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SARS-CoV-2 and Biosafety in Laboratory

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Abstract

The newly discovered coronavirus (Severe acute respiratory syndrome coronavirus-2, SARS-CoV-2) is the causative agent of the ongoing pandemic. Broad arrangement has been done to minimize virus spreading among population and to control the worldwide outbreak. Expanded biosafety measure specifically with respect to the work require using SARS-CoV-2 in laboratory (lab.) and a special consideration should be taken to protect researcher and lab. worker during handling of specimens. Therefore, the aim of this review is to help the scientists, researchers, lab. staff and biosafety specialists to respond to the current coronavirus disease (COVID-19) through discussion of effective biosafety practices that can prevent laboratory acquired infections and to lessen the spread of infection into community and environment.

Keywords : Coronavirus, COVID-19, biosafety, research, laboratories

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List of abbreviations: BSC = Biological safety cabinet, BSL = Biosafety level, COVID-19 = Coronavirus disease 2019, HEPA = High-efficiency particulate air, Lab. = Laboratory, PPE = Personal protective equipment, SARS = Severe acute respiratory syndrome

1- Introduction

Coronavirus is one of the respiratory viruses that infect human. Outbreaks of coronaviruses have been previously reported; in 2003, the severe acute respiratory syndrome (SARS) and in 2012, the Middle East respiratory syndrome (MERS) were considered as a serious public health threat. In 2019, a novel coronavirus was emerged in Wuhan, China that is now named SARS-CoV-2 that cause coronavirus disease-19 (COVID-19) [(1)]. World health organization (WHO) announced that COVID-19 is an international health emergency on Jan. 30, 2020, the natural history of this disease is still not fully known, however, infected person was shown to be able to transmit infection before they become symptomatic, and days after recovery [(2)]. An increasing number of cases is recorded every day, override 38 million cases worldwide were recorded till the time of writing this manuscript [(3)]. Researchers and laboratories accepting specimens from patients under investigation for SARS-CoV-2 must be aware about proper handling of these specimens poses a risk of exposure or infection, which could seriously affect the staff and the community. SARS-CoV-2 has been classified as a hazard group 3 human pathogen by the Centre for Biosecurity [(4)].

2- Types of transmission and precautions

Understanding the SARS-CoV-2 mode of transmission can help to select the most suitable personal protective equipment (PPE) and disinfection procedures. SARS-CoV-2 transmission occurs mainly via droplets

(aerosol) and also contaminated surfaces (fomites). In addition, the level of exposure i.e., the amount of virus that acquire by healthcare worker has an influence on the severity of disease. Transmission through direct aerosol spread from a patient to the health worker via talking, coughing or sneezing, however, a safe distance of approximately 2 meter is recommended to reduce the virus spreading [5]. van Doremalen et al. (2020) found that SARS-CoV-2 can be detected in aerosols for up to 3 h with a decrease in viral particles concentration from $10^{3.5}$ to $10^{2.7}$ TCID50 /L of air [6].

Also, SARS-CoV-2 indirect transmission has been reported via environmental, therefore contaminated surfaces can rising the risk of contact transmission and nosocomial infection [7]. van Doremalen et al. (2020) found that SARS-CoV-2 was more stable on plastic and stainless steel than on copper and cardboard and can detected up to 72 h on these surfaces, however, the virus concentration was decreased from $10^{3.7}$ to $10^{0.6}$ TCID50/ml of medium on plastic and stainless steel after 72 and 48 h, respectively. While, no viable SARS-CoV-2 was detectedon copper and cardboard after 4 and 24 hr, respectively [6].

3- Risk Assessment

There are different hazards may encounter in lab. including sharps, chemical, biological and radiation. A Biohazard is a biological agents or substance that can cause human or animal disease and may present a hazard to the health of an individual working with the agent. Biohazard include microorganisms such as bacteria, viruses, parasites and fungi. Biohazard also include samples from humans or animals that an individual will work with either for diagnosis or research. The infection control measures including both administrative and environmental control measures. The administrative strategies are creating policies, plan for emergency and provide instruction and trainings, while the environmental strategies include providing good ventilation, building up isolation rooms with negative pressure, and creating frameworks for sterilization, disinfection and biohazard waste disposal [8].

Several organizations developed guidelines that discuss methods for handling and processing SARS-CoV-2 suspected specimen [4, 9,10]. The biosafety level should be determined depending on the risk assessment and the hazard of pathogen, detection method or experimental technique used by authorized personnel [11]. Risk assessment is the detection of hazards and risks that could adversely affect individuals, and/or the environment, their likelihood and consequences. These assessments help to define the risks and include steps, protocols and controls to minimize the effect on work activities [12]. The results of this process may be expressed in a quantitative or qualitative fashion. Risk assessment is a part of a broader risk management strategy to help reduce any potential risk-related consequences and it need to be carried out before work/activity starts. All biological agents require risk assessment and approval before work can proceed. Work would be blocked if the suitable facilities not provide adequate protection to staff, students and environment [12,13].

4- Building facilities

Facilities for undergoing research for SARS-CoV-2 should strictly implement the appropriate biosafety practices inside the lab. Both biosafety level-2 (BSL-2) and biosafety level-3 (BSL-3) are the required lab. set-up when handling SARS-CoV-2. All inactivated specimens should be performed in a validated biological safety cabinet class-II (BSC-II). The diagnosis methods that not require growth of the virus, such as amplification of virus nucleic acid or sequencing and another routine lab. tests such as biochemical and serological tests should be performed in BSL-2, while diagnosis methods that require the growth of virus such as virus isolation, tissue culture or neutralization assays should be performed in BSL-3 [9,10].

In addition, procedure that require inoculation of virus in animals should be performed in BSL-3 animal facilities [11]. Any procedure generate aerosols should be performed at BSC-II [10]. WHO highly advises that national governments keep a list of licensed laboratories that keeping and working with suspected or confirmed specimens of SARS patients [14] and the laboratories are required to engage in external quality management programs [15] (Figure 1).

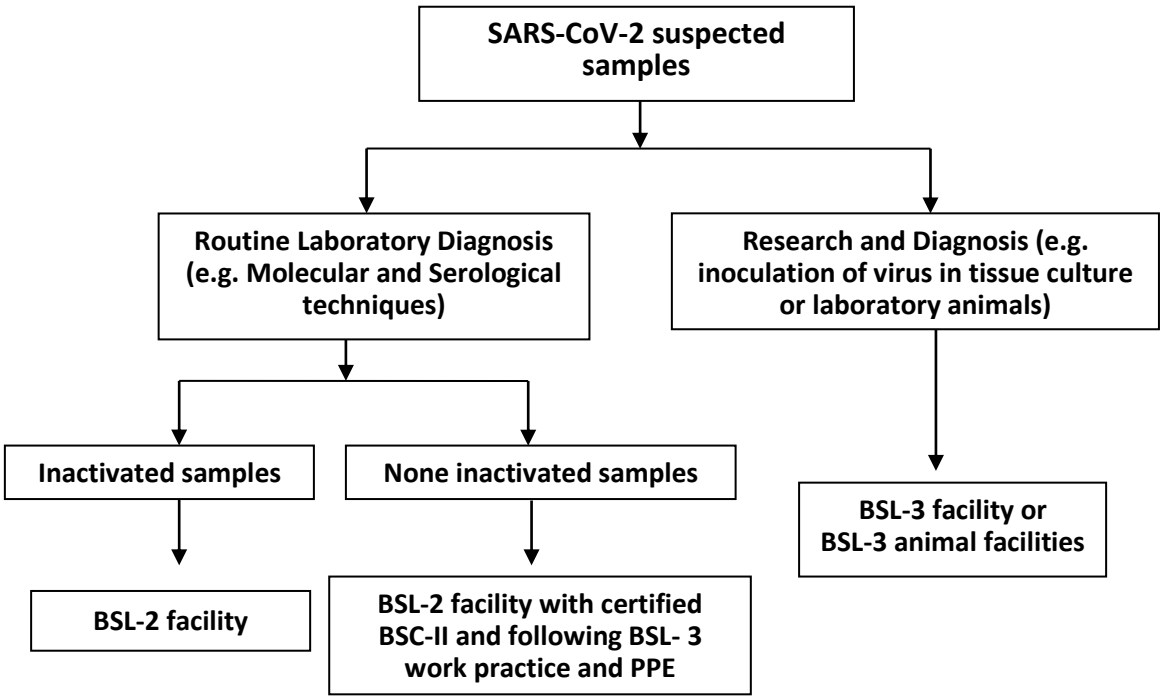

Figure 1. Building facilities required for SARS-CoV-2 suspected sample

The BSL-2 lab. should be physically segregated, with restricted access, from other operation areas in the same building. Staff should have access to a protective clothing dressing area. It is recommended to put the autoclave in a contained area [10,11], (Figure (2). The BSL-3 lab. should be physically isolated from other areas of operation or placed in a different building and have double doors that are self-closing. In the enclosed area, an electronically powered sink for hand washing and communication network with the outside should be present. There could be an emergency shower placed in the lab. Staff should have access to a protective clothing dressing area. To prevent the leakage of contaminants from inside to outside, the work area is kept at negative air pressure. After filtration via a high-efficiency particulate air (HEPA) filter, so air is exhausted from the lab. and can't be re-circulated inside the building unless a second HEPA filter is installed in the exhaust system [11,16].

5- Lab worker personnel

Laboratories should, as far as possible, encourage social distance within the workplace and monitor the likelihood of any daily exposure and health status of lab. staff [18]. Workers dealing with SARS-CoV or samples possibly carrying the virus should be educated on the signs of infection and recommended to report promptly to their boss any fever or respiratory symptoms [19]. After an observational time, personnel with prior BSL-3

expertise will be cleared to operate in the facility under supervision. The length of the training period depends on the skill and competence of the required techniques. In addition, those who working in the BSL-3 facility should be immunocompetent [16], therefore a high-risk group (e.g., over 70 years of age, pregnant, those with immune deficiencies or underlying medical conditions) should not work with SARS-CoV-2.

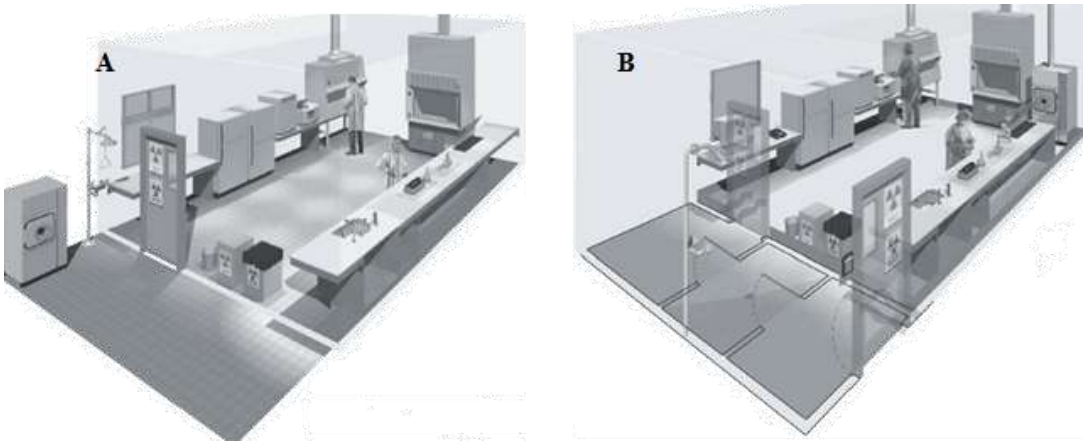

Figure 2. Biosafety level-2 (BSL-2) facility (A), Biosafety level-3 (BSL-3) facility (B) [17]

Institutions engaged in SARS coronavirus study should include the storage of a serum sample of individuals working with viruses or specimens containing viruses [19]. When real-time polymerase chain reaction (RT-PCR) findings are negative, the four-fold increasing in antibody titer between acute and convalescent phase sera could help COVID-19 diagnosis [20]. In addition, these organizations should establish and incorporate a clear occupational medical strategy. At a minimum, the strategy should include management procedures: identifiable breaks in lab. procedures; symptom-free employees; symptomatic employees within 10 days of exposure; and symptomatic lab. workers without known exposure [19].

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6- Personal Protective Equipment (PPE)

Due to less effectiveness compared to other approaches and high cost in the long term, the PPE is rated as the lowest in the infection control measure. PPE can only be used in combination with other regulatory and environmental control measures [8]. Full PPE including disposable gloves, lab. coat, class two filtering face-piece respirator and eye cover such as goggles or face shield should be worn by lab. staff while dealing with hazardous materials [7,10,21] and particular attention should be paid to hand hygiene after removal of gloves by regular hand washing with soap and water for at least 40 sec. and careful attention should be given to hand hygiene after removal of gloves [11,18]. Authorized filter respirator N-95 or higher standard (i.e., R95, P100, Powered air purifying respirator (PAPR) fitted with HEPA filters) should be used as recommended by the National Institute for Occupational Safety and Health (NIOSH); while the lower level of protection is not accepted [2,10,19] (Figure 3). However, it has been shown that the detection of influenza virus and coronavirus RNA in respiratory droplets and aerosols, respectively has been greatly reduced by surgical face masks [22].

Figure 3. Biosafety level-2 (BSL-2) facility (A), Biosafety level-3 (BSL-3) facility (B) [17]

There are various N95 mask brands from multiple manufacturers available. At 95 percent filtration efficiency, N95 is not resistant to oil. For oily solvents with 95 percent filtering efficiency, R95 is recommended. Higher designations like P100 mean that 99.99 percent of particles are flushed out. Fit checking for the N95 respirator is needed because it is a negative pressure respirator, so it should be perfectly shaved for N-95 users to match the mask fit test. However, fit checking for PAPR is not needed because the atmosphere of the breathing zone is under positive pressure and so a tight seal is not needed for proper operation, a PAPR can be used for users with beards [2]. After facing each patient, the instructions recommended the disposal of N95 respirators [23].

The lack of PPE forced hospitals to change infection control strategies after the COVID-19 pandemic. CDC endorsed N95 reuse techniques, even for the length of their shifts, workers reusing their own N95s. They tested decontaminate and reuse N95 at Duke University Health System using BSL3 facility Hydrogen Peroxide Vapor processing room to prolong their life and verified that the respirator already works for more than 50 times after 4 h of decontamination [24]. Also, Daeschler et al. (2020) observed that SARS-CoV-2 was inactivated by a single heat treatment for 60 min. at 70 ˚C at either 0 percent or 50 percent relative humidity in N95-respirators for more than 10 times. This is known to be a low-cost, rapid decontamination method for the secure reuse of disposable N95 respirators [25]. In addition, after disinfection with 1000 mg/L chlorine-containing disinfectant for 30 min, the PAPR could be reused after all sections repeatedly and uniformly cleaned with a soft cloth dipped in clean water [26].

7- Specimens collection, transport and storage

During sample collection, it iss crucial to adhere to the standard infection control practice and the use of complete PPE [20]. SARS-CoV-2 is an enveloped virus i.e., fragile, therefore care should be taken during sample collection and transportation. For molecular diagnosis, samples need to transported in viral transport medium (VTM). Specimens that would be directly sent to the lab. can be stored at 2-8 °C, but specimens will be frozen at -70 °C (dry ice) while there is a delay in specimens reaching the lab. [15,20]. Samples should be clearly labeled and stored frozen separately from other samples in restricted-entry locked freezers [11].

Specimens should be brought in special delivery tanks and boxes that satisfy the criteria for biosafety [26]. SARS specimens should be wrapped, secured and decontaminated as key containers for transportation within the facility [19]. But samples should be packaged in triple packs for shipment outside the facility in compliance with the WHO post-outbreak biosafety recommendations [14] and the International Air Transport Association (IATA) for hazardous

goods regulations [27]. The United Nation model regulations should be followed by international transportation [15].

8- Biosafety of laboratory diagnosis

While respiratory samples have the highest yield, in other specimens, including stool and blood, the virus can also be identified. Nucleic acid amplification test, such as RT-PCR, can used to screen the suspected cases for the virus [15], that targeting SARS-CoV-2 RNA-dependent RNA polymerase and E genes [7,28]. Serological testing could aid in the detection of a current pandemic of SARS-CoV-2, retrospective estimation of the occurrence rate of an outbreak, and could help the diagnosis of COVID-19 if the outcome of RT-PCR is negative [20,29].

Any operation with the ability to produce aerosols should be performed inside a certified BSC-II. Using enclosed centrifuge rotors for centrifugation, or a guard bowl with gasketed cover. Procedures carried out outside a BSC must be carried out in a way that minimizes the hazard of exposure to employees and release to the environment [19]. In the event of any accident during centrifugation, the lab. worker can pause the centrifuge, wear the BSL-3 PPE, wait half an hour for the centrifuge lid to release, and spray 75 percent ethanol. After that, the rotor with the specimen to be treated in the biosafety cabinet should be taken out [18].

After exposure to numerous widely used disinfectants and fixatives, a related coronavirus which causes SARS loses infectivity. However, acetone fixation for immunofluorescence assays at room temperature does not effectively kill the SARS virus until the acetone is cooled down to -20 ˚C [30]. Sera should be inactivated for 30 min. before analysis at 58 °C exposure. Tissue inactivated by formalin fixation for pathological analysis, fixation of smears for regular staining and microscopic tests and extraction of nucleic acid for PCR. Although it should be remembered that infectious RNA can be present in inactivated clinical samples [13]. Where appropriate, it is important to use automated instruments and analyzers. Aerosol-generating sample processing steps should be conducted in a BSC-II to manually treat non-respiratory specimens, wearing the recommended PPE [18].

9- Decontaminate equipment and surfaces

The virus sensitivity to inactivation is dependent on the environment and virus concentration [31]. Coronaviruses are enveloped viruses and generally thermo-labile [11]. Most disinfectants can easily affect the outer layer of the virus envelope [32]. The amount of time the virus is expected to survive depends on the sort of virus-containing substance or body fluid and different environmental factors, such as temperature or humidity [11]. It has shown that it is possible to kill a similar coronavirus that causes SARS at 56 °C at about 10000 units per 15 min [30]. Therefore, decontamination of work surfaces and disinfectant equipment is important using disinfectants at least every 3 h [10,32]. In addition, hazard specimens should be disinfected or autoclaved immediately [18].

It has been demonstrated that through disinfecting surfaces with 62-71 percent alcohol or 0.5 percent hydrogen peroxide bleach or household bleach containing 0.1 percent sodium hypochlorite, coronaviruses may be inactivated within minutes. Now a catalog of disinfectants that can be used against the SARS CoV-2 virus has been released by the US Environmental Protection Agency (EPA) [33]. The virus was destroyed in a report by Chin et al. (2020) by incubating a virus culture for 5 min with different disinfectants, such as 1:50 household bleach, 60-70 percent ethanol, 7.5 percent povidone iodine, 0.05 percent chloroxylenol or chlorhexidine and 0.2 percent to 0.4 percent benzalkonium chloride [34].

It is also recommended that an appropriate freshly prepared chlorine disinfectant (5500 mg/L) be used for > 30 min if the suspected specimen has leaked or generated BSC or

bench contamination. When lab. exposure is caused by positive specimens, the lab. room is closed to avoid contaminants from spreading, then the infected area should be cleaned with an appropriate chlorine containing towel (5500 mg/L) for > 30 min. Miscellaneous disinfectants (e.g., peracetic acid (2 g/m^3), H_2O_2 (3%), chlorine dioxide (100 mg/L)) may be used for overnight lab. fumigation, or aerosol disinfectants may be sprayed for 1-2 hours [18].

10- Waste management

Specimens and tissue culture should be disinfected or autoclaved and collected in leak-proof containers with their tops properly sealed prior to disposal [9]. Sharp items can be disposed of in a separate plastic box, sealed and sprayed with chlorine-containing 1000 mg/L disinfectant. Medical waste should be stored in a double-layer waste bag, wrapped in a gooseneck manner with cable ties, and sprayed with 1000 mg/L of chlorine containing disinfectant. Shift of waste to another facility with decontamination capability if decontamination is not feasible on site [9,26]. Bagged waste gathered into a collection box for hazardous waste, apply a special label for pathogen, completely enclose and transfer the box. Move the waste to a temporary medical waste collection point along the designated route at a set time point and store the waste separately at a fixed location until the licensed medical waste disposal contractor collects and disposes of it [26].

11- Biosecurity

There should be limits on entry to labs. A list of approved personnel engaged in the collection and archiving of COVID-19 samples should be preserved and circulated for bio-safety and biosafety purposes with the appropriate authorities. Sample storage zones, including those outside the main labs, should be guarded. Access to test databases, including storage locations and information, should be restricted to the relevant staff only [35]. It is desirable that two individuals work together while tissue culture is conducted in the BSL-3 lab. If it is not possible, however, it is mandatory that at least one other person be present directly outside the BL3 region of the lab. This person will be responsible for the continuous surveillance of the BSL-3 operation by means of internal video equipment [16].

12- Conclusion

It is essential to develop biosafety training and standard operative procedures for Iraqi laboratories, in addition establish emergency plans and practices for incidents that may occur in the lab. staff must be supplied with the necessary PPE that they need to comfortably do their work. A biosafety level must be determined based on the risk assessment in order to carry out research work on SARS-CoV-2. Moreover, due to special requirement needed to work with highly infectious viruses, the laboratories performing viral diagnosis in Iraq should be limited to only certified laboratories.

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