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Review Article

Biosafety Measures for the Laboratories Engaged in the Diagnosis/Research of SARS-CoV-2

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Abstract

Severe acute respiratory syndrome coronavirus 2, SARS-CoV-2 (COVID-19) infection emerged in Wuhan city of China, December 2019 and subsequently WHO announced COVID-19 pandemic. In the absence of effective antiviral drugs, change in genomic make-up which leads to evolution of new variant, effective biosafety measure in place, front line health care workers or laboratory personnel engaged in diagnosis and research are always at risk. As per the scientific risk assessments, the SARS-CoV-2 comes under Risk group 3 pathogens, and to prevent laboratory-acquired infections and disease transmission in the local population and environment, adequate biosafety containment levels are required. Therefore, non-propagative work and diagnosis of SARS-CoV-2 with inactivated samples should be performed at least under Biosafety Level 2 (BSL2), while diagnosis with non-inactivated samples should be carried out under BSL3 or BSL2 with inward unidirectional air flow along with BSL3 safety equipments and work practices. However, SARS-CoV-2 culture and isolation, as well as research and development activities, must take place inside the BSL3 containment facility. We attempted to establish adequate and efficient biosafety strategies for avoiding SARS-CoV-2 infections within the laboratory. This may be accomplished by conducting a systematic and comprehensive biosafety risk assessment on a continuous basis in order to cope with evolving risks in the laboratory setting. Furthermore, the healthcare workers in hospital or researchers in the laboratories may be unaware of the possibility of aerosols and droplets mediated infection in the laboratory during the process of centrifugation, vortexing, pipetting, and so on, or by SARS-CoV-2 infected individual during the process of breathing, coughing and sneezing and that aerosolized virus may travel up to 1- 6 m. Therefore, aim of this review is to describe the importance of biosafety measures against SARS-CoV-2 that should be introduced in laboratories undertaking diagnosis/research on SARS-CoV-2/any mutant form of SARS-CoV-2 like omicron suspected samples.

Keywords: Laboratory; Biosafety; Research; Diagnosis; Risk Assessment; SARS-CoV-2; COVID-19

1. Introduction

The novel coronavirus disease 2019 (COVID-19) is associated with manifestation of a broad clinical spectrum. It is a highly infectious and contagious viral disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a novel beta coronavirus. It's not the deadliest virus mankind has seen, but, spreads quicker than SARS-CoV and Middle East Respiratory Syndrome Coronavirus, MERS-CoV [29]. It was first identified and reported in December 2019 in Wuhan, China [14], and later the World Health Organization declared it a pandemic (WHO). The present COVID-19 pandemic has raised biosafety issues around the world and, especially regarding the risk assessment or evaluation of the contained use of SARS-CoV-2 for laboratory activities.

In hospitals where biosafety measures procedures have not been optimized and adopted, significant number of SARS-CoV-2 cases that have been identified in health care workers have been due to direct interaction with patients, although laboratory-acquired infections (LAI) were demonstrated to be very limited due to SARS-CoV-2. In the past, LAI was reported from various parts of the world, such as in 2003, when a doctoral student working in a virology laboratory who was not interested in SARS diagnostic work inadvertently manipulated SARS-CoV [41] and became infected with the virus in Singapore; and in Taiwan [43], where a Chinese laboratory-centered outbreak was recorded [45]. These are documented laboratory incidents that have posed concerns about biosafety in laboratories engaged in SARS-CoV-2 manipulation.

In this context, suitable biosafety measures are the key elements for the laboratories working with any infectious microorganisms like SARS-COV-2 or mutant of SARS-CoV-2 like Omicron which are having detrimental effects on health of laboratory personnel. Each laboratory should adhere good laboratory practices (GLP), such as the use of standard biological safety protocols, routine personnel training, and the use of standard operating procedures for handling and treating suspicious clinical specimens from various risk groups, which will aid in mitigating potential risks. Risk assessment is the first and most important part of biosafety measures. The procedures conducted, the identification of the hazards involved in the process and/or procedures, the proficiency level of the staff performing the procedures, the laboratory equipment and facility, and the resources available all influence risk assessments and mitigation steps, and they all vary from laboratory to laboratory. Therefore, all laboratories shall conduct their own risk assessments for handling of biological specimens from suspected or confirmed cases of SARS-COV-2 in order to prevent hazardous effect on life during laboratory works. Throughout the globe, categories of people like health care worker, police, housekeeping staffs, laboratory personnel are working on the frontline and often behind the scenes to ensure safe and secure diagnosis of SARS-COV-2 infection. COVID-19 is today's biggest threat to healthcare workers either in hospital or in the laboratory because they might not be aware about aerosols and droplets infection. Aerosol transmission was dismissed early in the pandemic, and it was assumed that transmission occurred primarily through droplets that travelled directly from emitter to receiver. Now, it is thought that a significant transmission mechanism is aerosolized droplets that remain suspended in the air, can accumulate in poorly ventilated areas, and that can travel further than the distances that droplets were thought to go [33]. Whereas droplets can be mitigated by distance and barriers; mitigating aerosols require facemasks; filters, or even more distance. As a consequence, there is need to address these issues in the COVID-19 pandemic, viz. how biosafety measures can be applied in laboratory? What type of personal protective equipment (PPE) should be used? How should general and biomedical waste be managed? How should laboratory equipment and work surfaces be decontaminated? Despite the fact that SARS-CoV-2 is a relatively new virus, the interim laboratory biosafety guidelines provided by the Centers for Disease Control (CDC) and the World Health Organization (WHO) address many concerns about sample safety in clinical, testing, and research laboratories. By adhering to these prescribed recommendations, laboratories will significantly mitigate the risk/harm to themselves and others to a large extent. In this context, the present review describes the importance of biosafety measures for SARS-CoV-2 to be adopted in the laboratory engaged in the diagnosis/research of SARS-CoV-2/COVID-19 suspected samples. The review is completed after testing of approx 70,000 (seventy thousand) COVID-19 suspected samples for rRT-PCR at ICAR- International Centre for Foot and Mouth Disease (ICFMD) having BSL3+ containment facility. The proficiency of ICAR-ICFMD in identifying SARS-COV-2 was confirmed by ICAR-Regional Medical Research Centre in identifying SARS-COV-2 with 100% precision, some of which included SARS-COV-2 previously detected with RT-PCR testing and some of which were virus-free samples. The duration of test was more than 7 months without a single positive case or LAI among the laboratory personnel. Therefore, it is advisable to adopt these biosafety measures in laboratory to avoid unintentional virus transmission within the laboratory or outside the laboratory i.e., local public and environment.

2. SARS-CoV-2 and its Threat

In the past the world has experienced several outbreaks due to various pathogens like severe acute respiratory syndrome-related coronavirus (SARS-CoV), Middle East respiratory syndrome-related coronavirus (MERS-CoV), bird flu, swine flu, Ebola, Zika, Nipah, and presently is confronting the challenge of a novel coronavirus (nCoV), a novel strain that has not previously been detected in humans. The SARS-CoV-2 was identified as the coronavirus strain responsible for causing COVID-19 pandemic [12,23]. CoV is a single-stranded positive sense, enveloped RNA virus with a genome size of 26-32 kb [24], while SARS-CoV-2 has a genome size of 29.8-29.9 kb

[16] and a diameter of SARS-CoV-2 virion could be varied between 60-140 nm [49], however, under electron microscopy, SARS CoV-2 with virus particle sizes varying from 70-90 nm was observed under a broad range of intracellular organelles [28]. The genome can function as mRNAs, and its purified genome is infectious in nature [17], which was also a concern in terms of biosafety both inside and outside the laboratory. The virus is thought to have evolved through recombination from an ancient bat virus, with which it shares 73% - 100% gene identity [5]. This virus generates its replication and transcription complex, as well as its RNA-dependent RNA polymerase (RdRp), from a single, large open reading frame known as ORF1ab [34]. Viral spike peplomer is a protein that populates the envelope surface and determines host tropism. As a result, epithelial cells in the respiratory and gastrointestinal tracts are the main target cells for SARS-CoV-2 (due to the presence of the receptor angiotensin converting enzyme 2, ACE-2, which facilitates viral entry into host cells). It could be one of the reasons for the virus being shed in respiratory exudates and faeces.

According to the WHO [40], SARS CoV pathogenicity is associated with lower respiratory infection and death from progressive respiratory failure. The incubation period for SARS-CoV-2 infection ranges from 4-5 days before the onset of clinical symptoms to 14 days [20], and patients may transmit infection during this time. The symptoms like fever, chill, dry cough, fatigue and myalgia, sputum production, headache, haemoptysis, nausea or vomition, diarrhoea and dyspnoea, anosmia, ageusia are manifested [14,31]. During clinical investigation, various parameters were observed as below: leukopenia, neutrophilia, lymphopenia, elevated serum alanine aminotranserase and aspartate aminotransferase levels, elevated lactate dehydrogenase, high C-reactive protein (CRP) and high ferritn [14,31]. While radiology examination showed CT chest abnormalities in all patients (bilateral in 98%); typically, bilateral lobular and sub segmental consolidation were observed [14]. Several workers reported that infection with SARS-CoV-2, may be asymptomatic (without any symptoms) and pre-symptomatic (before appearance of symptoms) [2,6,18]. Upon testing of about 70,000 referred SARS-CoV-2 suspected samples at ICAR-ICFMD, it was found that the proportion of asymptomatic infection is 55% (unpublished) and a similar finding was previously reported [11].

The contagiousness of any pathogen depends upon the route of its transmission. Till date several reports have discussed that SARS-CoV-2 viral RNA detected in multiple bodily fluids like urine [30], CSF [25], nasopharyngeal samples [35], saliva [37], fecal samples [35], semen [21], ocular fluid/tears [10] etc. Human SARS-CoV-2 infections are primarily spread through direct close contact with an infected person, exposure to infectious aerosols/droplets, the faecal-oral route [13], and mechanical transmission [44], ear [26], conjunctiva [8] etc. Furthermore, transmission of SARS-CoV-2 due to asymptomatic or pre-symptomatic infection is yet to be clearly understood, recent studies show that people who has asymptomatic or pre-symptomatic infection may transmit the virus [18]. Therefore, asymptomatic or pre-symptomatic laboratory workers with SARS-CoV-2 may be a threat for healthy laboratory staff if suitable and adequate biosafety measures are not routinely adopted/used.

Infectious aerosols, which are tiny liquid or solid particles floating in the air that contain infectious agents and may be produced during sample centrifugation and pipetting, are the main contaminating factors. They spread throughout the laboratory and remained infectious over time and space. These particles are of perfect size to reach the lower respiratory tract (< 5 µm in diameter) during the process of inspiration. Some organisms that may be spread by aerosols include *Aspergillus* spp. spores, *Mycobacterium tuberculosis*, rubeola virus (measles), and varicella-zoster virus (chickenpox) and also SARS-CoV-2. Droplets are larger contagious particles (>5 m in diameter) that quickly fall from the air, contaminating gloves, the surrounding work environment, and the mucous membranes of personnel. Bacteria *viz. Bordetella pertussis*, *Mycoplasma pneumonia, Neisseria meningitidis*, Streptococcus group A etc), and virus like influenza viruses, adenovirus, SARS-associated coronavirus (SARS-CoV) etc are the examples of infectious agents transmitted through droplets. Aerosols and droplets of particles less than 100 µm in diameter are not visible to the naked eye. Laboratory staffs may be unaware that such particles may be produced during several laboratory procedures and that they can be inspired/inhaled or contaminate work surfaces, materials, and various instruments.

According to an earlier published report, SARS- CoV-2 remained present in aerosolized form for up to 2 hours and was stable on surfaces of inanimate object like stainless steel, plastics for up to 72 hours [38]. It has also stated that SARS-CoV-2 is more stable on smooth surface than on rough surfaces [9]. As per WHO's re-

ports [42], the SARS-CoV remains infectious in excreta/stool and urine at room temperature for 1- 2 days. The virus will live for 48 hours on an exposed plastic surface. In stool from diarrhoea patients, the SARS-CoV-2 is steadier and more stable (up to 4 days). When exposed to widely used disinfectants and fixatives, the virus loses its infectivity. The process of fixing SARS coronavirus on glass slides for use in room temperature immunofluorescence assays does not inactivate the virus unless the acetone has been cooled to -20° C [42]. Some experiments have found SARS-CoV-2 RNA in air samples, with remnants of the virus identified by RT-PCR in microdroplets [33], while others have not [38]. The detection of RNA in air samples by PCR-based assays isn't indicative of viable virus replication, infection-competent virus that could be transmissible or appropriate inoculums to initiate invasive infection [48].

3. Laboratory Biosafety

As per the scientific risk assessments, the SARS-CoV-2 is classified as a Risk group 3 pathogen, requiring sufficient biosafety containment levels to prevent laboratory acquired infections and disease transmission in the local population and environment. Therefore, it is critical to maintain the highest biosafety measures in laboratories engaged in SARS-CoV-2 diagnosis from clinical samples, collected from COVID-19 suspected patients, as well as research and development in relation to SARS-CoV-2 and other risk group 3 and 4 pathogens. Biosafety laboratories consist a set of safety measures which are essential for dealing with hazardous biologicals in a safe and secure mode. Biosafety has several established definitions depending on the area of specialization *viz.* medical, health, veterinary, food, environmental, or space science etc. Biosafety refers to "the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release" [45]. This can happen, for example, if a laboratory staff is infected due to inadequate facilities or equipment, or if the virus is released due to inadequate air handling or waste decontamination systems [4]. The main objective of biosafety is to protect laboratory personnel, common public, animals, plant health and ultimately environment against LAI such as SARS-CoV-2, Influenza etc.

Several factors can affect biosafety containment for SARS-CoV-2 laboratory manipulations. The following considerations should be considered during risk assessment procedures *viz.* the laboratory activities planned with SARS-CoV-2, exposure time to the pathogens, virus concentration during exposure, the method and route of contact, the manipulations to be performed (creation of aerosols, types of needles used, lancets or sharp instruments), as well as the possibility of virulence changes in culture and in animals, as a result of which inoculated animals are capable of shedding live virus.

3.1. Risk assessment

A comprehensive biosafety risk assessment could be a key element of a successful biosafety program and may be a part of an all-hazards risk assessment; it should be conducted on a continual basis to deal with evolving risks within the laboratory atmosphere. The 5 'Ps'*viz.* procedures, personnel, protective equipment, place and pathogen, should be included while doing risk assessment and it should be followed in Plan-Do-Check-Act cycle (Figure 1) [32,36]. It may be a methodological approach of gathering information and assessing the likelihood and effect of introducing or releasing occupational hazard(s), as well as deciding appropriate risk control steps to reduce the danger to a suitable/acceptable degree. Hazards itself don't cause a risk to humans and animals however the work performed like sample assortment, sample reception, sample testing, PCR, virus isolation, decontamination procedures, and equipments used e.g., centrifuge, BSC, vortex etc. play an important role. Acceptable risk control procedures should be selected and applied for each known risk/hazard, such as aerosol exposures, eye splash, infectious culture substance spill, leak samples etc., in order to minimize residual risks to the lowest acceptable amount. Before handling any clinical sample either for diagnosis or research purposes, laboratory should carry out a site and activity-specific risk assessment to make sure the acceptable biosafety measures *in situ* with good laboratory practices (GLP) and good microbiological practice and procedure (GMPP). The risk assessment for each activity that may have a negative and harmful impact should be properly identified. The risk assessment should give a rating to all potentially negative results, or risks/threats, based on an assessment of the probability and effects of any of these identified risks. The risk assessment should confirm the most important appropriate control measures, as well as how the device will quantify the efficacy of these control measures, including the risk of contamination and the level of expected interaction with blood, bodily fluids, respiratory droplets, and/or open skin.

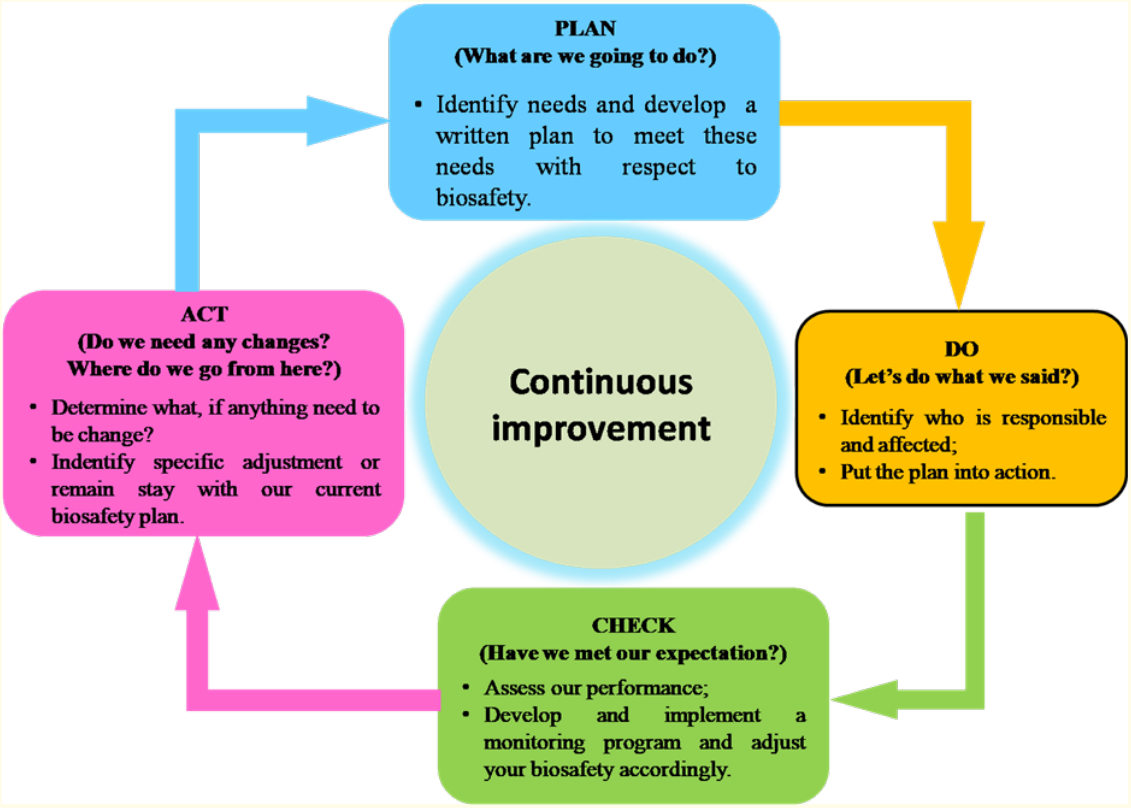

Figure 1: Plan-Do-Check-Act cycle (PDCA cycle) for the risk assessment of biosafety measures in the bio-containment laboratory.

3.2. Biosafety Level

Various queries arise while handling of either clinical sample suspected for identification of COVID-19 or R and D works inside the laboratory *viz.* are certified Class II biological safety cabinets (BSCs) needed to handle COVID-19 suspected or confirmed samples? Should laboratory workers engaged in laboratory to reduce personnel exposure if there is no specified Class II BSC?

According to the guidelines published for handling clinical specimen related to SARS by many organizations and countries, routine non-propagative diagnostic laboratory work like sequencing, nucleic acid amplification test (NAAT) for SARS-CoV-2 suspected cases should be conducted within BSL-2 facilities. A BSL-2 facility should have inward unidirectional air flow and a biological safety cabinet, as well as sealed centrifuge rotors or sample cups, PPE etc.

The propagative work like virus culture, virus isolation, neutralization assays, handling of large volume of infectious materials and inoculation of pathogens in animals for recovery and or its characterization should only be carried out by well trained and qualified professionals in a validated and calibrated biosafety cabinet class II B2 placed in dedicated laboratory room meeting the additional essential BSL3 containment with unidirectional airflow [4] preferably inward unidirectional airflow (negative pressure area because positive pressure area should mainly require for clean room production *viz.* vaccine, diagnostic kits etc.). Exhaust air from the laboratory room should exit through HEPA filters, and it should not be recirculated. If recirculated and reconditioned within the laboratory, it must be done through HEPA filter.

3.3. Biosafety Cabinet

SARS-CoV-2 is regarded as a risk group 3 microorganism; therefore, it should be handled in calibrated Class II B2 biosafety cabinet either in BSL3/BSL4 containment or in BSL2 with negative pressure with additional precautionary measures. These precautionary measures include PPE, sealed centrifuge rotors and centrifuge safety cups, aerosol resistant or barrier pipette tips etc used to minimize exposure risk of laboratory staff to the pathogens working inside the laboratory. Because of the infectious and contagious nature of the SARS-CoV-2 viral RNA [17], introduction of its genomic RNA into the permissive cells may allow SARS-CoV-2 infectious virus propagation; therefore, COVID-19 suspected clinical samples, genomic RNA, and downstream processes must be performed at least in Class II B2 biosafety cabinet placed in acceptable BSL facility. If unattainable, work like preparation of samples for molecular identification by real time PCR before samples inactivation, aliquoting should be carried out in Class II B2 biosafety cabinet. The suspected clinical samples like oropharyngeal swab, nasopharyngeal swab, faeces, urine, milk etc may also contain other potential source of infection to laboratory personnel and may lead to LAIs. If Class II B2 biosafety cabinet is not available, a Class II A2 biosafety cabinet (with external canopy to the environment for return air) may also be used for non-propagating work with additional precautions, such as the PPE mentioned above.

4. Standard Precautions and Measures

Excretion ad secretions (except sweat) of body, blood, mucus membranes, non intact skin, and various items used in research and development works may contain contagious infectious pathogens. So, standard precautions and measures play a vital role in maintaining biosafety in any work place and it could be achieved in the following ways

4.1. Use of calibrated equipment's and instruments

Always use certified and calibrated equipments to prevent any avoidable risk with respect to maintenance of biosafety in the laboratory.

4.2. Trained laboratory manpower

After proper risk assessment and acceptable biosafety measures in place, competent authority of the laboratory may take the decision that diagnostic or research work related with SARS-oV-2 should be carried out within a certified and calibrated Class II B2/A2 biosafety cabinet accommodated either in BSL3/BSL4 containment or in BSL2 laboratory with unidirectional air flow (outward to inward). The laboratory works should be carried out by competent person after getting adequate training on biosafety measures including introduction to biosafety, biosecurity, laboratory acquired infection, blood borne pathogens, Personal Protective Equipments (PPE) and standard operating procedures (SOP) specific for entry into laboratory, exit from laboratory, operation of BSC, dealing with spillage either in laboratory or in BSC, biomedical waste disposal etc. Laboratory personnel dealing with such type of pathogens must be conversant with all aspects of laboratory biosafety.

4.3. Packaging and Transport

Blood, urine, stool, and other conventional laboratory samples should be perfectly capped and placed in zip-locked biohazard bags, within a biohazard labeled, leak-proof cryobox, and sent to the appropriate laboratory for further processing. For transporting the infectious materials like patient samples, cultures/isolates, staffs must be adequately trained in the specific work designated to them as per the proper safety, packing, and shipping regulations for Division 6.2, UN 3373 Biological Substance, category B in compliance with WHO and the International Air Transport Association (IATA) guidelines [46]. Utmost care should be taken while transporting the clinical specimens from suspected or confirmed cases of COVID-19 within and between the laboratories for diagnosis which should be placed in a secondary packaging to minimize the potential breakage or a bio-spillage. Final packing of potentially infectious specimens (for example, to send to a reference laboratory) may be done at Containment Level 2 during sample packaging and shipment if the samples containing primary container are correctly sealed and decontaminated. Specimens leaving the BSC should be surface decontaminated with appropriate disinfectant. Depending upon nature of the samples, biological substance must be categorized as Category B (UN3373) and virus culture or isolates as Category A (UN2814, infectious substance, affecting humans) [46]. Early delivery preferably on same day by any means of transport for all samples types is recommended while samples like cultured/isolates/confirmed positive specimens for R and D or calibration work must be transported by an approved agency handling the category A carrier.

After collection of clinical samples for SARS-CoV-2 detection, the work on potentially infectious clinical materials (before inactivation) should be performed in a validated and calibrated biosafety cabinet class II A2 or B2 or primary containment with inward unidirectional airflow because that may lead to splashes, aerosols, droplet formation during opening of primary containers of infectious material, loading and unloading of sealed centrifuge cups, vortex etc. Prior to release from the BSC, the exterior surfaces of specimen containers and vials must be decontaminated using an appropriate disinfectant with proven action against the pathogen during sample movement inside the laboratory. Layout for handling of clinical samples for diagnosis and research and development (R and D) of SARS-CoV-2 suspected samples with proper biosafety measures is depicted in figure 2.

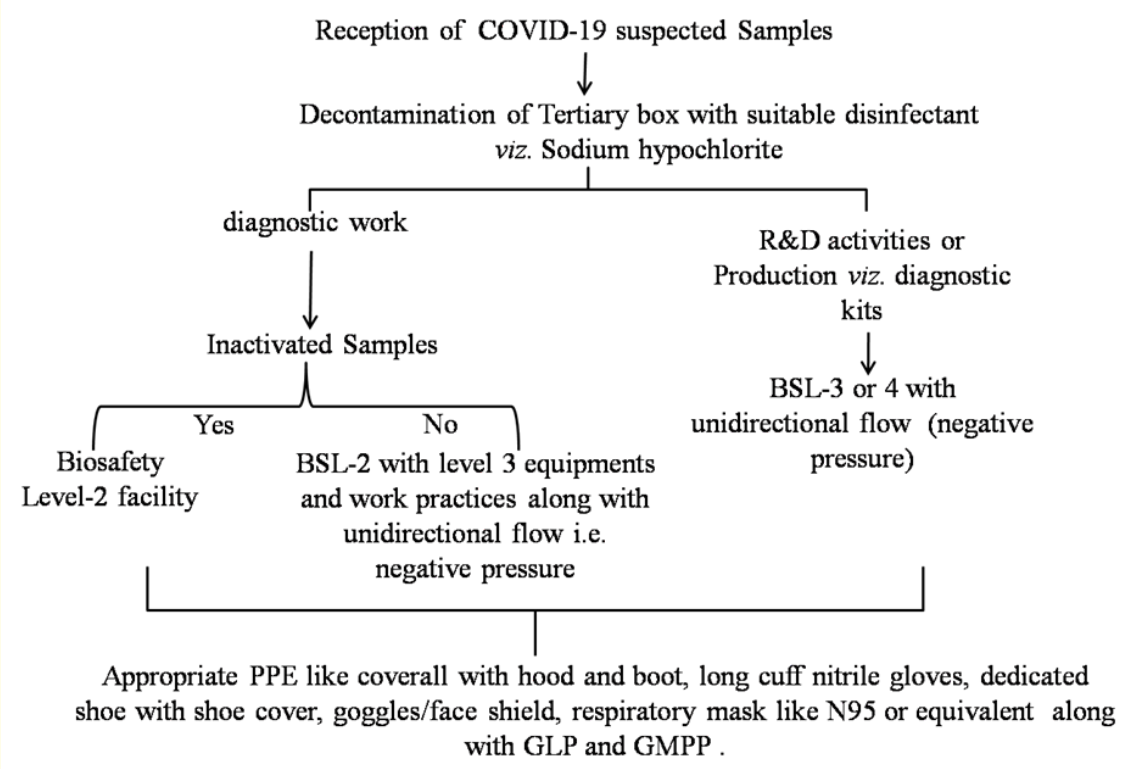

Figure 2: Flow diagram describing the required BSL facilities and biosafety measures for handling of SARS-CoV-2.

4.4. Laboratory standard operating procedures (SOPs)

All technical procedures should be carried out in accordance with laboratory SOPs to minimize the generation of aerosols and droplets [38] during sample manipulation [3]. Routine procedures like centrifugation, vortexing, pipetting, removing caps, vortexing, washing slide, aliquoting and loading specimens, manipulating needles during inoculation, aspirating and transferring body fluids like blood, sputum, collection of nasopharyngeal and oropharyngeal swab, and spills cleaning up may generate aerosols (infectious or noninfectious) and droplets which are undetectable and could be responsible for LAIs. This infection is mainly transmitted from one person to other through droplets, so, it could be minimized in laboratory by adopting GLP and GMPP. After vortexing the tubes containing samples, centrifugation of tubes should be carried out

in capped centrifuge rotors or sample cups and it could be kept either in BSC or on laboratory benches top. Similarly, routine hematology could be performed in auto analyzers using GLP at a specified level of biosafety after a suitable and sufficient risk assessment because it could be a source of infectious aerosols and infection transmission to healthy laboratory personnel while capping and uncapping sample tubes.

4.5. Personal protective equipment's (PPE)

Proper use of PPE is essential for effective maintenance of biosafety in the laboratory. Based on the risk factors, laboratory personnel should wear appropriate PPE like coverall with hood and boot, long cuff nitrile gloves, dedicated shoe with shoe cover, goggles/face shield (for eye protection as pathogens may be transmitted through the conjunctiva), appropriate respiratory mask like N95 or equivalent (respiratory protection) while working inside the laboratory. To reduce the risk of laboratory personnel exposure, use a certified Class II Biological Safety Cabinet (BSC) B2 or additional measures to provide a buffer, such as centrifuge safety cups and sealed centrifuge rotors, for procedures with a high probability of producing aerosols or droplets. It should be noted that masks or respirators are not a suitable substitute when processing clinically suspected samples for any hazardous microorganism such as SARS-COV-2 (COVID-19 pathogens) in a Class II B2 BSC, especially when aerosols are present. All laboratory personnel must adhere to PPE donning and doffing procedures and maintain GLP and GMPP in the laboratory.

- In labs, laboratory coats and gowns must be worn to prevent biological agents from splashing or contaminating open body parts or personal clothes. Splash-resistant laboratory coats should be long-sleeved, preferably with stretchable or fitted cuffs, and when worn inside the laboratory, it must be fastened; it should cover the knees only and not trail the laboratory floor. Rolling up your sleeves is never a good idea.

- For all procedures that may require expected or unintentional interaction with blood, body fluids, potentially infectious materials, or other biomedical waste, appropriate disposable nitrile gloves (powdered free gloves) must be worn. It should not be disinfected or reused in any way, as disinfectants and/or repeated usage undermine the glove's consistency and integrity, putting the user's safety at risk. Gloves should always be tested before use to ensure that they are in good working order. It is recommended that double gloves should be used while handling potentially infectious materials and after completion of work with infectious materials, superficial gloves must be removed in designated container containing recommended disinfectant before leaving the place.

- When protecting the eyes/face from splashes, impacting objects, or artificial ultraviolet radiation, safety glasses/goggles face shields (visors) or other protective equipment must be worn. Eye safety products may be reused, but they must be washed after each use with a disinfectant recommended by the manufacturer. If devices are splashed, they must be disinfected with the necessary disinfectant.

- Respiratory safety isn't usually one of the most important conditions. In the current COVID-19 context, use of masks in routine laboratory has been a debated issue because there is no direct contact with patient. However, a local risk assessment should be conducted to determine if the use of an appropriate respiratory mask, such as a surgical mask, N95, or equivalent, is essential. It will be used when procedures that generate aerosols and droplets outside the BSC are carried out, such as centrifugation, handling of leak samples, vigorous shaking and mixing, opening of infectious materials containers whose internal pressure which vary from the ambient pressure, and so on.

- Footwear must be worn in the laboratory, and it must be designed to prevent falls and trips, as well as injuries from dropping objects and biological agent exposure.

5. Decontamination and biomedical waste management

During this ongoing COVID-19 pandemic, safe handling of specimens and treatment of waste generated inside the laboratory during diagnostic and R and D activities is the responsibility of all the staffs because it could be the potential source of SARS-CoV-2 infection. Regardless of the extent of containment level, work surfaces (packaging and shipping, diagnostic, research and growth, and so on) and equipment must be decontaminated to monitor infectious risks with a suitable disinfectant after specimens have been treated or processed to avoid LAI. SARS-CoV-2 is an RNA virus with an envelope. The lipid membranes within the viral envelope are relative-

ly fragile, making it susceptible to most soaps, disinfectants, drying, and UV light. In compliance with local policies and following the manufacturer's instructions for use, an EPA licensed disinfectant solution or disinfectant wipe with proven activity against enveloped viruses or SARS-CoV-2 should be used. A suitable method of decontamination (cleaning and disinfection) should be used against this enveloped virus by using an appropriate disinfectant like sodium hypochlorite- 1000 parts per million (ppm) (0.1%) for general surface disinfection and 10000 ppm (1%) for spills; 62–71% ethanol; 0.5% H_2O_2; as per the manufacturer's guidelines. SARS-CoV-2 has been known to survive for up to 7 and 9 days on inanimate surfaces such as metal, glass, and plastic, respectively [9,15]. Regardless of the disinfectant used, for high efficacy of disinfectants on any pathogens, selection of disinfectants along with visible organic matter (removed from the surface), appropriate method of application, contact time, dilution (concentration of active ingredient), shelf life and expiry date of working solution, should be prioritized. Disinfectants of various types can never be mixed. Since certain disinfectants need more contact time at lower ambient temperatures depending on the mechanism of action, disinfectants should be chosen based on surface type and conditions, including temperature. Due to huge demand of disinfection on regular basis, disinfectants are in short supply during this COVID-19 pandemic at all the places, so, alternate option should be made ready. At our laboratory, 1% (V/V) sodium hypochlorite solution has been used for decontamination of tips and tubes, 5% (V/V) sodium hypochlorite solution was used for decontamination of COVID-19 suspected clinical samples (nasopharyngeal swab and oropharyngeal swab in virus transport medium) while work surface was wiped with 70% ethanol for decontamination on regular basis.

The clinical waste of suspected, confirmed COVID-19 cases or any biomedical waste ("Bio-medical waste (BMW)" means any waste, which is generated during the diagnosis, treatment or immunization of human beings or animals or research activities pertaining thereto or in the production or testing of biologicals or in health camps) generated within the laboratory should be disposed of according to the local, regional and national policies after its segregation and categorization of the waste. Before decontamination or disposal, SOPs should be followed to identify and segregate the waste in accordance with biomedical waste management disposal guidelines. Before disposal, solid and liquid waste generated within the laboratory must be treated. Solid waste should be properly autoclaved, and liquid waste should be routed to an effluent decontamination system (EDS) before being routed to a secondary effluent treatment plant (ETP) because infectious pathogens or their genomes may be present in untreated liquid waste/waste water/sewage [1] and the presence of viral RNA from the SARS 2002 outbreak was found in the sewage water of a hospital treating SARS-infected patients [39]. If decontamination facility for solid waste disposal is not available in the laboratory area, or onsite, solid waste must be packaged in a leak proof fashion as per SOPs of biomedical waste management disposal guidelines, for transfer to another facility with decontamination capacity or outsourced for BMW disposal. Furthermore, reusable laboratory dress must be autoclaved before exits from containment facility by using high temperature treatment (121°C for 15 minutes) followed by washing and ironing because surface of the laboratory dress may be positive for SARS-CoV-2 and it's likely that it's a cause of infection [27].

Laboratory biosafety lapses could cause an unintentional breach of biocontainment by any of the following: man (laboratory personnel), materials (liquid waste, solid waste, and other general waste), and air, which should be tested periodically at the exit level to preserve the biocontainment (Figure 3). Manpower working inside the laboratory should keep/remove the entire lab dress/lab coat/gloves/face shield etc at their designated place and take shower (if shower facility is available) before exit out of the laboratory. Materials generated within the laboratory, including solid and liquid waste, should be appropriately treated before leaving the laboratory (Figure 4). Solid waste (biomedical waste and general waste) generated inside the laboratory must be sorted, segregated and packed in biohazardous bags (Figure 5) as per the rules and regulations issued by local, regional and national body followed by autoclaving of these bags using high temperature treatment as appropriate cycle of time and pressure combination i. e. autoclaving at 121°C for 20 minutes (waste cycle) in double door autoclave. Before removing the BMW from the autoclave for disposal, the autoclave parameters should be checked and passed (self-contained biological indicator containing *Geobacillus stearothermophilus* 10^6 spores per ampoule and chemical indicator as depicted in figure 6). We also tested the confirmed positive SARS-CoV-2 samples, having different Ct Value of RdRp gene, N-gene and E-gene, with high temperature treatment, 121°C for 20 minutes (waste cycle) for the presence of RNA of SARS-CoV-2. We found that no SARS-CoV-2 RNA was detected by rRT-PCR and this result indicate that inactivation of samples by high temperature treatment reduced the quantity of detectable SARS-CoV-2 viral RNA or it could be due to fragmenta-

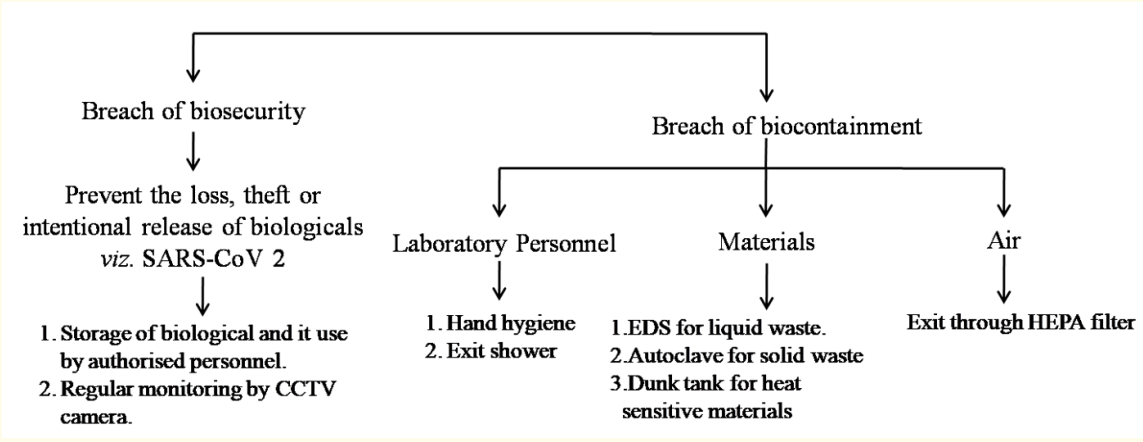

Figure 3: Figure 3 depicted laboratory biosecurity (security of biologicals, pathogens and toxins) management to deter pathogens from escaping the laboratory either through loss, theft, intentional release or due to breach in biocontainment (intentional or unintentional) by laboratory personnel, materials (which may be liquid waste, solid waste, contaminated equipments) and exhaust air to environment or local population. The first aspect of biosecurity, namely loss, theft, and deliberate release, could be handled via a lock and key arrangement or biometric entry, while the second aspect of breach in biocontainment, could be maintained through proper decontamination of biomedical waste induced onsite, exit showers for staff, decontamination of solid and liquid waste by autoclave and EDS and ETP, and exit of laboratory exhaust air via HEPA filter. Our primary purpose is to avoid the deliberate or accidental release of pathogens from the laboratory into the wider/public community, which includes the general people, local population and the environment.

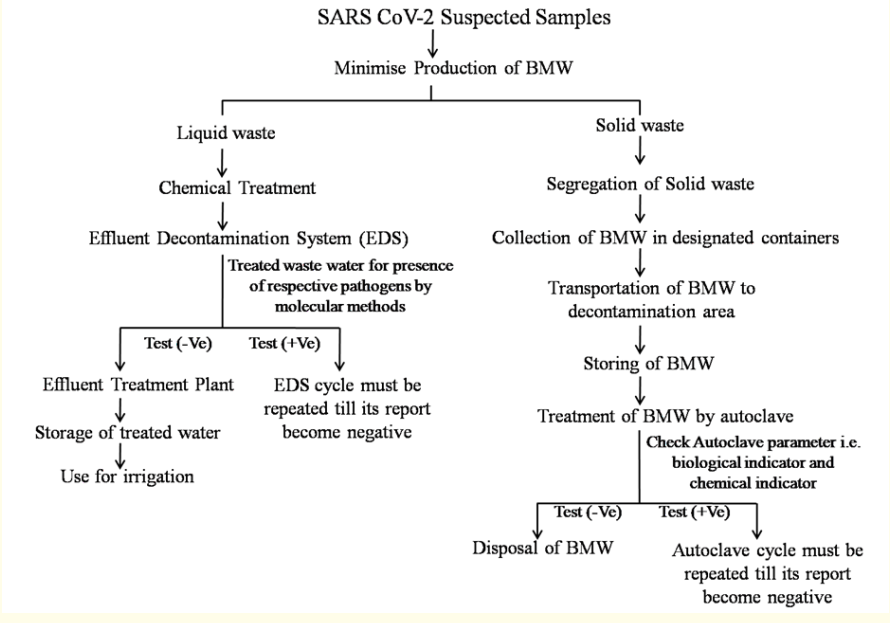

Figure 4: Flow diagram describing the standard biomedical waste management (BMW) protocol for disposal of liquid and solid waste generated inside the laboratory during the handing of SARS-CoV-2 suspected samples at ICAR-International Centre for Foot and Mouth Disease.

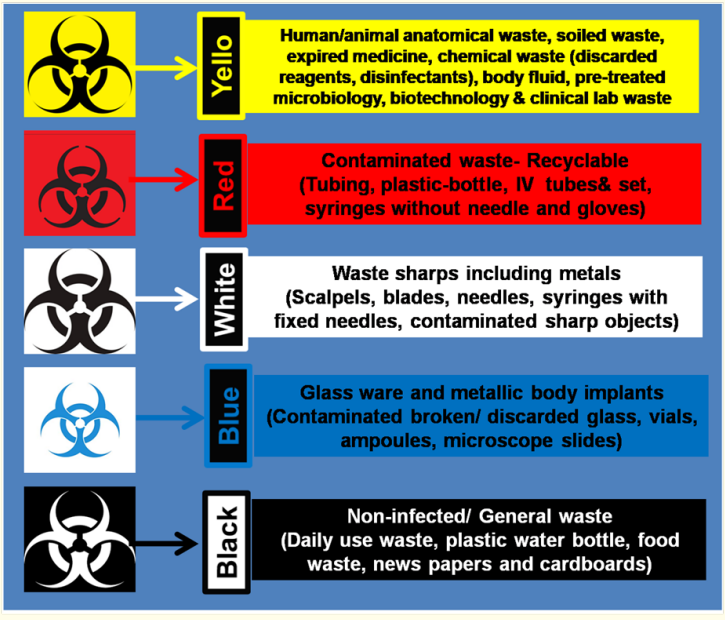

Figure 5: Segregation, sorting and packaging of solid waste generated inside the bio-containment laboratory as per the colour coding under biomedical waste management protocol.

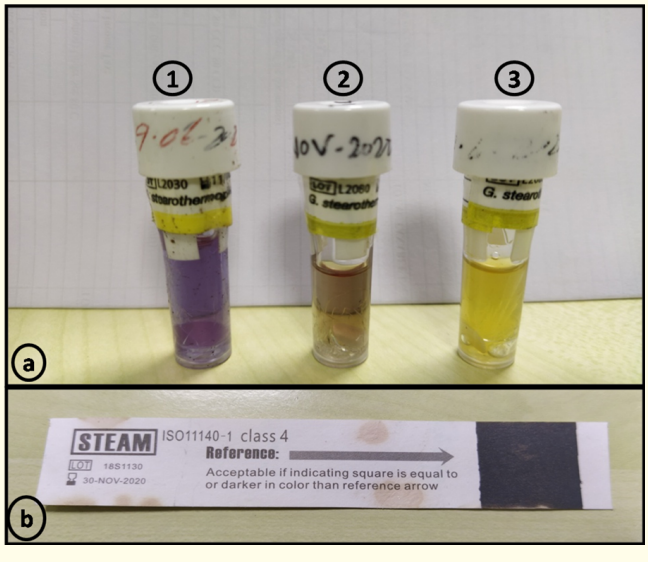

Figure 6: Biological and Chemical indicators used in the decontamination-autoclave for decontamination of biohazardous solid waste generated inside the laboratory. a) Biological indicators (Geobacillus stearothermophilus 106 spores per ampoule) were used to determine the fate of autoclave cycle i.e. inactivation of pathogens by the changing of colour of the ampoules, ampoule-1: purple colour indicates that autoclaving procedure is successful i.e. pathogens are completely inactivated, ampoule-2: light yellow/purple colour indicates that incomplete autoclaving i.e. pathogens are partially inactivated, while ampoule-3: yellow colour indicates that failure of autoclave cycle i.e. intact pathogens are present and autoclave cycle must be repeated. b) Chemical indicator, change in colour of strip from grey to black, indicates autoclave cycle are passed.

tion of nucleic acid which was undetermined during the process rRT-PCR. Similar finding was also reported by other researchers [7]. Laboratory/Return air should be propelled into the environment though exhaust containing HEPA filter.

6. Additional consideration for standard precautions for biosafety measures

- Regardless of biosafety standard, proper caution should be taken while handling to prevent accidental or unintended contamination of the exterior surfaces of all vessels and containers. All the equipments/instruments used during diagnostic and research and development procedures of COVID-19 should be disinfected with appropriate disinfectant (> 70% Ethanol) recommended by WHO only through wiping not through spraying [47] because spraying may lead to aerosol generation which could be potential source of infection for the laboratory personnel. In case of hand exposure or contamination, hand should be wiped immediately with wet towel containing 1% soap powder, 0.05% chlorine or 0.25% active chlorine from sodium hypochlorite to remove 90% of virus [22]. Ethanol and Isopropanol should be used immediately, as they efficiently inactive the virus in 30 sec [19].

- Since respiratory droplets may move up to 1m to 6m via the process of normal breathing, coughing, and sneezing [33], they may be aerosolized and present in the air due to a weak ventilation system. Therefore, to avoid the production of LAIs, good biosafety measures should be followed inside the laboratory.

- Laboratory staff should be routinely monitored for laboratory acquired infections (LAIs) against potential exposure and their health status. Laboratory head or head of the institution must take responsibility of ensuring health checkup of laboratory personnel/staff on routine basis. Each laboratory personnel/staff member may be required to undergo a health test/checkup to decide whether or not it is safe for them to work in the laboratory.

- Devices and equipment, laundry, food service utensils and biomedical waste can all be handled safely and routinely.

- Laboratory/facility must be equipped with a sink and eyewash station, first-aid kits and easily accessible to personnel/staff working in the laboratory at time of any accident. Items present in the first aid kits must be checked routinely by a biosafety officer to ensure that they are still functional and that there is enough stock.

- Emergency and contingency plan: To minimize the chances of being exposed to or releasing a biological agent, a contingency plan must be in place that outlines basic standard operating procedures (SOPs) to be followed in the event of an incident at work or in the surrounding area or local environment. Since it would not be possible to fumigate laboratory spaces if samples are spilled outside of BSL-3, thought must be given to what to do in the case of a spill and how effectively decontamination of the area will be done. Personnel/staff must be trained on a revised emergency plan and receive annual refresher training to ensure full competency.

- Incident and accident Plan: All incidents and accidents must be reported to the appropriate personnel (Biosafety officer and or in-charge of the facility) promptly for implementation of early necessary action. According to the guidelines, accident and incident must be reported and investigated as soon as possible, and taken into account while reviewing laboratory procedures and emergency response plans.

- Spill kits containing an adsorbent pad and disinfectant should be readily accessible to laboratory personnel and workers. Different protocols (as listed in the institute biosafety manual) can be followed depending on the scale, place, concentration, or volume of the spill. Written standard operating procedures (SOPs) for cleaning and decontaminating spills should be established for the BSC and or laboratory, followed by sufficient training of all laboratory personnel and annual refresher training to retain their competency.

7. Conclusion and Future Perspective

COVID-19 still has a worldwide problem due to its high transmissibility, morbidity and mortality in the elderly and those suffering from other chronic illnesses. Furthermore, the epidemic has caused significant socioeconomic disturbances throughout the globe. An efficient health-care system is the key in combating the global spread of COVID-19 and, finally, removing the virus infection. Laboratory biosafety, as well as containment principles, processes, and procedures, are critical components of an effective

health care system against COVID-19. Therefore, the laboratory biosafety measures outlined in this review would be beneficial in reducing SARS-CoV-2 risks to human health and the environment. Furthermore, the review's knowledge will be useful for the establishment of bio-risk-related standard operating procedures for the safe handling of SARS-CoV2 at the individual laboratory level.

Author Contributions

All of the authors contributed significantly to the conception, design, review, and evaluation of evidence, as well as reviewing and authorizing the final version of the manuscript, and consent to take responsibility for its contents.

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Conflict of Interest

All authors identified no possible conflicts of interest (s).

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