

Review Article

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Biorisk assessment for infrastructure & biosafety requirements for the laboratories providing coronavirus SARS-CoV-2/(COVID-19) diagnosis

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Novel coronavirus infection [coronavirus disease 2019 (COVID-19)] has spread to more than 203 countries of various regions including Africa, America, Europe, South East Asia and Western Pacific. The WHO had declared COVID-19 as the global public health emergency and subsequently as pandemic because of its worldwide spread. It is now one of the top-priority pathogens to be dealt with, because of high transmissibility, severe illness and associated mortality, wide geographical spread, lack of control measures with knowledge gaps in veterinary and human epidemiology, immunity and pathogenesis. The quick detection of cases and isolating them has become critical to contain it. To meet the increasing demand of the diagnostic services, it is necessary to enhance and expand laboratory capabilities since existing laboratories cannot meet the emerging demand. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a BSL-2 (Biosafety Level 2) agent and needs to be handled in biosafety cabinet using standard precautions. This review highlights minimum requirements for the diagnostic laboratories opting testing of material for the diagnosis of COVID-19 and associated biorisk to the individuals and to the community.

Key words Biorisk - biosafety - diagnosis - infrastructure - laboratories - novel coronavirus

Introduction

Coronaviruses are enveloped viruses with non-segmented positive-sense RNA, widely distributed in humans and animals^{1,2}. Initially, infections caused by several human coronaviruses (HCoVs) were only mild and hence were considered as neglected pathogens. After the emergence of highly pathogenic severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1)

(2002 and 2003) and Middle East respiratory syndrome coronavirus (MERS-CoV, 2012)³, it has become obvious that coronaviruses can cross the species barrier and cause life-challenging infections in human, thus needing greater attention than the initial HCoVs⁴.

Recently, another pathogenic HCoV was identified in Wuhan, People's Republic of China, which was initially named as 2019 novel

coronavirus (2019-nCoV)⁵. But later, it has been named as SARS-CoV-2 by the International Committee on Taxonomy of Viruses (ICTV)⁶. This virus causes coronavirus disease 2019 (COVID-19). The clinical presentation of infection ranges from asymptomatic to very severe pneumonia with acute respiratory distress syndrome, septic shock and multi-organ failure resulting in death⁷. As of March 2020, SARS-CoV-2 has spread to more than 203 countries of various regions including Africa, America, Europe, South East Asia and Western Pacific alarming public health authorities around the world⁸.

Since, the first case reported on December 31, 2019, the WHO has been notified with more than 100,000 confirmed cases including 3,380 deaths globally as on March 6, 2020, of whom 90 per cent (3,045) were from China itself, while the remaining 10 per cent (335) were from other countries⁹. The SARS-CoV-2 is now one of the top-priority pathogens to be dealt with, because of high fatality rate in severe cases, spread in a wide geographical area, lack of control measures and knowledge gaps in its epidemiology, immunity and pathogenesis. Currently, there are no licensed vaccines or therapies specific to COVID-19. Hence, the WHO has initially declared COVID-19 as the global public health emergency¹⁰ and subsequently as pandemic¹¹.

Because of the rapid spread of this virus, it has become necessary to enhance laboratory capabilities to provide immediate diagnostic assistance as the load of samples from suspected patients is increasing on a daily basis and existing laboratories cannot meet the demands. The objective of this review is to highlight various requirements for the diagnostic laboratories involved in the testing of SARS-CoV-2 and also describe measures to mitigate the risk factors involved in laboratories that are providing molecular diagnosis, so that more laboratories become available for providing quick diagnosis under all safety precautions.

Sample of choice

Coronaviruses are mainly responsible for respiratory tract infections resulting in symptoms such as common flu. Choice of sample for detection will be respiratory samples including clinical material from the upper and lower respiratory tracts depending on the symptoms and condition of the patient^{12,13}. For SARS-CoV-2, shedding patterns are not well understood and further investigations are required to understand the timing, compartmentalization and magnitude of

virus shedding. However, the virus may be detectable in other specimens including blood and urine as in cases of SARS-CoV-1 and MERS-CoV¹⁴⁻¹⁶. The mean incubation period for SARS-CoV-2 is 5.2 days; however, it may vary widely depending on severity of illness¹⁷.

Specimen collection

Only trained staff should be allowed for appropriate specimen collection, storage, packaging and transport, ensuring that adequate standard operating procedures in consonance with the national or the WHO guidelines¹⁸ are in use, and all specimens should be treated as potentially infectious.

Diagnosis

Suspected cases should be tested for the virus with nucleic acid amplification tests, such as real-time reverse transcription - polymerase chain reaction (RT-PCR) with confirmation by nucleic acid sequencing when needed. Viral RNA extraction should be done in a biosafety cabinet in a BSL-2 or equivalent facility, which will be used further for amplification of genes targeted including nucleocapsid (*N*), spike (*S*), envelope (*E*), and RNA-dependent RNA polymerase (*RdRp*)¹⁸. Serological tests are still under development, and once these become available, field surveys will aid in better understanding of the outbreak, implementation of control measures and also understanding cross-reactivity with other viruses.

Infrastructure needed

Suspected samples should be handled at initial phase in a biosafety cabinet by well-trained staff with respect to standard BSL-2 facility. National guidelines on laboratory biosafety should be followed in all circumstances¹⁹. At present, very limited information is available on the risk posed by COVID-19, therefore, all procedures should be undertaken based on risk assessment.

Specimen handling for molecular testing of COVID-19 would require BSL-2 or equivalent facilities. These facilities include separate hand and eye wash sinks, and these also need to have automatic door locking systems. The BSL-2 laboratories should have access to facility of decontamination, including an autoclave¹⁹.

It is recommended that good microbiological laboratory practices and universal precautions must be followed in all laboratories where primary specimens

(such as sputum, throat swab, nasopharyngeal swab, oropharyngeal swab, and stool) that may contain SARS-CoV-2 virus, are handled. While working with suspected patient's samples, laboratory personnel should be supervised by staff competent in handling infectious agents and related standard procedures¹⁹. The list of basic laboratory equipment and reagents required for providing laboratory diagnosis for COVID-19 is provided in Table I.

Biosafety measures

In most cases, SARS-CoV-2 is transmitted from human to human through inhalation or deposition on mucosal surfaces of large respiratory droplets. Other routes identified are contact with contaminated fomites and inhalation of aerosol, generated during handling of large volumes, *etc*²⁰. For the laboratories involved in the diagnosis of COVID-19, it is necessary that staff should be well trained in the implementation of appropriate biosafety measures. The rational, correct and consistent use of available personal protective equipment (PPE) and appropriate hand hygiene help to reduce the spread of the pathogens. Though PPE is considered as a primary prevention strategy, it should not be completely relied upon for complete prevention for virus transmission. The effectiveness of PPE depends upon proper handling of PPEs by trained staff, hand hygiene practices and human factor²¹⁻²⁴. Immunization policy for influenza would also help in

Table I. List of laboratory equipments and reagents required for laboratory diagnosis of coronavirus disease 2019 (COVID-19) and personal protective equipment (PPE) for carrying out COVID-19 molecular test

Sr. No.	Details of PPE and equipment
1.	Disposable, back closure laboratory gowns
2.	Face mask and head cap
3.	Disposable gloves
4.	Closed-toe footwear
5.	Protective eyewear
6.	Protective laboratory coats
7.	Disposable shoe covers
8.	Centrifuge tube 15 ml sterile (250 tubes/pack)
9.	Centrifuge tube 50 ml sterile (150 tubes/pack)
10.	Microcentrifuge tube (1.5 ml)
11.	Micropipettes of variable volumes
12.	Sterilized filter tips
13.	Vortex
14.	Mini spin
15.	Small high-speed centrifuge for RNA extraction process
16.	Cold centrifuge for sample processing
17.	Plate spinner
18.	Real-time PCR machine
19.	Biosafety cabinet class 2 type II

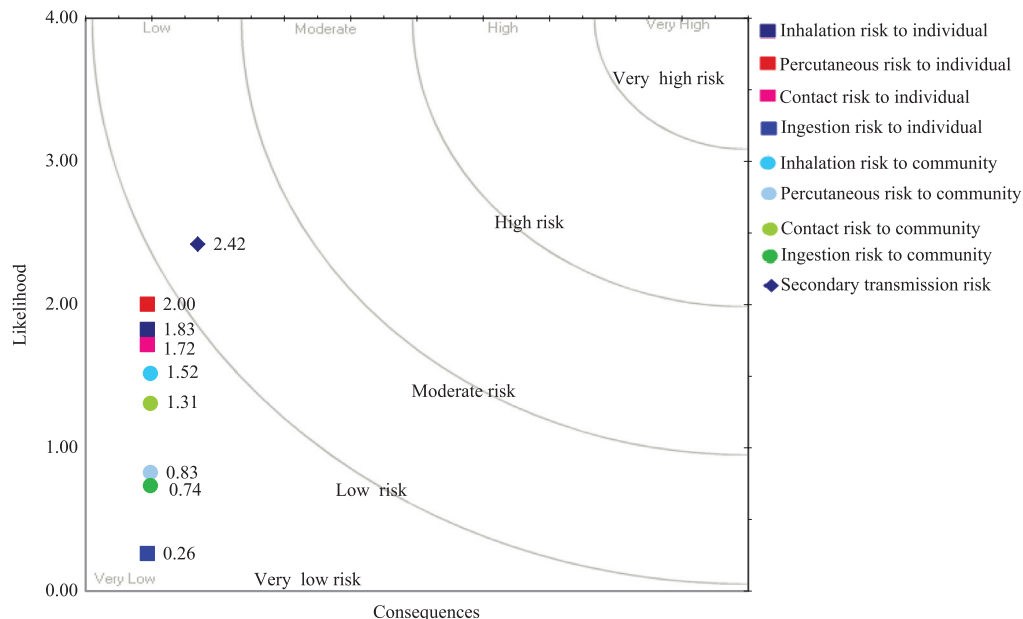


Figure. Biorisk assessment for individual laboratory personnel and community with regard to providing molecular diagnosis for coronavirus disease 2019. Likelihood of secondary transmission to human is on moderate risk, in case laboratory personnel get infected while handling infected material.

Table II. Basic biosafety requirements for the laboratories (some important features of procedures and processes to be followed during processing samples for coronavirus disease 2019 laboratory diagnosis)

Sr. No.	Requirements
1.	Personnel wear dedicated laboratory clothing (<i>e.g.</i> , scrubs) which should not be worn outside the laboratory, anteroom or change room
2.	Primary containment devices should always be used in this procedure, these should be validated/certified and well-maintained and there are procedures in place for proper use
3.	Type of material to be used in this procedure for diagnostic samples should be up to 250 ml volumes. Absorbent materials should be used on the bench or BSC to contain spills and reduce splashing
4.	Proper practices for reducing/eliminating aerosols should be identified in the laboratory procedures; should be taught and verified on a regular schedule
5.	The measures should be in place to reduce infectious aerosols exiting the laboratory, all the aerosolization procedures and processes should be conducted in the biosafety cabinets and, during open bench, proper PPEs should be worn; depending on the risk assessment, respirators (<i>e.g.</i> , N95, N100 and PAPR), goggles and face shield should be used
6.	Since all such procedures will be performed in biosafety cabinets, and being small volumes of samples handled, there will be very low potential and extent of a splash or spill in this procedure, however, personnel must be trained on biosafety and should have laboratory procedures in place during spill or splash
7.	Biosafety cabinets should always be used, these should be routinely validated/certified and well-maintained and there are procedures in place for proper use
8.	Contaminated waste should be safely and efficiently treated within laboratory and should be stored in the laboratory, till disposed properly
9.	No sharps should be used in these laboratory procedures
10.	All surfaces in the laboratory should be easy to clean and decontaminated. No equipment should be maintained or repaired without decontamination, and the process should be documented and validated
11.	The laboratory should have a complete and well-maintained inventory system. It should also have an active shipping and receiving programme and well-defined procedures and plans in place
12.	There should be medical surveillance programme in place
13.	Laboratory should implement standard laboratory practices for safety
14.	There should be defined procedures in place for entry into the laboratory
15.	Institution/laboratory should have defined roles and responsibilities for biosafety and should also be commitment to safety as well as comprehensive biosafety documentation and should conduct biosafety drills or exercises

BSC, biological safety cabinet; PAPR, powered air-purifying respirator

giving protection to laboratory workers and reduce the suspicion of the staff to be getting infection in such emergency situation. Basic biosafety requirements for the laboratories that include important features of procedures and processes to be followed during processing the samples for COVID-19 laboratory diagnosis are provided in Table II.

Biorisk assessment carried out for individual laboratory personnel and to the community with regard to providing molecular diagnosis for COVID-19 is provided in the Figure. This biorisk assessment will also provide guidance for the future laboratories that are opting to provide laboratory diagnosis for this infection.

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Conflicts of Interest: None.

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