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Laboratory biosafety measures of SARS-CoV-2 at containment level 2 with particular reference to its more infective variants

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ABSTRACT

The novel betacoronavirus (Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2) is a pathogen that causes deadly respiratory disease named coronavirus disease 2019 (COVID-19). The incidence of this disease has increased in the last few months affecting 257,832,881 people in 221 countries and 51,68,069 deaths worldwide according to Worldometer at 04:03 GMT on November 22, 2021. Thus, the emergence of this disease creates a challenge for health care providers in handling this pathogen and reducing its risk of transmission. In developing countries, this virus is treated in biosafety level 2 laboratories, where a high concentration of pathogen can easily affect the laboratory staff and cause the spread of this disease. Based on the epidemiology and characteristics of the SARS-CoV-2 virus already discussed in recent studies, we will provide biosafety guidelines and suggestions for safe handling and transportation of the SARS-CoV-2 virus in dealing with the current pandemic situation with a focus on increased infectivity of emerging new variants.

1. Introduction

Coronavirus disease 2019 (COVID-19) outbreak is the third coronavirus pandemic in the last 20 years after other respiratory diseases like Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS) [1]. This disease was initially reported in Wuhan, China, in December 2019 [2], having an unknown etiological agent. The etiological agent was then identified and named the Novel Beta Coronavirus (Severe Acute Respiratory Syndrome Coronavirus 2, SARS-CoV-2). After more than 100 million people were affected by this disease, which declared a global pandemic on March 11, 2020 by WHO [3] Although in initial reports, coronavirus disease was considered as less severe than respiratory disease SARS, there is a progressive increase in coronavirus disease cases especially in Italy (13.53%) and Spain (11.07%) in 2020 and now around the world [4].

The risk assessment of novel beta coronavirus was based on the available information reported from various studies regarding etiological agents, characteristics, and molecular features. It is classified in Risk Group 3 organism by WHO and CDC [5]. Initially, it was diagnosed by direct pathogen detection. Nowadays, polymerase chain reaction (PCR) is used for viral genome detection in biosafety level 2 laboratories [6].

An essential aspect of COVID-19 is that the health care professionals who work in COVID-19 handling laboratories are at the highest risk of this deadly disease [7].This statement was proved by studies reported from China and Italy that 3.8% of infected Chinese people were from health professionals, increasing up to 63% [8]. In Italy, the total figure of COVID-19 cases is as high as 10.7% among health care professionals [9]. This alarming situation needs to be addressed by biosafety considerations in laboratories. This review aims to highlight the current biosafety guidelines in clinical laboratories for preventing and controlling the spread of COVID-19.

2. Leadership/Administrative controls and personal protection equipment

The laboratory where COVID-19 cases are handled is a high-risk area for the spread of laboratory-acquired infections. The biosafety rules and regulations are selected by leaders based on risk assessment study of coronavirus [10]. The pathogen is considered in Risk Group 3 [10] according to their characteristics and genome analysis. Elimination of every risk and addressing the laboratory personnel is of utmost importance. The laboratory management staff and supervisors are considered leaders. The leaders should formulate proper SOPs for the workers keeping in mind the practical issues of the workers and workplace. The leaders should arrange awareness training sessions for the workers about the risk and safe handling of COVID-19 samples. They should train their staff about the proper use of Personal Protective Equipment (PPE) according to their workload and experiment context

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because the selection of inappropriate PPE can have a disastrous effect on the health status of workers (Table 1) [11]. These responsibilities of leaders will strengthen administrative management and control, leading to the practical application of biosafety guidelines in the laboratory.

3. Lab biosafety guidelines for COVID-19 sample analysis

Specimens are obtained from blood, respiratory tract, nasopharyngeal swabs, body secretions, body fluids, feces, and sputum. These all might be considered infectious until the result is reported [13].

Assessment specific to the site and activity must be performed to identify and reduce the threats of biological hazards. The estimates of risk and their way of reduction depending on the following [14] that are the methodologies being performed, determining the threats being involved in methodologies being performed, the level of competency the person is dealing with specimen the apparatus of lab and facilities being provided and the availability of resources. The specimens being handled may have highly infectious material, so standard precautionary measures must be followed the use of personal protective equipment that is gowns, lab coats, gloves, eye protectors, and hygiene of hand [15]. For disinfection of working areas and lab management, lab practices on a routine basis must be followed. The following procedures are performed for regular diagnostic testing, using standard preventive measures at the BSL-2 lab when [16] automated analyzers and instruments are used initial samples are to be processed, fixed smears are to be stained and microscopically analyzed, bacterial cultures are to be examined, formalin-fixed inactivated tissues pathologic examination and formalin-fixed processing. A nucleic acid that is extracted is prepared and analyzed molecularly. Packaging, sealing, and final transportation of sample and the sample must be in a disinfectant container. In addition, specimens must be in the inactivated form that is in nucleic acid extraction buffer. Electron microscopic grid analysis is performed.

4. Specimen collection, storage, packaging, and shipping

As per the World Health Organization's recommendations, all specimens collected for laboratory investigations should be viewed as potentially infectious, and hence the individuals tasked with sample collection should use appropriate PPE [17]. If the aerosol-generating procedure is followed for sample collection, protective as a NIOSH-certified N95, an EU standard FFP2, or the equivalent is worn by personnel [18]. Sample handling personnel must be trained in the decontamination of spillage. After the sample is received, it should be placed in leak-proof specimen bags that are secondary containers containing a separate sealable pocket for sample keeping: a plastic biological hazard bag with proper labeling on specimens container and lab request form [19]. Biosafety guidelines need to be followed in handling and transporting depending on the type of specimen being managed. Pneumatic tube systems should not be used in delivering the sample. Complete biodata of the patient must be entered in lab request form name, date of birth of the patient, age, etc. and lab must be notified as soon as possible when a sample is transported.

Table 1
The selection of personal protective equipment.

Containment Levels	PPE [11,12]
Level 1	Lab coveralls/ uniform, surgical mask, gloves
Level 2	Lab coveralls, gloves, safety glasses, face shields, eye protector, full covered footwear
Level 3	Solid front gowns, scrub suits, respiratory protectors, head covering, shoe covering

To reduce the chances of breakage and spill while transferring the materials from one lab to another, they should be considered biological threats kept in individual containers. The surface of the specimen should be disinfectant when it is out from BSC [19].

5. Receiving and processing of COVID-19 samples

Containment level 2 biosafety guidelines should be followed in the handling of COVID-19 samples. The laboratory receiving staff should wear proper PPE like lab coveralls, gloves, N95 masks, face protectors. They should check that the sample is appropriately coded and labeled according to their pathogenicity and prevent the storage material's integrity. If the storage material is damaged or leaked, the staff should immediately follow the emergency guidelines according to containment level 3 [20]. The surface of the storage material should be disinfected by alcohol, sodium hypochlorite, or any other disinfectant before handling and processing the sample [21]. The sample should be opened and processed in biosafety cabinet class II (A1/A2 or higher containment equipment) because they give personal, product, and environment protection [22]. Sealed rotors or cups should be used in centrifugation which is filled and emptied in a biosafety cabinet. Wear [23]an eye protector, mask, and gloves and wait until 15 mins after completing the centrifugation process. Check the centrifuge for any damage or spill of the sample, clean it with 75% ethanol and remove the waste in a separate coded/labeled container [23]. The biosafety cabinet must be validated before working with COVID-19 samples. If the sample needs time in diagnosis, it must be stored in a separate area or refrigerator to avoid contamination [17].

6. Biosafety measures in diagnostic tests of COVID-19

The following procedures make COVID-19 diagnosis:

In the pre-analytical phase, the sample collected from the respiratory tract at the proper site and time is essential for diagnosing novel beta coronavirus. PCR remains the best and most reliable technique in the analytical phase, and antibody-based diagnosis is also used. In the post-analytical step, the results are analyzed and compared using both molecular and serological procedures [24].

According to WHO Biosafety considerations in the pre-analytical phase, all samples should be considered as infectious, and proper protective equipment must be used for obtaining these samples. These PPEs include an N95 mask, face covers, and full-toed shoes.

In performing procedures like centrifugation, which can produce aerosols, a respiratory protector must be used [25].

Biosafety measures in the analytical phase reduce the chance of aerosol production in procedures like sample dilution, centrifugation, smear fixation, fungal/ bacterial media preparation, and vortexing. These procedures must be performed in biosafety cabinet class II, and containment level 3 guidelines will be used for personnel protection. COVID-19 samples must be kept in different positions and keep the lid closed tightly. Use a separate analyzer for each sample to avoid mixing of COVID-19 sample with other specimens. In automatic analyzers, there is a risk of breakage of vessels and spillage of the hazardous pathogen, so laboratory staff should check the integrity of containers before the experiment. For this type of spillage, enhanced containment level 2 protection is recommended [22,26].

In the case of real-time PCR, nucleic acid extraction, reaction mixture preparation, and amplification should be performed in different areas. In nucleic acid, the extraction sample is kept in lysis buffer to extract viable coronavirus but keeps the viral RNA integrated. Before inactivation, the staff must use safety goggles, an N95 mask, face, and respiratory protectors. The amplification process generates amplicons that are not hazardous by themselves but can contaminate the surrounding area, leading to false-positive results if it reaches to patient swab sample at the time of the test. Therefore, it should be per-

formed in a biosafety class II cabinet or class III if a high pathogen concentration is available [27].

The working area must be disinfected before and after the experiment. All the equipment should be validated before use. The used PPE and laboratory waste must be autoclaved before removing from the laboratory. The laboratory air pressure should be set at negative pressure to avoid aerosol contamination. Leaks, injuries, or reactions to hazardous agents (COVID-19 samples) must be reported to the laboratory supervisor. A written record of all emergencies must be preserved [28].

7. Biosafety measures and methodologies after testing

Experimental studies have shown that coronavirus remains viable after releasing from a sample on plastic surface and stainless steel for about 72 h and remains viable in air for three days after aerosol generation in experimental analysis. Depending on the surfaces, coronavirus may remain infectious for about nine days at temperatures ≥30 ˚C (86˚F). Disinfection of surfaces with 0.5% hydrogen peroxide solutions, diluted household bleach solutions (0.1% sodium hypochlorite), or alcohol solutions (i.e., >70% ethanol) may be used for inactivation of coronaviruses <1 min [29].

8. Disinfection

8.1. Incineration

The high-temperature combustion range (800 ˚C –1,200 ˚C) can potentially kill pathogens and burn almost all of the organic matter (up to 90%). Therefore, COVID-19 wastes are subjected to incineration at a temperature >1,100 ˚C. Treatment with flue-gas is also facilitated.

8.2. Using alternative thermal techniques

Biohazardous contents potentially containing COVID-19 can be accurately dealt with using alternative thermal technologies, i.e., (i) high-temperature pyrolysis technique and (ii) medium-temperature microwave technique.

8.2.1. High-temperature pyrolysis technique

Pyrolysis is the most crucial technique that employs high-temperature incineration (540 ˚C – 830 ˚C). Different variants of this methodology are induction-based pyrolysis, plasma pyrolysis, pyrolysis-oxidation, and laser-based pyrolysis.

8.2.2. Medium temperature microwave technique

The temperate range of temperature (177 ˚C – 540 ˚C) employed in this technique results in polymerization or degradation of organic matter occurs by breaking organic matter. High energy microwaves and an inert atmosphere is applied.

8.3. Chemical disinfection technique

Pre-treated COVID-19 waste in combination with mechanical shredding is subjected to chemical disinfection. During shredding, high efficiency particulate air (HEPA) filters are used to pass exhausted air to prevent the formation of an aerosol. The broken-down material is mixed with a fixed volume of disinfectants at a negative pressure for a while. The chemical treatment of COVID-waste involves chlorine- and non-chlorine-based systems. Disinfectant media used in such systems is NaOCl or ClO2. Peptide links and denatured proteins are oxidized by chlorine negativity, and denaturing proteins penetrates cells at neutral pH. Dioxins, Halo acetic acid, and chlorinated aromatic compounds are released by NaOCl chemical disinfectants. ClO2 is a strong biocide and its use greatly increased. It yields less toxic and nonreactive products after degradation. The non-chlorine-based treatment system includes hydrogen peroxide.

8.4. Chemical solutions

Inactivation of SARS-CoV-2 is carried out by isopropanol (>70%), povidone iodine (>0.23%), ethyl alcohol (>75%), and formaldehyde (>0.7%) [30,31].

9. Standard safety measures on the disinfection process

The causative agent of laboratory-acquired infection spread is mainly COVID-19 waste. Several preventive measures need to be followed. The neighboring environment (including floor, tables, working area, etc. of the contaminated area) must be sprayed with 2 g/l of chlorine for a minimum of 30 min. COVID-waste must be decontaminated with double-layered, tightly closed yellow bags [30,31].

10. The accidental exposure management plan

ASOPs-related emergency plan must be established to reduce exposure and accidents, providing the SOPs to deal with such circumstances. The laboratory staff must be trained in following emergency guidelines, and all equipment must be validated according to their need in an emergency. Medical facilities like eyewash kits, bandages must be available and insufficient amounts for the laboratory staff. They should be checked for their quality and expiry regularly. In case of accidental spillage of COVID-19 samples, the guidelines will switch from containment level 2 to level 3 protection. More safety is required in COVID-19 sample spills outside the biosafety cabinet. The laboratory staff should immediately leave the area and label the door with an indicator to warn others about the spillage. Wait outside for 30 mins till the aerosols settle down. All the equipment should be disinfected after the spillage, and gaseous disinfection is also done in the affected area. Trained staff will be required for this operation [32].

11. Conclusion

This article provides a detailed description of biosafety guidelines in handling COVID-19 samples around the world. Although from country to country, there must be some changes or enhancements in guidelines according to their environment and laboratory setup. According to all the previous reports, SARS-CoV-2 is considered as a Risk Group 3 organism. Therefore, proper biosafety guidelines must be followed to handle, process, and transport these samples to reduce the risk of transmission in health professional staff. Government or other related authorities should ensure the application of these guidelines in the laboratories. Furthermore, they should provide special training and awareness programs for health care workers to properly use PPE and biosafety measures according to their work context and working environment.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Author contributions

Wafa Naeem: Writing – Original Draft. **Habiba Zeb:** Writing – Original Draft. **Muhammad Ibrahim Rashid:** Writing – Review & Editing.

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