samples is not subjected to heating or ultraviolet treatment, the STR profile will likely be lost. However, for the high template DNA biological samples, there is no loss of STR typing sites. Judging from the effect of the above five cases on processing samples, the safety precautions did not have any significant impact on the STR typing of biological samples (Box1). We still need more trials to conclude whether the above-mentioned safety measures lead to a statistical difference of detection rate in low template DNA biological samples. In following the protective measures described above, our colleagues handling the test materials did not develop COVID-19 after two weeks.

Usually, 60 °C for 15–30 min is enough to reduce SARS-CoV in cell-free plasma [8]. In another study, heating at 56 °C for 25 min reduced the MERS virus by 4 times (log_{10} TCID50)/mL [9]. In addition, the internal research of inactivated coronavirus mainly focuses on heating and solvent/detergent (S/D) treatment [10]. After 30 min of treatment with S/D produced by Octaplas, the virus was reduced by more than (5.75 ± 0.3 log_{10} TCID50)/mL [11]. However, the WHO has not released data on how long the virus, COVID-19, stays on surfaces. The emergencies facing the world today require urgent and effective measures to protect people who are more susceptible to COVID-19. The protective measures that we have adopted are based on the knowledge of other viruses. The virus survives on the surface for a short time. After leaving the host, the spread of the novel coronavirus will slow down until it loses effect. When detecting respiratory viruses, including coronavirus, the consistency of saliva and naso-pharyngeal specimens are as high as...
90%. Positive virus cultures indicate that saliva contains live viruses that may spread [12]. COVID-19 and its sister branches SARS-CoV and Middle East Respiratory Syndrome (MERS)-CoV have been confirmed to be transfusion transmissible [13]. Usually, coronaviruses are vulnerable to acid-pH, basic-pH, and heat but seem to be more stable at low temperatures [14]. Wang Lunan’s group summarized the different methods of SARS-CoV and MERS-CoV inactivation and laboratory tissue culture [10]. COVID-19 can be detected on copper for up to 4 h, on plastic and stainless steel for up to two to three days, and on cardboard for up to 24 h (https://www.bignewsnetwork.com/news/264345044/new-coronavirus-can-survive-on-some-surfaces-for-days-study).

During the epidemic, medical doctors and forensic pathologists have faced direct occupational exposure. Similarly, we cannot evaluate whether COVID-19 was present in the biological samples that have not been destroyed. Many infected people are asymptomatic, but they can also become a source of infection [4]. These asymptomatic people also increase the risk of contracting the virus during the outbreak. The Royal College of Pathologists recommends sending standard samples (such as respiratory swabs and tissue samples) to the local microbiology department according to the autopsy practices associated with possible cases of COVID-19 for differential diagnosis of pathogens. However, it is difficult to require all domestic DNA extraction laboratories to achieve the same biological safety standards as microbiology departments. But the crime and biological samples collection continues. Therefore, when collecting on-site evidence or processing biological samples in the laboratory, we have to follow the standards for protection from coronaviruses that are used in clinical and forensic research to reduce the health hazards to staff.

### 4. Conclusion

COVID-19 has a longer failure time than other coronaviruses as previously mentioned. With reference to the protective measures taken by healthcare professionals who had contact with COVID-19 patients during the pandemic, these protective measures could be useful in reducing the risk of infection. It only has a negligible effect on STR typing following our recommendation to preprocess COVID-19 related high template DNA forensic biological samples. In the coming months, a lot of new information about COVID-19 will be provided, which will enable us to make more detailed decisions concerning safe collection and disinfection procedures of forensic biological samples.

### Declaration of competing interest

We declare that we do not have any interest that represents a conflict of interest in connection with the work submitted.

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