ISTANBUL UNIVERSITY
CERRAHPAŞA

Enhanced Significance of Laboratory Biosafety and Biosecurity During COVID-19 Pandemic

Mustafa Sencer KARAGÜL[1], Fahriye SARAÇ[2], Mustafa HASÖKSÜZ[3]

[1]Kartepe Vocational School of Equine Science, Kocaeli University, Kocaeli, Turkey
[2]Pendik Veterinary Control Institute, İstanbul, Turkey
[3]Department of Virology, İstanbul University-Cerrahpaşa, Faculty of Veterinary Medicine, İstanbul, Turkey

Cite this article as: Karagül, M. S., Saraç, F., & Hasöksüz, M. (2021). Enhanced Significance of Laboratory Biosafety and Biosecurity During COVID-19 Pandemic. *Acta Veterinaria Eurasia*, *47*(2), 108-116.

ORCID IDs of the authors: M.S.K. 0000-0001-7215-5229; F.S. 0000-0001-5854-1676; M.H. 0000-0003-3185-6453.

Abstract

Seven coronavirus species, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), cause respiratory diseases in humans. Four of these species, namely, HCoV 229E, HCoV NL63, HCoV HKU1, and HCoV OC43, typically cause mild upper respiratory infection in babies, kids, and the elderly. Severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus, and SARS-CoV-2 lead to more serious diseases in humans by infecting the lower respiratory tract. Epidemic and pandemic situations are the consequences of the rapid transmission of SARS-CoV-2 and large number of deaths. Evaluations related to the recent COVID-19 pandemic have revealed some requirements, which are not well known but essential for fighting against this disease. These requirements include the obligation to work at laboratories with high biosafety level (BSL) and conduction of studies under the guidance of biosafety and biosecurity simultaneously. Likewise, to overcome the hazardous microorganisms, laboratory research is required alongside therapeutic and diagnostic services in the field. This article aims to explain biosafety and biosecurity practices at BSL-3 laboratories, which are necessary for the studies of SARS-CoV-2 as the causative agent of COVID-19.

***Keywords*:** Biorisk, biosafety, biosecurity, COVID-19, pandemic

Introduction

The word "pandemic" stems from the Greek words, *pan* and *demos*, which means all people as a phrase (Lacroix, 2012). Pandemic is an epidemic infection that influences a large number of people in different continents of the world or even the whole world (Kelly, 2011; Lacroix, 2012). Seasonal epidemics that cross international boundaries are not considered to be pandemic (Kelly, 2011).

Through the history of mankind, there have been several serious pandemics such as pox, influenza, cholera, tuberculosis, and plaque, some of which had zoonotic origin owing to the domestication of animals. One of the most recent deadly pandemics was the Spanish flu, which emerged in 1918 and caused the death of about 50 million people (Lacroix, 2012). It should also be taken into consideration that 60% of the infections and 70% reemerging diseases of humans have zoonotic origins for this reason; humans and veterinary health experts should collaborate against zoonoses that threaten both human and animal health (Ahmad et al., 2020). In addition, "One Health" approach should be followed in the studies focusing on COVID-19 (Ahmad & Hui, 2020). Nowadays, a new pandemic named as COVID-19 is targeting humankind, the causative agent of which was identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Following the first case detected in Wuhan, China, the virus has spread all around the world rapidly. Together with SARS-CoV-2, there are six coronavirus species infecting humans. Although HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 cause mild upper respiratory diseases, SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) might be responsible for serious respiratory disorders by infect-

Address for Correspondence: Mustafa HASÖKSÜZ • **E-mail:** mhasoksuz@gmail.com
Received Date: October 27, 2020 • **Accepted Date:** February 26, 2021 • DOI: 10.5152/actavet.2021.20078
Available online at actavet.org

ing lower respiratory tract (Hasöksüz et al., 2020). Studies focusing on the less dangerous ones that cause mild respiratory diseases could be carried out at biosafety level 2 (BSL-2) laboratories with appropriate biosafety cabinets. However, studies including SARS, MERS, and COVID-19, which cause contagious and fatal diseases, should be carried out at BSL-3 laboratories.

Diagnostic, therapeutic, and prophylactic practices are the components that support and complete each other during the struggle with pandemic. However, knowledge provided by studies focusing on animal models, vaccine, and drug development at suitable laboratory circumstances should not be neglected.

Several research or clinical laboratories have critical roles against the global struggle with emerging and re-emerging diseases such as Avian Influenza, SARS, Nipah, Chikungunya, New variant Creutzfeldt–Jakob disease, Lyme borreliosis, Hantavirus, West Nile, Rift Valley fever, and multidrug-resistant *Mycobacterium tuberculosis*, among others. Even though the work in these laboratories is vital for humankind, effective management of safety and security practices in these laboratories is essential (Salerno & Gaudioso, 2007). Work with hazardous pathogens poses risks to laboratory workers because of the laboratory-associated infections (Astuto-Gribble & Caskey, 2014; Salerno & Gaudioso, 2007; Şeker & Yardımcı, 2003). The SARS laboratory-associated infection in Singapore in 2003 created international awareness about laboratory biosafety (Salerno & Gaudioso, 2007). High-level biosafety laboratories must be our priority to be able to conduct proper studies safely and securely against biological risks. The main aims of high-level biosafety laboratories are the reliable containment of the pathogen, prevention of the release of the microorganisms, and protection of the researchers against the laboratory-acquired infections (OIE, 2012).

Microorganisms are classified into four different risk groups (RGs). The common property of RG-4, which includes the most hazardous agents such as Ebola and Lassa virus (NIH, 2016), poses high risk to individuals and community. Although they are highly transmissible, there is no effective prophylaxis and therapy (OIE, 2012; WHO, 2004). *Brucella abortus*, *Brucella suis, Burkhalderia mallei, Coxiella burnetii, Venezuelan equine encephalomyelitis virus*, SARS-CoV, and MERS-CoV are some of the agents belonging to RG-3 (NIH, 2016). These pathogens cause serious diseases for humans and animals. Although these agents pose high individual risk and low community risk, generally, there are shared effective prophylactic and therapy methods (OIE, 2012; WHO, 2004). The required biosafety level of the laboratory should be determined in accordance with the characteristics of the agents belonging to different RGs. In this sense, classification of the laboratories depending on biosafety levels provides the safest working area. Identification of RGs is essential to determine the laboratory biosafety level. Some of the microorganisms in different RGs are listed in Table 1 (Abacıoğlu & Sönmez, 2014; BVL, 2013; Council Directive, 2000 Frey, 2013; HSE, 2013; NIH, 2016; Wittek et al., 2013).

Some of the organisms such as *Salmonella typhi,* classified in RG 3, are not transmitted by air and pose a limited risk to laboratory workers (Council Directive, 2000).

Countries may have their own RG classification; therefore, the same agents might be classified in different groups depending on the local regulations. Classification of the pathogens generally follows the risk-based approach, which is parallel to the definitions of the World Health Organization (WHO) (OIE, 2018; Silman, 2014). There is not always a one-to-one compatibility between the RG of the microorganisms and the biosafety level of laboratories (OIE, 2018). Laboratories are also grouped under four categories according to biosafety level. BSL-1 laboratories that are serving for basic training and research should have suitable working principles for good microbiology techniques (GMTs). It should be clarified that good laboratory practice, which is related to practices for the safety test practices of preclinical search, is different from the GMT in this sense (OECD, 1998).

GMTs indicate the laboratory techniques and practices that enable safer working conditions at the laboratories. Most of the laboratory accidents and occupational infections are the consequences of using equipment wrongly and poor laboratory techniques. Selection of a suitable sample box and a proper storage area for samples and even the specific attention paid to the handling of the microbiological transfer loop so that it would not be longer than 6 cm are good practices of GMTs (WHO, 2004). In addition, the GMT is a basic way to reduce aerosolization risk (WHO, 2012). Laboratories that provide primary health care, diagnostic services, or research opportunities are grouped under the BSL-2 laboratory category, which involves personnel protective equipment, biohazard signage, and GMTs (WHO, 2004).

BSL-3 and BSL-4 laboratories are identified as biocontainment and maximum biocontainment laboratories having their capacities that restrict and enclose the biological risks (Ceyhan, 2005; Yücel et al., 2014). BSL-3 laboratories, whose mission is specific diagnosis and research activities, have additional measures such as specific personnel protective equipment, access control, one-way air flow, and necessity of doing all practices in the biosafety cabinet (Abacıoğlu & Sönmez 2014; WHO, 2004).

There are no clear-cut distinctions between RGs and biosafety levels (WHO, 2004). For instance, BSL-2 laboratory conditions might generally give sufficient protection for practices with RG-2 agents. However, it should not be accepted that BSL-2 conditions are adequate for protection against all the practices related to RG-2 agents such as handling a large number of agents. This possible misconception related to RGs and biosafety levels may lead to biosafety and biosecurity gaps.

Table 1
Risk Group 3 and 4 Agents

Risk group 3 agents		
Bacillus anthracis	*Yersinia pestis*	*Lymphocytic choriomeningitis virus* (neurotrophic type)
Brucella melitensis	*Orientia tsutsugamushi*	*Rift Valley fever virus*
Brucella abortus	*Coccidioides immitis*	*Hantaan Virus*
Brucella suis	*Histoplasma capsulatum*	SARS-CoV
Brucella canis	*Salmonella paratyphi*	MERS-CoV
Burkhalderia mallei	*Shigella dysenteriae*	Japanese encephalitis virus
Burkhalderia pseudomallei	*Mycobacterium bovis (except BCG strain)*	*Monkeypox virus*
Coxiella burnetii	*Mycobacterium leprae*	*Transmissible spongiform encephalopathies TSE*
Chlamydia psittaci (avian type)	*Orientia tsutsugamushi*	*Human immunodeficiency virus*
Escherichia coli, Verotoxigenic strains (O157:H7, O103)	*Mycobacterium caprae*	*Vesicular stomatitis virus*
Francisella tularensis (Type A)	*Chikungunya virus*	*West Nile virus*
Rickettsia akari	*St. Louis encephalitis virus*	*Yellow fever virus*
Salmonella typhi	*Venezuelan equine encephalomyelitis virus*	*Influenza virus* (1918 H1N1, H2N2 1957–1968, H5N1)
Risk group 4 agents		
Guanarito virus	*Junin Virus*	*Equine Morbillivirus*
Lassa virus	*Crimean-Congo hemorrhagic fever virus*	*Whitepox virus (Variola virus)*
Machupo virus	*Ebola virus*	*Equine morbillivirus*
Sabia Virus	*Marburg virus*	*Variola (major and minor) virus*

Note. SARS-CoV = severe acute respiratory syndrome coronavirus; MERS-CoV = Middle East respiratory syndrome coronavirus.

Biosafety level that seems proper for the initial practices should be re-evaluated when there is a large number of microorganisms or a high concentration of aerosol is generated (Abacıoğlu & Sönmez, 2014; Silman, 2014; WHO, 2004). Therefore, the tendency toward determining the biosafety level based on the RG of microorganisms does not always present the optimum solution. RGs might just be a guide in initial stages for determination and implementation of laboratory biosafety (Silman, 2014). The containment level can be estimated based on a combination of physical conditions and practices (OIE, 2012).

Risk analysis and assessment are cornerstones to maintain biosafety (WHO, 2004). Biological risk analysis is a process composed of identification and characterization of the risk-related health, safety and security, implementation of control measures to reduce the risks to an acceptable level, and measurement of the effectiveness of control assessments (OIE, 2018). Risk analysis should be conducted by the experts who are familiar with the microorganisms in the study, procedure and equipment to be used, possible animal model, and other facility conditions. The director of the laboratory and the principal investigator are supposed to carry out proper risk assessments regularly (WHO, 2004). Risk analysis is used not only for laboratory biorisk management, but it is also a beneficial tool for finance, engineering, energy, and health industries (OIE, 2018).

The risk assessment of the COVID-19 pandemic has outlined the procedures necessary to be performed in order to prevent the spread of the disease . BSL-2 conditions are found to be sufficient for non-propagative practices such as handling samples for molecular analysis, sequencing, and nucleic acid amplification. However, applications such as cell culture, isolation, and propagation, which might be necessary for laboratory search related to SARS-CoV-2, should be carried out at facilities having at least BSL-3 requirements (WHO, 2020a; WHO, 2020b).

Relationship between BSL-3, Biosafety, Biosecurity, and Pandemic

In the fight against pandemics, knowing the biological hazard and threat, being aware of risk posed by hazard or threat, and implementing safety–security measures to reduce the risk to an acceptable level are the initial stages that should be followed. The biological hazard of COVID-19 pandemic is known to be SARS-CoV-2, which is also the causative agent of the disease (WHO, 2020a). Some effects of the virus are well known and demonstrated through clinical studies. However, there are still some unclarified issues such as virus interaction with immune system, reaction in the animal model, and in vitro or in vivo tri-

als of therapeutic chemicals. These issues require handling the virus at laboratories under BSL-3 conditions.

BSL-3 and animal biosafety level-3 (ABSL-3) laboratories, which support research and animal experiments, are the laboratories that are the most difficult to design and operate. These laboratories need certification before initial use, after renovation of replacement of critical heating, ventilation, air conditioning (HVAC) system components and on a regular basis (Wilson & Memarzadeh, 2006).

Laboratory biosafety and biosecurity should be established in the facility to meet the basic necessities for biological hazards (CWA, 2011). In some countries, the term biosecurity is sometimes used synonymous to biosafety (CDC, 2009). Laboratory biosafety includes the principle of containment and necessary practices, which are used to avoid unintentional exposure to biological agents and toxins and their accidental release. By contrast, laboratory biosecurity refers to the ways to prevent loss, theft, misuse, unauthorized access, and intentional release of the pathogens in the laboratories (CWA, 2011). The important issues of biosafety include the presence of the hazard due the biological agent and emergence of the undesirable events unintentionally. Contrastingly, intentional activities by people, namely, threats to cause unwanted situations, are the foci of the security approach (Salerno & Gaudioso, 2007). In this context, the research studies carried out in the struggle with pandemics might present benefits, and they might also be identified as gain of function research because of the risks they include. Therefore, such kind of studies should be evaluated based on their sufficient capacity of biosafety and biosecurity (Johnson & Casagrande, 2016).

Even though biosafety and biosecurity represent different concepts, there is an undisputable engagement and complementary interaction between them (CDC, 2009). A biosafety program cannot be considered as safe and steady in the absence of biosecurity. The same holds true for biosecurity program in the absence of biosafety. For this reason, the risks stemming from pathogens should be analyzed with an integrated approach under the guidance of both biosafety and biosecurity (CDC, 2009; Salerno & Gaudioso, 2015; Weidmann, 2014; WHO, 2006). All of the organizations working with biological agents or toxins are supposed to establish safety and security (Gribble et al., 2015). As a consequence, laboratory studies focusing on uninvestigated topics related to SARS-CoV-2 should be carried out in laboratories with minimum BSL-3 under the guidance of biosafety and biosecurity.

Biosafety Measures: HVAC System

Compatibility of laboratory to biosafety practice is an essential approach for COVID-19. All the practices related to this disease should be conducted at laboratories equipped with proper device and equipment under the guidance of relevant technique and safety procedures by trained personnel (WHO, 2020b). To implement the studies safely within the working plan, every laboratory should conduct its own risk analysis to decide on suitable risk control measures (Astuto-Gribble & Caskey, 2014).

Risk assessment is a process that evaluates and collects data about the consequences and likelihood of hazardous pathogen release or exposure to hazard worked on. It is aimed to reduce the risk to an acceptable level by taking risk control measures determined by risk assessment. In this context, equipment used and procedures applied play a significant role in the emergence of risk at laboratories (WHO, 2020b).

The biosafety law in Turkey is more about risks coming from genetically modified organisms and products rather than laboratory biosafety (Resmi Gazete, 2010). There are many international guides to create the biosafety system and to identify biosafety measures (CDC, 2009; Solerna & Gaudioso, 2015; WHO, 2004; YALE University, 2019). Several guiding documents such as standards, handbooks, or manuals are aimed to explain and clarify the topic of biosafety. However, decision makers for a biosafety system should have their own understanding of the concept of biosafety before giving a final decision. This understanding consists of not only following rules but also harmonization of control measures to reduce risks. Therefore, the biosafety system has to be evaluated continuously, the system performance has to be determined based on the effectiveness of risk mitigation strategies, and the system has to be maintained in terms of assessment, mitigation, and performance model (Karagül, 2019). It is known that COVID-19 pandemic causes moderate to severe respiratory diseases and death. Transmission of the virus is possible through droplets, fomites, and air (WHO, 2020c). When the virus transmission route is taken into consideration, the HVAC system becomes significant for the BSL-3 laboratory studies of COVID-19. HVAC systems are the critical components of the initialization and certification of the laboratory. The convenient design and operation of the system are also very important for the proper use of biosafety cabinets (FAO, 2018). In COVID-19, being exposed to respiratory droplets (>5–10 μm) through eyes, nose, or mouth results in the disease. Airborne transmission might happen through droplet nuclei (aerosols < 5 μm). Transmission of the virus through air might occur during aerosol-generating procedures (WHO, 2020c). Equipment that might generate infectious aerosols must be kept in primary barrier devices such as a class 2 biosafety cabinet that will discharge air into the laboratory with high-efficiency particulate air (HEPA) filtration (CDC, 2020).

Presence of possible aerosol-generating procedures such as pipetting, vortexing, centrifugation, and mixing (WHO, 2012) at the BSL-3 laboratory indicates the importance of biosafety conditions deriving from the HVAC system for laboratory workers' health and the exhausted environment. It is possible to increase the reliability of central HVAC systems with the help of multiple air handling units and exhaust fans as they will provide redundancy (NIH, 2019).

The primary functions of the HVAC systems are to provide safe and comfortable working areas for all workers. These systems also protect the personnel, laboratory animals, and the community outside from hazardous agents and chemicals (NIH, 2019). Clean air supply into laboratory, discharge of the exhaust air, creating negative pressure differences between zones, establishment of proper air conditions, and ensuring inward directional air flow are among the other essential functions of the HVAC systems (Yücel et al., 2014).

HVAC systems should have the following characteristics to meet the criteria, such as maintaining the temperature and humidity at the required levels, serving without interruption, having an appropriate control system, preventing off-limit background noises and vibrations, and removing fumes, odors, and airborne contaminants (NIH, 2019).

Notifying the personnel with audible alarms and visual signs is as important as the HVAC system itself (WHO, 2006). Safety measures are built on shell-in-shell approach at BSL-3 laboratories, which provide multilayer barriers for the risky areas (Yücel et al., 2014). Although biosafety cabinets are thought to be the primary containment barriers, the laboratory itself constitutes the secondary containment barriers for practices with infection agents (CDC, 2009).

Dedicated Air Supply and Exhaust System

Having a dedicated ventilation system composed of supply and exhaust parts separately facilitates the isolation of the risk area in that it does not pose a risk through ventilation (UCOP, 2020). BSL-3 and ABSL-3 laboratories should have such kind of supply air systems, which specifically serve the risk area, and in this way, they do not cause cross contamination to other spaces outside the containment laboratories. Even BSL-3 and ABSL-3 laboratories should not use a common supply air system (NIH, 2014). The laboratories that require the same level HVAC systems may use common ventilation systems on the condition that isolation of each laboratory is provided by gas-tight dampers and HEPA filters (FAO, 2018). It should not be forgotten that there is always a necessary risk analysis and the result of this analysis behind these recommended actions.

It is not obligatory to conduct the HEPA filtration process to supply air to BSL-3 laboratories and ABSL-3 facilities; however, depending on the working program, using HEPA filtration for supply air should be considered (NIH, 2014). It is necessary to exhaust laboratory air of BSL-3 and BSL-4 laboratories without recirculation as it is considered to be potentially contaminated (UCOP, 2020; WHO, 2004). This kind of exhaustion of air is called dedicated, single-pass exhaust system (CDC, 2009; UCOP, 2020).

The exhaust air of BSL-3 laboratory must be released away from inhabited buildings and air intakes or it must be discharged with the help of HEPA filters (WHO, 2004). If high level of aerosol containment is required, HEPA filtration is a necessity for BSL-4, but it can be optionally carried out at BSL-3 as well (CDC, 2009). Even though HEPA filtration is not essential in all cases, the air system should be appropriate for revisions for the prospective work requiring HEPA-filtered exhaust air (NIH, 2014). The changing of HEPA filters must be carried out based on the requirements of bag-in bag-out principle. Moreover, the ventilation lines should have separate flaps so that changing filters will not shut down the whole laboratory ventilation (CDC, 2020; Hufert & Weidmann, 2014; UCOP, 2020). Only discharging laboratory exhaust air is not a sufficient precaution for the safety of the laboratory and the environment. Therefore, the exhaust laboratory air should be released away from supply air intakes so that it will not re-enter into the building air supply system (CDC, 2009; CDC, 2020; UCOP, 2020; Y.U., 2019). The exhaust stacks of the laboratories must be located at a minimum of 3 m above off the roofline and horizontally, the radius should be 4 m. At the discharge point, exhaust velocity must be at least 15–20 m/s (3000 ft/m) (UCOP, 2020; Wilson & Memarzadeh, 2006).

The exhaust air system dedicated for the laboratory should have pressure-independent constant-volume air terminal units, roof-mounted exhaust fans, and variable-frequency drives for filter installation. Exhaust air fans should be *N*+1, which refers to at least one more than what is needed. The same is also recommended for air supply fans (FAO, 2018; UCOP, 2020). All the exhaust lines must be gas tight for proper decontamination. There must be independent exhaust and supply air units for each laboratory room. This design provides constant pressure differences and room isolation during decontamination (NIH, 2014).

BSL-3 laboratories must possess supply and exhaust fans with interlock. With help of these interlocks, it can be possible to overcome reversing of air flow in the case of exhaust fan breakdown. When there is supply air fan failure, interlocks might prevent decreasing pressure dramatically (FAO, 2018; UCOP, 2020). Air system capacity should be appropriate for a 20% increase for a possible need in the future (NIH, 2019; UCOP, 2020).

Another function of the HVAC system is the dynamic interaction with the other components of the laboratory biosafety such as decontamination. For instance, the hood of the double-door autoclave located on the dirty side should be attached to the exhaust air system of BSL-3 (UCOP, 2020). This simple attachment shows that the laboratory biosafety, which includes HVAC and decontamination measures, should be managed via a complementary and supporting approach between all components of the safety system.

Directional Air Flow

Safety measures of the facility should support avoiding the release of agents unintentionally from the laboratory. Application of safety measures is very important, particularly for BSL-3 and BSL-4 laboratories, because fatal pathogens that need high-biosafety-level standards during the work might be transmitted by inhalation. Directional air flow, which is one of the

mentioned measures, prevents the spread of aerosol from the laboratory to other areas of the building. Directional air flow is associated with the integration of the HVAC system component (CDC, 2009). The air ventilation system provides the flow of air from the entrance toward the working area directly (Y.U., 2019). In this way, the air moves from the cleanest areas to the most possibly contaminated rooms (CDC, 2009; Y.U., 2019), which directs the air from the safest to the potentially most dangerous areas (UCOP, 2020). The airflow into the laboratory is built by release of the air, which is greater in volume than the supply air (CDC, 2009). Laboratory staff must be able to confirm the direction of the airflow (CDC, 2009; Y.U., 2009). This confirmation must be verified with the help of visual monitors. In addition, audible alarms must inform the stuff in the case of a possible air flow disruption (CDC, 2020; Wilson & Memarzadeh, 2006). Air flow alarms should inform the staff when the room pressure changes from negative to positive or when the door stays open for more than 20 s (Wilson & Memarzadeh, 2006). Digital or analog pressure indicators should be placed at the entrance of each pressure zone (UCOP, 2020). The directions of the laboratory air flow should be checked regularly with smoke tests (UCOP, 2020; Wilson & Memarzadeh, 2006; Y.U., 2019).

Pressure Differences between Zones

The air flow of the BSL-3 and ABSL-3 facilities that moves from the expected clean areas to contaminated areas is related to the pressure differences between zones (NIH, 2014; Wilson & Memarzadeh, 2006). There should be an ideal –12.5 Pa pressure difference between clean and contaminated areas (NIH, 2014; UCOP, 2020; Wilson & Memarzadeh, 2006). However, the pressure difference should not be less than 7.6 Pa under any conditions (Wilson & Memarzadeh, 2006). The aim of the ideal pressure difference is to avoid spread of the pathogens to the other areas in case of the pathogen release from biosafety cabinets (UCOP, 2020).

When there are a lot of containment areas, more negative pressure values are supplied sequentially in the laboratory. In this way, more negative pressure is kept steady in the more contaminated areas compared with the clean areas. Visual indicators and alarms should be in use in order to maintain the pressure difference. These devices should be present at the entrance of the containment area, in anterooms, and in every single room of the containment area (NIH, 2014; Wilson & Memarzadeh, 2006). To provide the negative pressure sequencing, an airflow difference at the level of 47 L/s (100 cfm) is needed (NIH, 2019; UCOP, 2020).

In short, the supply and exhaust units of the ventilation system should keep the laboratory at negative pressure to provide gradual pressure differences between neighboring zones and directional air flow, which is the expected outcome (CDC, 2009; FAO, 2018). For this outcome, the exhaust system capacity has to be 15% more than the supply air (Y.U., 2019).

Makeup Air Capacity and Ventilation Rates

BSL-3 laboratories have to supply at least 6 air changes per hour (ACH) (NIH, 2014; NIH, 2019; UCOP, 2020, Wilson & Memarzadeh, 2006), while 12–15 ACH is also one of the recommendations (Y.U., 2019). For instance, 6–12 ACH is the recommended value for Tuberculosis Laboratory (WHO, 2012). This value rises up to 10–15 ACH in animal facilities. Required makeup air at a minimum rate should be generated at all times even when the laboratory is not fully active (NIH, 2014; NIH, 2019; Wilson & Memarzadeh, 2006). These ventilation rates are not only required to remove airborne contaminants safely, but they also support managing heat and odor issues (NIH, 2014; Wilson & Memarzadeh, 2006).

ACH indicates the required duration for the effective discharge of the airborne contaminants. For instance, the required duration for the removal of aerosols by 99% is 46 min with 6 ACH. This duration will decrease to 23 min with 12 ACH (Buchan et al., 2019; CDC, 2019). These rates primarily state the duration before entering the evacuated laboratory after a large amount of spill occurs.

There is a correlation between ACH and airborne pathogens at the laboratory. If ACH rates increase, it directly affects the possibility of airborne pathogen transmission. There are some factors involving humidity, aerosol diameter, infective dose, and air turbulence that should be taken into consideration for the determination of the best ACH for the laboratory (Buchan et al., 2019). There are other issues such as fume hood need, cooling load, pressurizing the area, and getting rid of fumes and odors, which are used to calculate the ventilation rate. Therefore, the device infrastructure and cooling need for the heat generated from equipment are also significant for the ventilation rate (NIH, 2019).

Biosecurity System

Contagious diseases are still responsible for death at the global level, and they constitute one-third of the death causes all around the world (Salerno & Gaudioso, 2015). At present, humanity is facing the intercontinental impact of the agent of another transmissible disease. Apart from causing diseases, biohazard agents also have the potential to be biological weapons. This unwelcome potential is warning people about the risk of bioterrorist attacks via these agents. A recent case, which is known as Anthrax attack or "Amerithrax," occurred in 2001, could be considered as an example for such kind of biological attacks (Salerno & Gaudioso, 2015). The necessity of biosecurity measures on farms because of the potential role of microorganisms in agroterrorism has also been stated (Kelly, 2005; WHO, 2004). The recent incidents in the global level have indicated the need to protect the laboratories and biological materials (WHO, 2004). High-level biosafety laboratories might be the target of bioterrorist attacks because of the pathogen handling in them. Therefore, biosafety and biosecurity measures should be followed strictly at BSL-3 laboratories, which are being used to struggle against COVID-19.

Laboratory biosecurity has got a shorter history than biosafety (Astuto-Gribble & Caskey, 2014). Estimated biosafety level is generally thought to be equal to the biosecurity level. However, this approach is not considered as correct, particularly for biosecurity (Salerno & Gaudioso, 2015). Laboratory biosecurity measures are aimed to avoid theft, misuse, loss, and intentional release of microorganisms (Buchan et al., 2019; CDC, 2009). In this respect, biological waste should not be lost or stolen either (Buchan et al., 2019).

Biosecurity is a discipline, which focuses on the security of microbiological agents and toxins and on their intentional misuse or release. Moreover, it addresses the things threatening humans, animals, environment, and economy (CDC, 2009). Unlike biosafety, biosecurity aims to prevent people with bad intentions from reaching biological materials rather than avoiding the transmission of hazardous and also valuable biological materials to humans (CDC, 2009; Salerno & Gaudioso, 2007).

All hazardous biological materials have got dual uses: they have both medical and research-related uses, and they might be exploited to cause infectious diseases deliberately (Salerno & Gaudioso, 2007). Briefly, biosafety mainly aims to protect people from microorganisms. By contrast, biosecurity intends to protect microorganisms from people with bad intentions. Biosecurity, which is the starting point of the need to protect the microorganisms that scientists investigate, is the basic component of the biorisk management.

Biosecurity Precautions

A risk analysis should be conducted for biosecurity as well as for biosafety (Salerno & Gaudioso, 2007). In some cases, there might be conflicts between biosafety and biosecurity practices. The warning signs at the entrance of the laboratories might serve as examples for these conflicts. These biohazard signs are used to warn people against the hazards in laboratories; however, they might not be in line with the security objectives all the time (CDC, 2009).

High-level biosafety laboratories might be the target of the bioterrorist attacks; therefore, such kind of informative signs could be considered to be a biosecurity gap by showing the risky areas. In this sense, both biosafety and biosecurity approaches should be managed simultaneously without unbalancing biorisk management. Taking combined safety and security measures, which are identified through risk assessment, might be the best recipe against conflicts (CDC, 2009).

Laboratory biosecurity is composed of five essential items: physical security, personnel security, material control, transport security, and information security (Caskey & Sevilla-Reyes, 2015; CDC, 2009; Salerno & Gaudioso, 2007; WHO, 2006). Some of the components are common for both biosafety and biosecurity (CDC, 2009). However, as they focus on different risks, the aim, content, and performance of their mitigation measures may differ from each other. Biosecurity measures must be practiced more strictly for high-risk scenarios. In other words, higher risk pathogens need more security applications than low risk ones (Salerno & Gaudioso, 2007).

Physical security aims to minimize the risk of access to containment areas without authorization. To achieve this, several different elements such as putting physical barriers, access control, and detection of trespassing and alarms must be evaluated (Salerno & Gaudioso, 2007; WHO, 2006). Physical biosecurity includes engineering and the personnel responsible for the structure of the building and its security (WHO, 2006).

Personnel security is a measure focusing on the insider threat. It does not aim to provide the security of the personnel but rather secures the system against threats stemming from the personnel. In this way, only the trusted staff is allowed to enter the restricted areas (Salerno & Gaudioso, 2007). Appointing the laboratory staff at suitable positions may mitigate the risks that might stem from unintentional and intentional actions (Salerno & Gaudioso, 2007; WHO, 2006).

The aim of material control and accountability is to establish a discouraging environment for the insiders who intend to steal or use agents for harmful reasons (Salerno & Gaudioso, 2007). Regular updates of inventories, selection of access personnel, documentation of material transfers, inactivation, and disposal of the material are among the applications of material control and accountability (WHO, 2006). Even though it is not possible to keep track of every single microorganism in the laboratories, it might be possible to take the necessary precautions to prevent the theft of these materials from the laboratories (Salerno & Gaudioso, 2007).

Transport security is a mechanism that reduces the theft risk stemming from insider and outsider threats via material control and accountability during transfer of the material from one restricted area to another. Transportation of the material could be carried out both in the facilities and between national and international institutions. The exchange of biological materials could be made for different reasons by scientists, health institutions, and diagnostic laboratories (Salerno &Gaudioso, 2007; WHO, 2006). The materials become more prone to theft and tampering when transported out of the restricted area (Salerno & Gaudioso, 2007).

Information security includes some policies and practices used to protect the critical knowledge (Salerno & Gaudioso, 2007; WHO, 2006). When such kind of information is accessible to people, it will reveal the ways of overcoming the laboratory biosecurity system in the case of theft attempts for these biological materials. Therefore, the protection of this critical and sensitive information is an important security measure (Salerno & Gaudioso, 2007). The first step of establishing information security is to define the sensitive information. Such kind of information should be confidential both for the public and for unauthorized bodies (CDC, 2009; Salerno & Gaudioso, 2007).

Laboratory security plans, inventories, and the locations of biological materials might be given as examples for such sensitive information (CDC, 2009; WHO, 2006).

Biosecurity measures should be a part of the usual practices of laboratories like other microbiological work. These measures should not prevent the effective sharing of reference materials, clinical, and epidemiological samples for studies related to clinical and health studies (WHO, 2004).

Conclusion

Both managing biosafety and biosecurity at BSL-3 laboratories and fighting against pandemic might be thought to have similar workflows. In both of them, the success depends on the constant and complete implementation of all the necessary duties without showing tolerance. Nowadays, the world is at a critical time that sharing knowledge at national and international levels provides tremendous health benefits while fighting against COVID-19 pandemic. In this sense, while struggling against this pandemic at laboratories under the biosafety and biosecurity barriers, sharing of all details related to possible remedies is a necessity for humanity to win this fight.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.S.K., M.H., F.S.; Design – M.S.K., M.H., F.S.; Supervision – M.H.; Resources – M.S.K., F.S.; Materials – M.H., F.S.; Data Collection and/or Processing – M.S.K., M.H., F.S.; Analysis and/or Interpretation – M.S.K., M.H., F.S.; Literature Search – M.S.K., M.H., F.S.; Writing Manuscript – M.H., M.S.K.; Critical Review – M.S.K., M.H., F.S.; Other – M.S.K., M.H., F.S.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: The authors declare that this study has received no financial support.

References

Abacıoğlu, Y. H., & Sönmez, C. (2014). *Ulusal Mikrobiyoloji Standartları Laboratuvar Güvenliği Rehberi*. Türkiye Halk Sağlığı Kurumu, Sağlık Bakanlığı Yayın No: 937, Ankara. https://hastane.ksu.edu.tr/depo/belgeler/Ulusal%20Mikrobiyoloji%20Standartlar%C4%B1.compressed_1710261701460340.pdf (Accessed on 25.10.2020)

Ahmad, T., Haroon, Dhama, K., Sharun, K., Khan, F. M., Ahmed, I., Tiwari, R., Musa, T. H., Khan, M., Bonilla-Aldana, D. K., J Rodriguez-Morales, A., & Hui, J. (2020). Biosafety and biosecurity approaches to restrain/contain and counter SARS-CoV-2/COVID-19 pandemic: a rapid-review. *Turkish Journal of Biology*, *44*(3), 132-145. [Crossref]

Ahmad, T., & Hui, J. (2020). One health approach and coronavirus disease 2019. *Human Vaccines & Immunotherapeutics*, *16*(4), 931-932. [Crossref]

Astuto-Gribble, L. M., & Caskey, S. A. (2014). *Laboratory Biosafety and Biosecurity Risk Assessment Technical Guidance Document*. United States. doi:10.2172/1171429. https://www.osti.gov/biblio/1171429-laboratory-biosafety-biosecurity-risk-assessment-technical-guidance-document (Accessed on 25.10.2020) [Crossref]

Buchan, B., Relich, R. F., & Mahlen, S. D. (2019). *Interim Clinical Laboratory Guideline for Biological Safety the American Society for Microbiology*. https://asm.org/ASM/media/Policy-and-Advocacy/Biosafety-white-paper-2019.pdf

BVL, 2013. *BVL-Federal Office of Consumer Protection and Food Safety*, Bekanntmachung der Liste risikobewerteter Spender- und Empfängerorganismen für gentechnische Arbeiten BVL 112/2013/4 (Accessed on : 25.10.2020). http://www.bvl.bund.de/SharedDocs/Downloads/06_Gentechnik/register_datenbanken/organismenliste_pdf.pdf?__blob=publicationFile&v=7.

Caskey, S., & Sevilla-Reyes, E. E. (2015). Risk assessment. In R. M. Salerno, J. Gaudioso (Eds.), *Laboratory Biorisk Management, Biosafety and Biosecurity* (pp.45-63). CRC Press, New York. [Crossref]

CDC, (2009). *Biosafety in Microbiological and Biomedical Laboratories*. HHS Publication No. (CDC) 21-1112. https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF (Accessed on 25.10.2020).

CDC, (2019). *Guidelines for Environmental Infection Control in Health-Care Facilities Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC).* https://www.cdc.gov/infectioncontrol/pdf/guidelines/environmental-guidelines-P.pdf (Accessed on 25.10.2020).

CDC, (2020). *Inspection Checklist forBSL-3 Laboratories* (7 CFR 331; 9 CFR 121; 42 CFR 73; BMBL 5th Edition). https://www.cdc.gov/selectagent/resources/Checklist-BSL3.pdf (Accessed on 25.10.2020).

Ceyhan, İ. (2005). Biyogüvenlik Laboratuvar Seviyeleri ve Biyogüvenlik Kabinlerinin Seçimi Kullanımı ve Bakımı. In M. Günaydın, A. Saniç, B. Gürler (Eds.), *Proceedings of 4. Ulusal Sterilizasyon Dezenfeksiyon Kongresi* (pp.608-633). https://das.org.tr/kitaplar/kitap2005/57-05.pdf (Accessed on 25.10.2020).

Council Directive, (2000). *On the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC),2000/54/EC* Official Journal L262, 43, 21-45.

CWA, (2011). *European Committee for Standardization Workshop Agreement 15793, Laboratory Biorisk Management Standard.* https://internationalbiosafety.org/wp-content/uploads/2019/08/CWA-15793-English.pdf (Accessed no 25.10.2020).

FAO, (2018). Biosafety Primer 2018. Bangkok. pp. 120. http://www.fao.org/3/i6800en/I6800EN.pdf (Accessed on 25.10.2020).

Frey, J. (2013). Classification of Organisms Part 1: Bacteria. Federal Office for the Environment FOEN, Federal Office of Public Health FOPH. FOEN/FOPH 2013. https://www.bafu.admin.ch/bafu/en/home/topics/biotechnology/publications-studies/publications/classification-of-organisms.html. (Accessed on: 25.10.2020).

Gribble, L. A., Tria, E. S., & Wallis, L. (2015). The AMP Model. In R. M. Salerno, J. Gaudioso (Eds.), *Laboratory Biorisk Management, Biosafety and Biosecurity* (pp. 31-43). CRC Press New York. [Crossref]

Hasöksüz, M., Kiliç, S., & Saraç, F. (2020). Corona viruses and SARS-CoV-2. *Turkish Journal of Medical Sciences*, *50*, 549-556. [Crossref]

HSE, (2013). HSE-Health and Safety Executive. *The Approved List of Biological Agents Advisory Committee on Dangerous Pathogens*. https://www.hse.gov.uk/pubns/misc208.pdf (Acessed on: 25.10.2020)

Hufert, F. T., & Weidmann, M. (2014). Technical and Practical Aspects of BSL-3 Laboratories In M. Weidmann, N. Silman, P. Butaye, & M. Elschner, (Eds.), *Working in Biosafety Level 3 and 4 Laboratories:*

A Practical Introduction (pp 21-29). Wiley Blackwell, Menheim. [Crossref]

Johnson, B., & Casagrande, R. (2016). Comparison of International Guidance for Biosafety Regarding Work Conducted at Biosafety Level 3 (BSL-3) and Gain-of-Function (GOF) Experiments. *Applied Biosafety*, *21*(3), 128-141. [Crossref]

Karagül, M. S. (2019). *Assessment, Mitigation, Performance Model in Biorisk Management [Biyorisk Yönetimin Değerlendirme, Azaltma, Performans Modeli]*. In A. Özkan, S. Biroğul, Ö. Güngör, M. Of, B. Demir, Ç. Taşdemirci (Eds.), *Proceedings of International Marmara Sciences Congress* pp.133-138.

Kelly, A. M. (2005). Veterinary Medicine in the 21st Century: The Challenge of Biosecurity. *ILAR Journal*, *46*(1), 62-64. [Crossref]

Kelly, H. (2011). The classical definition of a pandemic is not elusive. *Bulletin of the World Health Organization*, *89*(7), 540-541. [Crossref]

Lacroix, V. (2012). *All about Pandemics (Epidemic Infectious of Disease)*. University Publishing, Delhi.

NIH, (2014). *BSL-3 and ABSL-3 HVAC System Requirements - Part I*. The National Institues of Health 1, 51. Division of Technical Resources https://www.orf.od.nih.gov/TechnicalResources (Acessed on: 25.10.2020)

NIH, (2016). *National Institute of Health Guidelines: Guidelines for research involving recombinant or synthetic nucleic acid molecules*. https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.pdf (Accessed on 25.10.2020).

NIH, (2019). *Design Requirement Manual. Division of Technical Resources*. https://www.orf.od.nih.gov/TechnicalResources/Documents/DRM/DRM1.503262020.pdf (Acessed on: 25.10.2020) [Crossref]

OECD, (1998). *Series on Principles of Good Laboratory Practice and Compliance Monitoring*. ENV/MC/CHEM(98)17. http://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?doclanguage=en&cote=env/mc/chem(98)17 (Accessed on 25.10.2020)

OIE, (2012). *Biosafety and biosecurity in the veterinary microbiology laboratory and animal facilities*. Terrestrial Manual Chapter 1.1.3. https://caninerabiesblueprint.org/IMG/pdf/1.01.03_biosafety_biosecurity_vet_microbiology_lab_new.pdf (Accessed on 25.10.2020)

OIE, (2018). *Standart for managing biological risk in the veterinary laboratory and animal facilities.* Terrestrial Manual 2018 Chapter 1.1.4. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/1.01.04_BIOSAFETY_BIOSECURITY.pdf

Resmi Gazete, (2010). *Biyogüvenlik Kanunu* 5997. https://www.resmigazete.gov.tr/eskiler/2010/03/20100326-7.htm (Accessed on 25.10.2020)

Salerno, R. M., & Gaudioso, J. (2007). Laboratory Biosecurity Handbook. CRC Press, Taylor & Francis Group, New York. [Crossref]

Salerno, R. M., & Gaudioso, J. (2015). *The Case for Biorisk Management*. In R. M. Salerno, J. Gaudioso (Eds.), *Laboratory Biorisk Management, Biosafety and Biosecurity* (pp. 01-30). CRC Press New York. [Crossref]

Şeker, E., & Yardımcı, H. (2003). Mikrobiyoloji laboratuvarlarında biyogüvenlik. *Orlab On-Line Mikrobiyoloji Dergisi*, *01*(04), 3-32.

Silman, N. (2014). Hazard Criteria and Categorization of Microbes Classification Systems. In M. Weidmann, N. Silman, P. Butaye, & M. Elschner, (Eds.) Working in Biosafety Level 3 and 4 Laboratories: A Practical Introduction (pp. 13-20). Wiley Blackwell, Menheim. [Crossref]

UCOP, (2020). *University of California Office of the President. Biosafety Level 3 (BSL-3) Laboratory Design Standards*. https://www.ucop.edu/safety-and-loss-prevention/_files/hcloc/uc-bsl3-design-standards-final.pdf (Accessed on 25.10.2020).

Weidmann, M. (2014). Introduction. In M. Weidmann, N. Silman, P. Butaye, & M. Elschner, (Eds.), *Working in Biosafety Level 3 and 4 Laboratories A Practical Introduction* (pp. 1-3). Wiley Blackwell, Menheim. [Crossref]

WHO, (2004). Laboratory Biosafety Manual. 3rd ed. Geneva. https://www.who.int/publications/i/item/9241546506 (Accessed on 25.10.2020)

WHO, (2006). Biorisk management Laboratory biosecurity guidance. WHO/CDS/EPR/2006.6 https://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2006_6.pdf (Accessed on 25.10.2020).

WHO, (2012). Tuberculosis Laboratory Biosafety Manual. WHO/HTM/TB/2012.11, WHO Press, Geneva. https://www.who.int/tb/publications/2012/tb_biosafety/en/ (Accessed on 25.10.2020).

WHO, (2020a). Interim Guidance. Laboratory testing for corona virus disease 2019 (COVID-19) in suspected human cases. WHO/COVID-19/laboratory/2020.4 https://apps.who.int/iris/handle/10665/331329 (Accessed on 01.03.2020).

WHO, (2020b). Interim Guidance. Laboratory biosafety guidance related to corona virus disease (COVID-19). WHO/WPE/GIH/2020.3 https://apps.who.int/iris/handle/10665/332076 (Accessed on 25.10.2020).

WHO, (2020c). Scientific brief. Transmission of SARS-CoV-2: implications for infection prevention precautions. https://www.who.int/publications/i/item/modes-of-transmission-of-virus-causing-covid-19-implications-for-ipc-precaution-recommendations (Accessed on 25.10.2020)

Wilson, D. E., & Memarzadeh, F. (2006). National Institutes of Health Biosafety Level 3 Laboratory Certification Requirements. https://www.orf.od.nih.gov/TechnicalResources/Bioenvironmental/Documents/BSL3CertificationGuidelinesFINAL_508.pdf (Accessed on: 25.10.2020)

Wittek, R., Dorsch-Häsler, K., & Link, J. (2013). Classification of Organisms Part 2: Viruses. Federal Office for the Environment FOEN, Federal Office of Public Health FOPH. FOEN/FOPH 2013. https://www.bafu.admin.ch/bafu/en/home/topics/biotechnology/publications-studies/publications/classification-of-organisms.html. (Accessed on: 25.10.2020)

Y.U. - YALE University, (2019). Yale Environmental Health & Safety Biological Safety BSL3 Laboratory Manual. https://ehs.yale.edu/sites/default/files/files/bsl3-lab-manual.pdf (Accessed on: 25.10.2020)

Yücel, F., Ülbeği Polat, H., Akçael, E., & Deniz, T., (2014). Establishment of biosafety level 3 laboratory infrastructure to study with highly pathogen organisms and principles of operation: Review. *Turkiye Klinikleri Journal of Medical Sciences*, *34*(1), 120-36. [Crossref]