Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-COV-2) is a virus responsible for the outbreak of COVID-19, which began in Wuhan, China in December 2019. It is a disease-causing human pathogen. Common symptoms of COVID-19 in human include fever and respiratory symptoms (e.g. cough, shortness of breath or difficulty of breathing). Other less common symptom includes diarrhea. The symptoms can range from mild to severe. Most cases present mild symptoms except for elderly and immunocompromised which constitute a higher percentage of mortalities due to pneumonia related complications. Travel associated cases from China was initially the primary source of exposure which eventually spread to local exposure primarily due to human-to-human transmission. The statistics is scoring every hour in an alarming record while the scientific community is pooling their resources to characterize the extent of the pathology and mitigation strategies of management including diagnosis, containment and treatment models including vaccine development.

Laboratory diagnosis primarily from clinical specimens, contain lower concentration of pathogens than found in cultures. These include blood, urine, feces, swabs and tissues collected from patients (PUI and confirmed COVID-19 positive). For routine diagnosis, nasopharyngeal swabs (NPS) and oropharyngeal swab (OPS) are collected. It is recommended that, at a minimum, laboratory must strictly enforce its compliance with Biosafety Level 2 guidelines based on WHO Biosafety Manual 3rd Edition, specifically in work areas where primary specimens are handled and tested. Enhancements are added based on local risk assessment.

Laboratory manipulation of clinical specimen for in vitro and in vivo propagative activities (e.g. viral culture, animal inoculation, viral isolation and concentration techniques) from Person Under Investigation (PUI) and laboratory confirmed COVID-19 positive patients is strictly prohibited and strongly discouraged. These laboratory procedures require a certified and properly maintained and functional BSL 3 facility and authorization for purposes of biosecurity.

Routine diagnostic tests and other non-viral propagative research procedure can be safely conducted in BSL 2 facility with enhancements based on local risk assessment. Enhancements include a directional airflow, clean to dirty work flow, working in buddy system, strict monitoring of body temperature, equipment and facility, training and proficiency, restriction of work to authorized personnel only and use of fit tested NIOSH-approved filtering face piece respirator that provides a level of filtration of 95% or greater, e.g. N-95 or its equivalent.
A combination of engineering, administrative, practices and PPE mitigates the risk of exposure, infection, release and unintentional spread of contamination. Below are the mitigation measures recommended for laboratories handling and testing specimen for laboratory diagnosis of COVID-19 based on risks identified per procedure.

1. Engineering

1.1. Air Handling

The air must flow from an area of lower contamination to an area of higher contamination (inward directional airflow). Supply air must be located away from BSC where it may disrupt the curtain of air at the front grille of the cabinet. This must be checked daily prior to use as part of the maintenance record.

1.2. Biological Safety Cabinet

BSC provides effective primary containment for work with infectious materials when they are certified, properly maintained and used in conjunction with good laboratory techniques. A continuous stream of inward air known as the inflow prevent aerosols from escaping through the front opening the exhaust air is HEPA filtered to protect the environment. High Efficiency Particulate Air (HEPA) filters are capable of filtering greater than 99.97% of airborne particles smaller or larger than 0.3μm in diameter. BSC is efficient in providing both the user and product protection if used according to guidelines.

Biosafety Cabinets must be located away from areas where movements that affects airflow pattern could disrupt the fragile air curtain at the front of the cabinet. Adequate clearance should be provided between the exhaust outlet on top of the BSC and any overhead obstruction (12-14 inches) and between BSC, on each side (at least 12 inches) to allow access. BSC should not be located directly opposite other BSC or chemical hood. Safe distance must be considered to avoid operator collision.

BSC must be supported by emergency power supply to ensure containment is maintained during electrical emergency situations.

BSC must be duly maintained and certified by authorized agencies or vendors. Certification of BSC must be conducted upon initial installation, annually, after any repairs and relocations done to ensure that they are operating as designed.

When working with BSC, proper placement of materials and movements of hands must be observed. Worker must avoid instances that cause unnecessary blockage of the front and back grille. A smoke pattern test must be conducted to ensure correct flow of air.
1.3. Facility

The laboratory must be well lighted and of adequate space. There must be a sink with adequate supply for clean water for hand washing located near the exit door. In addition, emergency eyewash must be installed inside the laboratory. The laboratory must be designed for easy access to supplies, communication, first aid and spill response kit. Autoclave and other means of decontamination must be available and located near the laboratory.

1.4. Laboratory Access

Access must be restricted to laboratory personnel and support service providers only. Laboratory must at least have door and lock / key. There must be a system to monitor and record transaction within the facility.

2. Administrative

Laboratory must always be aware on current events related to COVID-19 and must update their local biorisk assessment accordingly. No laboratory activities and examinations shall be conducted without approved and established mitigation control measures based on risk assessment and quality assurance program or validation system. Personnel involved must be trained and competent.

Approved Standard Operating Procedures specific for COVID-19 must be in place. There must be established and maintained system for emergency response and personnel health management including provision of health services (e.g. vaccination), medical surveillance and evaluation. All related activities must be documented. Important records must be kept on file.

3. Practices

Biosafety Level 2 standard and special practices are written on WHO Biosafety manual and the BMBL. For emphasis the following must be considered:

3.1. Work with respiratory specimen must be inactivated prior to processing. Other clinical specimens from confirmed COVID-19 patients and PUI (e.g. stool other body fluids) may be inactivated based on risk assessment. Inactivation must be done in a certified Class II Biological Safety Cabinet inside a BSL 2 laboratory. For diagnostic procedures that require bacterial / fungal culture of respiratory specimen, processes may be done in BSL 2 with enhancements, specifically in PPE and practices.

3.2. Blood, serum, stool and urine for routine testing hematology, microscopy, serology, bacterial and fungal culture can be conducted in BSL 2 laboratory following BSL 2 practices. A closed system automation and point of care testing must be considered.
3.3. Procedures with high potential of generating fine-particulate aerosols must be performed in a certified Class II Biological Safety Cabinet (BSC II). These procedures include vortex mixing the specimen, pipetting, inoculation using loops, opening primary containers after vigorous mixing and other procedure that applies pressure to the viral suspension. Appropriate primary containment devices like safety centrifuge buckets or sealed rotors should be used for centrifugation. Safety centrifuge buckets must be sealed and should be loaded and unloaded only in a BSC II. Use of filtered / aerosol resistant tips must be considered when obtaining aliquots, dispensing fluids, adding and mixing potentially contaminated solutions.

3.4. Packaging, shipping and transport of specimens must comply with the local and international requirements for transportation of infectious substances.

3.4.1. For local land transport, patient sample must be shipped following the basic triple packaging system
Reference: DOH Manual on Packaging and Transport of Laboratory Specimen for Referral and DOH DM 2018-0413 - Interim Guidelines on Transportation of Biological Specimens

3.4.2. For air shipments, sample from confirmed and/or suspected patient must be shipped as Category B
UN Number and Proper Shipping Name: UN 3373, “Biological Substance, Category B”
Reference: ICAO Technical Instructions for the Transport of Dangerous Goods
WHO Guidance on regulations for the transport of infectious substances 2019-2020

3.4.3. For air shipments, of COVID-19 viral culture or isolates must be shipped as Category A
UN Number and Proper Shipping Name:
UN 2814, "Infectious substance, affecting humans”
Reference: ICAO Technical Instructions for the Transport of Dangerous Goods
WHO Guidance on regulations for the transport of infectious substances 2019-2020

3.5. The agent being investigated may likely be susceptible to disinfectants with proven activity against enveloped viruses (e.g. hypochlorite, alcohol, hydrogen peroxide, quaternary ammonium compounds and phenolic compounds) just like other coronaviruses. Bleach or sodium hypochlorite solution, alcohol and hydrogen peroxide are readily available in laboratories and are generally safe and less toxic if used according to manufacturer’s recommendations. Working solutions (diluted from the stock solution) must be freshly prepared and must be used immediately. Observe 10-15 minutes contact time. Contact time may be extended up to 30 minutes based on assessment.

Bleach (usually 5.25% or 6.00%-6.15% sodium hypochlorite depending upon manufacturer) is usually diluted in water at 1:10 or 1:100. Approximate dilutions are 1-1/2 cups of bleach in a gallon of water for a 1:10 dilution (~6,000 ppm) or 1/4 cup of bleach in a gallon of water for a 1:100 dilution (~600 ppm). Reference: Disinfection with Bleach
https://multimedia.3m.com/mws/media/7359760/disinfection-with-bleach-tech-talk.pdf
3.5.1. For general surface disinfection
  3.5.1.1. Sodium hypochlorite (prepare 1:100 dilution)
  3.5.1.2. 62-71% ethanol / alcohol
Note: Sodium Hypochlorite is corrosive. Wiping surfaces with alcohol or water after complete surface disinfection with Sodium Hypochlorite reduces the risk of corrosion of BSC and other equipment.

3.5.2. For decontamination of blood spills
  3.5.2.1. Sodium hypochlorite (prepare 1:10 dilution)

3.5.3. For space decontamination
  3.5.3.1. Hydrogen peroxide (e.g. 3% or greater concentration) is prepared according to manufacturer's recommendation, taking into consideration the space area to be decontaminated and the fumigation equipment to be used. Exposure time ranges from 30 minutes to one hour or more. Assistance of a qualified biomedical engineer is essential.

Other commercially available solutions like Lysol and quaternary ammonium compounds can be used and prepared according to manufacturer's recommendation.

3.6. All potentially contaminated laboratory wastes must be autoclaved at 121°C, 15 psi for 30 minutes prior disposal. Monitoring the performance of autoclave is essential and must be documented. Biological Indicators shall be used in conjunction with chemical indicator and physical monitor. Waste shall be disposed according to institutional policy and guidelines.

3.7. Laboratory must prepare for accidental spills and related laboratory emergencies while working with COVID-19 specimens.

3.7.1. Biological Spill Response
In areas with anticipated potential risk for spills, biological spill kits must be available and strategically located in the laboratory. A 1:10 dilution of Sodium Hypochlorite solution must be freshly prepared and absorbent cloth, gauze or paper towels must be available to cover the spill. A contact time of at least 30 minutes must be observed prior cleaning the spilled area.

In case of large / unmanageable spill, remove and dispose the outer contaminated PPE (e.g. gloves), proceed to the anteroom, and then remove protective clothing (gown, face / eye protection and lastly the inner layer gloves). Do not remove PPE that provide respiratory protection (N95, P100, PAPR) yet; and hand rub with 70% alcohol. Close the room, put signage (Do not Enter!) and leave the room. To remove the respirator, wear a new set of gloves, doff the respirator and then doff the gloves. Wash hands with soap and water. Do not touch potentially contaminated PPE and surfaces without PPE. Inform the immediate supervisor and Biosafety Officer. Plan succeeding spill response procedures with identified (internal and/or external) responders.
3.7.2. Emergency

In case of an emergency that requires evacuation of the area, the laboratory personnel are encouraged not to panic. If possible, secure all open primary specimen, isolate and culture media containers. Dispose outer layer of gloves and leave the laboratory. If the scene is safe, proceed with proper donning of PPE and hand washing. Proceed with the emergency response procedure according to established institutional guidelines.

4. Personal Protective Equipment

Respiratory protection is essential since the virus is naturally transmitted via inhalation routes. It is recommended to use a properly fit-tested, NIOSH-approved filtering face piece respirator that provides a level of filtration of 95% or greater (N-95 or its equivalent). A powered air-purifying respirator (PAPR) equipped with high-efficiency particulate air (HEPA) filters must be used in case of failed Respirator Fit Test. Personnel must be evaluated properly by a Medical Doctor prior use of a respirator. Selection and use of gloves, gown, face shield, dedicated laboratory shoes and other PPE must be based on risk assessment.

Attached is a recommended PPE Matrix per laboratory procedure specific for COVID-19 and can only be used as a reference.

References:

A. WHO Biosafety Manual
   https://www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf?ua=1


C. Canadian Biosafety handbook

D. CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019 Novel Coronavirus (2019-nCoV)

E. CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)
F. SARS COV 2 Biosafety Advisory

G. Disinfection with Bleach
   https://multimedia.3m.com/mws/media/7359760/disinfection-with-bleach-tech-talk.pdf

H. WHO Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV)
### Common Laboratory Procedures

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Gloves (preferably Nitrile)</th>
<th>Scrub Suit</th>
<th>Laboratory coat</th>
<th>Lab Gown Disposable (impermeable/breathable, long sleeves, back enclosure)</th>
<th>Cover All Gown Disposable (impermeable/breathable)</th>
<th>Hair Cover / Foot Cover</th>
<th>Dedicated Lab Shoes / Boot</th>
<th>Face shield / visor / or goggles</th>
<th>Fit Tested N95, N100, P100 (PAPR must be used in case of failed respirator fit test)</th>
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<tbody>
<tr>
<td>Specimen Collection from a Suspected / Confirmed Patient</td>
<td>Double Gloves Recommended</td>
<td>Recommended</td>
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<td>Recommended</td>
<td>Recommended</td>
<td>Optional Use of surgical mask or N95 as respiratory protection must be based on local risk assessment</td>
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<td>Specimen to be collected:</td>
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<td>Upper and Lower Respiratory Specimen</td>
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<td>Blood and other body fluids</td>
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<td>Specimen receiving transport boxes and documents</td>
<td>Recommended</td>
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<tr>
<td>Specimen receiving, sorting, and verification of specimen</td>
<td>Double Gloves Recommended</td>
<td>Recommended</td>
<td>Recommended</td>
<td>Optional / maybe required based on local risk assessment</td>
<td>Recommended</td>
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<tr>
<td>Decontamination of Transport boxes (within similar laboratory facility, where samples are received, sort and verified)</td>
<td>Double Gloves Recommended</td>
<td>Recommended</td>
<td>Recommended</td>
<td>Optional / maybe required based on local risk assessment</td>
<td>Recommended</td>
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<td>Transport of specimen collected from ward to laboratory and from laboratory to laboratory within the institution</td>
<td>Recommended</td>
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<td>Specimen Testing</td>
<td>Double Gloves Recommended</td>
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<td>Optional / maybe required based on local risk assessment</td>
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<tr>
<td>Routine analysis of Blood, Stool, Urine and other body fluids except Respiratory, secretions, fluids and tissues for routine clinical Hematology, Chemistry, Blood Banking, Serology, Microscopy and Microbiology</td>
<td>Double Gloves Recommended</td>
<td>Recommended</td>
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<td>Optional / maybe required based on local risk assessment</td>
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<tr>
<td>Common Laboratory Procedures</td>
<td>Prescribed Personal Protective Equipment</td>
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<td>Inactivation of Respiratory specimen (e.g. OPS/ NPS) with potential risk of aerosol production during the process of Cell Lysis / inactivation</td>
<td>Double Gloves (preferably Nitrile) Recommended</td>
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<td>Fit Tested N95, N100, P100 (PAPR must be used in case of failed respirator fit test)</td>
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<td>Vortex mixing and mechanical concentration techniques prior inactivation</td>
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<td>Nucleic Acid Extraction of Respiratory specimen source</td>
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<td>Vortex mixing, centrifugation and mechanical concentration techniques of inactivated material</td>
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<td>PCR Analysis of inactivated Respiratory secretions, (NPS / OPS)</td>
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<tr>
<td>Shipping of Specimen for confirmation to external laboratory</td>
<td>Double Gloves (preferably Nitrile) Recommended</td>
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<td>Packaging Original Specimen (not inactivated)</td>
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<td>Shipping Extracts for confirmation to external laboratory</td>
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<td>Procedure conducted at the Mortuary Section / Pathology</td>
<td>Double Gloves (preferably Nitrile) Recommended</td>
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