BRAP-PAMET Biosafety Guidance on COVID-19 Testing for Laboratorians

A joint project by the
BioRisk Association of the Philippines 2015, Inc.
and the
Philippine Association of Medical Technologists, Inc.

2020 Edition

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U.S. Department of State, Biosecurity Engagement Program
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FOREWORD

It was in early January when word of a novel virus started to spread throughout the country. A coronavirus, described as SARS-like and MERS-like was later named SARS-CoV-2. The World Health Organization (WHO) called the flu-like disease COVID-19. It has an alleged long incubation period (14 days), and during this time, the patient becomes an infective carrier. At this point, the WHO declared COVID-19 as pandemic since cases started to be diagnosed worldwide.

It is because of this long incubation period, and most important, that the early signs and symptoms of COVID-19 were non-specific, diagnosis by conventional means seemed lacking, thus, confirmation by molecular biology techniques seemed necessary to make a good and specific diagnosis. However, due to this new technology quite different from conventional protocols, laboratorians needed to be introduced and updated to these molecular biology techniques. Because of this, the BioRisk Association of the Philippines 2015, Inc. (BRAP) and the Philippine Association of Medical Technologists, Inc. (PAMET) discussed a possible molecular biology guidance or guideline for laboratorians handling COVID-19 specimens.

In late March 2020, both BRAP and PAMET rounded up all international standards, guidance documents and guidelines (i.e., WHO, Centers for Disease Control and Prevention, and the International Federation of Biosafety Associations) plus the most recent issuances of the Philippine Department of Health (DOH) and the DOH Research Institute for Tropical Medicine. The two associations wanted to simplify all these documents and create an updated document for ease of use following the laboratory steps.

This handbook collates all aforementioned standards, guidance documents, guidelines, and issuances and incorporated these in the entire handbook we created. In the event that there are different concerns on an issue, the DOH issuances supersede all other resources.

BRAP, PAMET, the contributors, and peer reviewers wish that through this handbook, we help lower the biosafety and biosecurity risks of the workplace by giving the laboratorians a safe and secure guidance on handling valuable biological materials from molecular biology laboratories handling COVID-19 specimens and by following a standard procedure/protocol for testing.

PREFACE

COVID-19 pandemic is one of the biggest battles the world has ever fought. Millions were infected and hundreds of thousands have died. It definitely changed and is still profoundly changing our lives. It manifested the urgency to address the gaps on various aspects of healthcare services, highlighted by the concerns of laboratorians on matters pertaining to biosafety, biosecurity, and biorisk management. It also focused on the needed support and interventions to assure everyone's safety in dealing with the deadly virus.

Such situation paved the way for the BioRisk Association of the Philippines 2015, Inc. (BRAP) and the Philippine Association of Medical Technologists, Inc. (PAMET) to step up and ponder beyond the challenges that made them decide to embark on a meaningful and practical endeavor that could help defeat the pandemic. It provided an opportunity to explore the possibility of coming up with a biosafety guidebook through the combined knowledge and expertise of its proponents, collaborators, and partners.

With the concerted efforts and unwavering support of those who envisioned it, this "BRAP–PAMET Biosafety Guidance on COVID-19 Testing for Laboratorians", came into reality. Well-respected and highly-esteemed healthcare professionals from BRAP and PAMET, together with high-caliber reviewers, joined together and willingly became part of the team. They unselfishly and painstakingly worked to complete this guidebook, which we now consider as a little masterpiece.

It took half a year to complete and publish this piece of work. It is divided into five (5) parts following the standard laboratory stages of pre-preanalytical, preanalytical, analytical, postanalytical and post-postanalytical. It was designed in such a way that laboratorians will find ease in using this handbook as it is patterned after the usual cycle in the laboratory testing. Related topics were grouped accordingly to make it user-friendly and for better referencing. We are looking forward that this will help and serve its users as intended.

The task was definitely demanding, challenging, and exhausting. There were many days spent on writing, researching, and consolidating. There were series of meetings, discussions, and deliberations. There were sleepless nights of layouting, editing, and proofreading, which made the job even more arduous and backbreaking.

Regardless how difficult the journey was, this end-product, borne out of a noble purpose, is more than a reward because this represents BRAP's and PAMET's humble contribution to all laboratorians and laboratories in keeping them safe, as we pay homage to their boundless heroism and to the country as it moves toward recovery and rebuilding itself as a nation.

PART 1: PRE-PREANALYTICAL STAGE

Section 1: Basic Biosafety Principles

Introduction

Laboratory biosafety and biosecurity are fundamental biorisk management practices that shall be employed in all diagnostic (clinical), academic, and research laboratories. While they are each separate concepts, it is important to recognize that both are complementary and share a common goal: to keep the laboratorians, the laboratory, the community, and the environment safe and secure.

Abiding by appropriate and adequate biosafety and biosecurity practices is essential to ensure the safety and security of diagnostic, academic, and research laboratories. This document focuses on severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent for COVID-19. Testing of samples from patients suspected of, or being monitored for SARS-CoV-2, shall be performed in adequately and appropriately equipped laboratories by competent staff trained in relevant technical and safety procedures.

In the basic concepts of laboratory biosafety, the risk of exposure to biological agents in a diagnostic, research, or academic environment depends on four (4) main principles according to importance:

1. Engineering controls.
2. Administrative controls.
3. Good microbiological practices and procedures.
4. Personal protective equipment.

Moreover, good laboratory techniques are of major importance in laboratory biosafety. Both are based on comprehensive technical knowledge, experience, common sense, and an attitude of courtesy and consideration for others. Risk assessment and mitigation will also be discussed in this stage of the process.

Note: Since this will just be a compilation of local and international guidance made simple and within reach for the laboratorians, referencing will all be in the references section for this section. Most of the articles here are lifted verbatim from the references cited to avoid misinterpretation.

Objectives

1. To provide laboratorians interim guidance on laboratory biosafety related to the testing of clinical specimens of patients that meet the case definition of COVID-19.

2. To gather, summarize, and make applicable to the Philippine setting the international guidance, guidelines, and best practices (from WHO, Centers for Disease Control and Prevention (CDC), and the International Federation of Biosafety Associations) for laboratories handling and working on the SARS-CoV-2, which is the causative agent for COVID-19.
3. To highlight the Department of Heath's issuances on laboratory biosafety for the implementation in laboratories handling and working on SARS-CoV-2.

Definition of terms

Aerosol-generating procedure: any procedure that intentionally or inadvertently results in the creation of liquid or solid particles, which become suspended in the air (aerosols).

Aerosol/airborne transmission: the spread of infection caused by the inhalation of aerosols or their deposition on mucosal surfaces of an exposed subject.

Biological agent: a microorganism, biological toxin, protein (prions), or human endoparasite, either naturally occurring or genetically modified, which may have the potential to cause infection, allergy, toxicity, or otherwise create a hazard to human health, animals, or plants.

Biosafety: containment principles, technologies, and practices that are implemented to prevent unintentional exposure of laboratory personnel to biological agents or their accidental release.

Biosecurity: principles, technologies, and practices that are implemented for the protection, control, and accountability of biological materials and/or the equipment, skills, and data related to their handling. Biosecurity aims to prevent their unauthorized access, loss, theft, misuse, diversion, or release.

Consequence (of a laboratory incident): the outcome of an incident (exposure to and/ or release of a biological agent) of varying severity of harm, occurring in the course of laboratory operations. Consequences may include a laboratory-acquired infection, other illness or physical injury, environmental contamination, or asymptomatic carriage of a biological agent.

Containment: the combination of physical design parameters and operational practices that protect personnel, the immediate work environment, and the community from exposure to biological agents. It is, of note, however, that the levels of containment, such as primary and secondary containment, are relative, subject to the actual usage and combination of devices and apparatuses. The term "biocontainment" is also used in this context.

Core requirements: a set of minimum requirements to describe a combination of risk control measures that are both the foundation for, and an integral part of, laboratory biosafety. These measures reflect international standards and best practice in biosafety that are necessary to work safely with biological agents, even where the associated risks are minimal.

Engineering controls: risk mitigation measures that are built into the design of a laboratory or preinstalled in laboratory equipment in order to minimize the risk of exposure to and/or unintended release of biological agents.

Exposure: an event during which an individual comes in contact with, or is in close proximity to, biological agents with the potential for infection or harm to occur. Routes of exposure can include inhalation, ingestion, intravenous injection and absorption and are usually dependent on the characteristics of the biological agent. However, some infection routes are specific to the laboratory environment and are not commonly seen in the general community.

Good microbiological practices and procedures: a basic laboratory code of practice applicable to all types of activities with biological agents, including general behaviors and aseptic techniques to reduce the creation of aerosols that shall always be observed in the laboratory. These practices and procedures serve to protect laboratory personnel and the community from infection, prevent contamination of the environment, and provide protection for the work materials in use.

Hazard: an object or situation that has the potential to cause adverse effects when an organism, system, or (sub)population is exposed to it. In the case of laboratory biosafety, the hazard is defined as biological agents that have the potential to cause adverse effects to personnel and/or humans, animals, and the wider community and environment. A hazard does not become a "risk" until the likelihood and consequences of that hazard causing harm are taken into account.

Heightened control measures: a set of risk control measures that may need to be applied in a laboratory facility because the outcome of a risk assessment indicates that the biological agents being handled and/or the activities to be performed with them are associated with a risk that exceeds an acceptable tolerance level.

Inactivation: a process to reduce the ability of biological agents to grow and/or multiply and/or have pathogenic functions.

Incident: an occurrence that has the potential to, or results in, the exposure of laboratory personnel to biological agents and/or their release into the environment that may or may not lead to actual infection.

Inherent risk: risk associated with laboratory activities or procedures that are conducted in the absence of mitigation measures or controls.

Laboratory-acquired infection (LAI): any infection acquired as a result of exposure to a biological agent in the course of laboratory-related activities, including secondary or tertiary infections.

Likelihood (of a laboratory incident): the probability of an incident (i.e., exposure to and/or a release of a biological agent) occurring in the course of laboratory work.

Maximum containment measures: a set of highly detailed and stringent risk control measures that are considered necessary for laboratory work where a risk assessment indicates that the activities to be performed pose very high risks to laboratory personnel, the wider community, and/or the

environment, and therefore an extremely high level of protection must be provided. These are especially needed in the case of work with biological agents that may have catastrophic consequences, including emerging and re-emerging infectious agents and unknowns, if an exposure or release were to occur.

Pathogen: a biological agent capable of causing disease or infection in humans, animals, or plants.

Personal protective equipment (PPE): equipment and/or clothing worn or held, by a personnel to provide a barrier against biological agents, thereby minimizing the likelihood of exposure. PPE includes, but is not limited to, laboratory coats, gowns, full-body suits, gloves, protective footwear, safety glasses, safety goggles, masks, and respirators.

Primary containment device (equipment): a contained workspace designed to provide protection to its operator, the laboratory environment, and/or the work materials for activities where there is an aerosol hazard. Protection is achieved by segregation of the work from the main area of the laboratory and/or through the use of controlled, directional airflow mechanisms. Primary containment devices include biological safety cabinets, isolators, local exhaust ventilators, and ventilated working spaces.

Residual risk: risk that remains after carefully selected control measures are applied. If residual risk is more than the predetermine/defined risk tolerance levels, it may be necessary to apply additional control measures or to stop the activity.

Risk: the combination of the likelihood of an incident and the severity of the harm (consequences) if that incident were to occur.

Risk assessment: a systematic process of gathering and evaluating information to support a risk management process.

Risk evaluation: part of risk assessment where the likelihood of exposure to a hazard is weighed against the potential severity of harm under a set of predefined circumstances, such as a specific laboratory procedure. The goal of a risk evaluation is to determine whether the assessed risk is acceptable or whether further targeted control measures shall be implemented to prevent or reduce the risks to within a tolerance level.

Risk control: use of a combination of tools, which include communication, assessment, training, and physical and operational controls to reduce the risk of an incident/event to an acceptable level. The risk assessment framework will determine the strategy that shall be taken to control the risks and the specific types of control measures required to achieve this.

Risk tolerance: the level of risk that is considered acceptable and allows work to proceed bearing in mind the expected benefit of the planned activities.

Standard operating procedures: set of well-documented and validated stepwise instructions outlining how to perform laboratory practices and procedures in a safe, timely, and reliable

manner, in line with institutional policies, best practices, and applicable national or international regulations.

Validation: systematic and documented confirmation that the specified requirements are adequate to ensure the intended outcome or results. For example, in order to prove a material is decontaminated, laboratory personnel must validate the robustness of the decontamination method by measurement of the remaining biological agents against the detection limit.

Verification: confirmation that a given item (product, process or system) satisfies the specified requirements. For example, verification that the performance of an autoclave meets the standards specified by the manufacturer shall be performed periodically.

Scope

Primary hazard of concern: SARS-CoV-2
Laboratory specimens collected from patients with COVID-19 may harbor other biological hazards including, but not limited to, other respiratory viruses. Non-biological hazards, such as chemical reagents used for diagnostic testing, shall be considered during the site-specific risk assessment process.

Pathogenicity: disease=COVID-19

- Currently no known treatment or vaccine.
- Mild to severe illness and may cause death.
- Common symptoms: pneumonia and severe respiratory disease.
- Symptom onset 2–14 days postexposure.

Communicability:

- Human-to-human transmission.
- Exposure to contaminated surfaces.

Primary laboratory hazards:

- Droplet exposure of the mucous membranes of the eye, nose, and/or mouth.
- Inhalation of airborne particles during aerosol-generating procedure.
- Exposure to contaminated surfaces.
- Ingestion.

Survival outside the host:

- Not specifically known for SARS-CoV-2.

Four (4) main biosafety principles from WHO Highlights and Key Points of the WHO laboratory biosafety guidance related to coronavirus disease (COVID-19):

1. Engineering controls

Initial processing (before inactivation) of all specimens, including those for sequencing and nucleic acid amplification test (NAAT)

- NAAT shall take place in an *appropriately maintained and validated biological safety cabinet* (BSC) or primary containment device.
- Handling of material with high concentrations of live virus (such as when performing virus propagation, virus isolation, or neutralization assays) or large volumes of infectious materials shall be performed only by properly trained and competent personnel in laboratories meeting additional essential containment requirements and practices, that is, biosafety level 3 (BSL-3).
- Non-propagative diagnostic laboratory work, including sequencing and NAAT, on clinical specimens from patients who are suspected or confirmed to be infected with COVID-19, shall be conducted adopting the practices and procedures of *core requirements* and an appropriate selection of *heightened control measures* as informed by the local risk assessment. In the interim, basic biosafety level 2 (BSL-2) is suitable for diagnostic services.

2. Administrative controls governing the laboratory

- Each laboratory shall conduct a local (institutional) risk assessment to ensure it is competent to safely perform the intended testing with appropriate risk control measures in place.
- All technical procedures shall be performed in a way that minimizes the generation of aerosols and droplets and halts the chain of infection via the route of entry of a pathogen (see table 1).
- Appropriate disinfectants with proven activity against enveloped viruses shall be used for the recommended contact time at the correct dilution and within the expiry date after the working solution is prepared.
- Point of care (POC) or near-POC assays can be performed on a bench without employing a BSC, when the local risk assessment so dictates and proper precautions are in place. They could be performed on a bench without employing a BSC, when the local risk assessment so dictates and the following conditions are fully met:
    - Performed on a diaper or large paper towel in a well-ventilated area free of clutter, where there are no documents, computers, or personal stuff.
    - Appropriate personal protective equipment (PPE) worn similar to other manual testing, such as but not limited to a full-length long (elastic) sleeved lab coat, safety goggles or glasses, and suitable disposable gloves.
    - Risk assessment should inform the use of respiratory protection as a supplementary precaution.
    - Staff well trained on good microbiological practice and procedure (GMPP).
    - No rush or increased pressure for test turnaround time.

- A validated infectious waste process including excess specimens.

Biosafety practice	Blocked routes of exposure
1. Do not mouth pipette.	Inhalation, ingestion, skin, and mucous membrane contact.
2. Manipulate infectious fluids carefully to avoid spills and the production of aerosols.	Inhalation, skin, and mucous membrane contact.
3. Restrict use of needles, syringes, and other sharps to those procedures for which there are no alternatives; dispose sharps in leak-proof and puncture-proof containers.	Percutaneous and inhalation.
4. Use lab coats, gloves, safety eyewear, and other personal protective equipment.	Skin and mucous membrane contact.
5. Wash hands after all laboratory activities, following the removal of gloves, and immediately following contact with infectious agents.	Skin and mucous membrane contact.
6. Decontaminate work surfaces before and after use and immediately after spills.	Skin and mucous membrane contact.
7. Do not eat, drink, store foods, or smoke in the laboratory.	Ingestion, skin, and mucous membrane contact.

Table 1. Biosafety practices and blocked routes exposure.

3. Good microbiological practices and procedures

- The handling and processing of specimens from cases with suspected or confirmed COVID-19 infection that are intended for additional laboratory tests, such as hematology or blood gas analysis, shall follow standard guidelines without additional measures.
- When handling and processing specimens, including blood for serological testing, laboratory practices, and procedures that are basic to GMPP shall be followed.
- Patient specimens from suspected or confirmed cases shall be transported as UN3373, "*Biological Substance Category B.*" Viral cultures or isolates shall be transported as Category A UN2814, "*infectious substance, affecting humans.*"
- The external lysis buffer of the listed common RNA extraction kits is effective in inactivating the COVID-19 virus without heat or other additional means.

4. Personal protective equipment

- Appropriate PPE, as determined by the institution's detailed risk assessment, shall be worn by all laboratory personnel handling these specimens (see annex 1).

Standard biosafety practices and basic containment for SARS-CoV-2

Containment refers to the minimum physical facility, equipment, and operational practices needed to contain infectious microorganism.

1. Practices

A. Standard biosafety practices

These practices were lifted from the best practices of the Department of Health Research Institute for Tropical Medicine Biorisk Management Office (DOH RITM BRMO) with permission.

- Access to the laboratory is limited or restricted in COVID-19 testing laboratory. No minors should be allowed in the laboratory. Animals and plants are not permitted in the laboratory. Laboratory doors are always kept closed.
- Laboratory personnel wash their hands or at least decontaminate using alcohol-based hand rub prior to donning PPE and wash hands with soap and water immediately after complete doffing of PPE and before leaving the laboratory.
- Prohibited practices like storage and consumption of food and water, smoking, handling contact lenses, applying cosmetics, mouth pipetting, and keeping animal and plants in the laboratory are not permitted in laboratory premises.
- Personal protective equipment is used to protect personnel from contact with hazardous materials and infectious agents. Appropriate clothing may also protect the materials from contamination.
- Personal protective devices and safety equipment as well as training in the proper use of those devices and equipment must be provided to all employees under the appropriate circumstances, for example, avoiding gloved hands coming into contact with the face.
- Protective clothing is not worn outside the laboratory. Clothing is changed when contaminated. Potentially contaminated reusable PPE is decontaminated before being laundered.
- Precaution must always be taken with any contaminated sharp items, including needles and syringes, broken glass wares, pipettes, and scalpels.
- All procedures are performed carefully to minimize the creation of splashes or aerosols from the original specimen.
- The laboratory should be kept neat and free of clutter; surfaces should be clean and free of infrequently used laboratory materials, supplies, and equipment.
- Work surfaces are decontaminated with appropriate disinfectant against coronavirus and ensure appropriate contact time after completing the

procedures and/or at least once a day and/or after any spill of viable material.

- All original specimen and swabs and other potentially contaminated material must be decontaminated (using an autoclave) before disposal.
- Materials to be decontaminated outside of the immediate laboratory using an autoclave are placed in a durable, leak proof, puncture proof container and closed for transport from the laboratory.
- An effective insect and rodent control program in place and are being implemented.

B. Special biosafety practices

- Procedure and pathogen based local biorisk assessment must be conducted specific for COVID-19. Personnel are advised of special hazards and risk as identified by local risk assessment and are required to cooperate and follow institutional guidelines and procedures to mitigate the risks.
- Laboratory personnel and support staff who are at increased risk of acquiring infection (immune suppressed and immune compromised) and/or for whom infection may have serious consequences are not allowed in the laboratory rooms.
- There are established and maintained policies and procedures whereby personnel must meet specific immunization requirements prior entering the laboratory and training requirement prior starting laboratory works. Personnel must receive updated flu vaccination and COVID-19 specific biosafety training and SARS-CoV-2 diagnostic training for conducting nucleic acid amplification test and serology. All personnel demonstrate proficiency in standard microbiological practices and techniques and in the practices and operations specific to the laboratory facility.
- A biosafety manual adopted specifically for COVID-19 testing laboratory must be available.
- A biohazard sign must be posted on the entrance to the laboratory. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the laboratory head and biosafety officers' name and telephone number, any PPE that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- A baseline sample for laboratory and other at-risk personnel is collected and stored. Additional specimens may be collected periodically, depending on the agents handled or specific circumstances development of signs and symptoms following documented unprotected exposure and/or accidental exposure.
- Specimens of body fluids or potentially infectious wastes are placed in a container with a screw cap cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant (e.g., 70% alcohol) on a routine basis, after work with infectious materials is finished.

- Equipment must be surface decontaminated before it is removed from the facility for repair or maintenance.
- Biological spills, splashes, or other contamination by infectious materials must be decontaminated according to spill response procedure. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory head and designated biosafety officer.
- Medical evaluation, surveillance, and treatment are provided as appropriate, and written records are maintained and monitored.
- All personnel wearing respirators need to be included in respiratory protection program that includes a medical evaluation, initial training and fit-testing, and annual retraining.
- Properly maintained biological safety cabinets, Class II, in combination with appropriate personal protective equipment or physical containment devices, are used whenever high concentrations or large volumes of infectious agents are used and procedures with a potential for creating infectious aerosols or splashes are conducted. These procedures include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, and opening containers of infectious materials. All open manipulations involving infectious materials (respiratory samples) are conducted in biological safety cabinets or other physical containment devices within the containment module. No work with respiratory samples in open vessels is conducted on the open bench.
- When a procedure or process cannot be conducted within a biological safety cabinet, then appropriate combinations of PPE (e.g., respirators and face shields) and physical containment devices (e.g., centrifuge safety cups or sealed rotors) are used.
- Blood specimen may be centrifuged in the open laboratory in combination with appropriate PPE and physical containment device (sealed rotor heads or centrifuge safety cups). These rotors or safety cups must be opened only in a certified and properly maintained BSC.
- Laboratory personnel are responsible for cleaning laboratory benches, equipment, and areas that require specialized technical knowledge.
- All biological wastes must be decontaminated prior to disposal.
- Completeness of waste decontamination process using physical, chemical, and/or biological indicator must be monitored and validated.
- There must be a system to document inventory and monitor accountability and traceability of all COVID-19 specimens.

2. Equipment (primary containment)

A. Biological safety cabinet

BSC provides effective primary containment for work with infectious agents when they are properly maintained in conjunction with standard and special

practices. There are various types of BSC, and they operate in similar basic principle. Personnel protection is achieved through a continuous stream of inward air that prevents infectious aerosol from escaping the front opening. The exhaust air is HEPA filtered and released to the immediate laboratory facility or directly outside the facility depending on the BSC model. Most BSCs provide product protection by using HEPA-filtered downflow to flush the cabinet interior of airborne contaminants and to prevent unfiltered inflow air from entering the work area. For work with COVID-19, most laboratories use BSC Class 2 A2 with a minimum average inflow velocity of 0.51 m/s through the front opening.

B. Autoclave

Autoclaving, when used correctly, is the most effective and reliable means to sterilize laboratory materials and decontaminate waste materials by killing or inactivating biological agents.

The following general safety precautions must be taken when using steam autoclaves.

- Operation and maintenance of autoclaves must be assigned to trained, competent individuals.
- Operating instructions for the autoclave must be available. Sterilization programs with application area (e.g., solids and liquids), and the parameters to be maintained (temperature, pressure, and time) must be defined.
- A loading plan (with information on the contents, number, volume, and mass of the sterilized product) should also be available. Large and bulky material, large animal carcasses, sealed heat-resistant containers, and other waste that impedes the transfer of heat must be avoided.
- A preventive maintenance program must be developed, including regular visual inspection of the chamber, door seals, gauges, and controls. This should be conducted by qualified personnel.
- A reliable steam source must be used to provide appropriately saturated steam, uncontaminated by water droplets or chemicals that inhibit the function of the autoclave or may damage the pipes or chamber of the autoclave.
- Waste or materials placed in the autoclave must be in containers that readily allow removal of air and permit good heat penetration.
- The chamber of the autoclave must be loosely packed so that steam can penetrate evenly.
- Hazardous chemical waste (e.g., bleach), mercury, or radioactive waste must not be treated in an autoclave.
- Operators must wear suitable thermally protective gloves, protective clothing, and eye protection when opening the autoclave, even when the temperature has fallen to levels appropriate for opening the chamber.

- Care should be taken to ensure that the relief valves and drains of autoclaves do not become blocked by paper, plastic, or other materials included in the waste or materials for decontamination.
- For the decontamination of volatile hazardous material (e.g., spores of pathogens), the air relief of the autoclave must be equipped with an appropriate filter.
- More information on the various types of autoclave and their validation, maintenance, and specifications can be found in the monograph: Decontamination and Waste Management.

C. Personal protective equipment

These practices were lifted from the best practices of the DOH RITM BRMO with permission.

PPE is used to protect personnel from contact with hazardous materials and infectious agents. PPE are designed to minimized the risk of exposure to various hazards. Appropriate clothing may also protect the materials from contamination. Personal protective devices and safety equipment as well as training in the proper use of those devices and equipment must be provided to all employees under the appropriate circumstances. The employees have the responsibility of properly using the equipment.

Head, eye, and face protection	Safety glasses	Must be worn in the lab whenever procedures are underway involving a low probability of splash, work with low hazard chemicals, or an impact hazard.
	Goggles	
	Face visor	Full face protection must be used for procedures that have anticipated splashes or sprays of infectious or other hazardous materials to the face or if there is a high potential for droplet and/or aerosol generation.
	Face shield	
	Head cover	It protects hair and scalp when contaminating from sprays, splashes, droplet, and airborne.
Full body protection	Scrub suits	These are worn as inner uniform and not functioning as PPE.
	Laboratory gown – lab coat Reusable	The most common type of PPE used to minimize the contamination of skin or street clothes with biological or other hazardous materials. In circumstances where it is anticipated that splashes may occur, the garment must be resistant to liquid penetration to protect clothing from contamination. If the garment is not disposable, it must be capable of withstanding sterilization, in the event it becomes contaminated.
	Disposable laboratory gown	Are usually solid-front or wrap-around laboratory gowns provide protects skin and street clothing from hazardous materials in laboratory including biological hazards. It must be breathable, fluid resistant, and with long sleeves.

	Cover all	Full body suits and coveralls provide further protection and are available in disposable and reusable materials.
		Coveralls must be made of high-density polyethylene fibers, polyvinyl chloride, and neoprene to provide good barrier to prevent penetration of hazardous material including chemicals.
	Apron	Aprons are worn over laboratory coat or gowns to provide additional layer of protection when there is anticipated gross contamination, spills, and splashes due to open procedures that includes necropsy.
Hand and foot protection	Power free Latex or nitrile gloves	Gloves protect the hands from contamination and reduce the risk associated with ingestion or absorption through the skin and mucus membrane.
		Must be selected based on the hazards involved and the activity to be conducted.
		They are susceptible to punctures, rips, and tears. Should be changed as soon as possible after they have become contaminated and when their integrity has been compromised or when necessary.
		Disposable gloves must not be washed or reused.
	Laboratory shoes, and foot covers	Footwears reduce the risk of exposure to infectious agents, toxins, or chemicals in the event of an accident. Use of dedicated laboratory shoes prevents cross-contamination from one facility to the other.
		Footwear should protect the foot from hazardous liquids. It should be completely enclosed with no heels. It must be easy to disinfect. It must be slip resistant.
Mask and respiratory protection	Respirator	Respirators are used when there is risk of exposure to infectious aerosols that can be transmitted through inhalation route.
	Mask	Surgical masks are loose fitting and offers little protection from inhalable hazards. It protects mucus membrane of the nose and mouth from spills and splashes.

3. Physical containment/facility requirements

No.	Requirement	Additional notes
1	There must be a door to separate laboratory facility to public areas. Approximate the size of door openings to allow ingress and egress of anticipated equipment. Door must be lockable.	Door serves physical barrier that separate the containment zone from public and administrative. It protects against the release of infectious material or toxins and provides a security barrier to limit access to the zone. Lockable doors provide a basic security barrier to prevent unauthorized access to the containment zone and to safeguard the infectious material and toxins stored inside.

		Doors must have appropriate signage including biohazard sign, containment level, contact information, and specific entry requirements.
2	A dedicated paper and computer work station must be separated from bench and other areas where samples are handled and manipulated.	There must be a dedicated room within the containment zone or a physical partition between bench and paper/computer workstation or locating paper/computer work stations in a space inside the containment zone but away from benches.
3	There must be a dedicated facility for non-laboratory activities.	Designate an office area, meeting room, and pantry outside the laboratory.
4	Provide an anteroom before entry to laboratory.	
5	The design and choice of materials used for walls, ceilings, floors, and barrier devices are critical for ensuring that the containment zone has the structural stability to withstand both internal stresses, such as the negative or positive pressures that result from a failure of the supply air or exhaust air fan, and external stresses, such as external weather conditions.	Provide non-absorptive doors, frames, casework, working surfaces, and bench tops. Provide scratch, stain, moisture, chemical, and heat resistant surfaces and impact resistant in accordance with laboratory function. Provide gas and chemical resistant interior coatings in accordance with laboratory function (e.g., will withstand chemical disinfection and fumigation). Provide cleanable interior coatings. Interior surfaces (i.e., walls, floors, and ceiling) made of materials that limit the penetration of gases and liquids (e.g., stainless steel, epoxy resin or laminate covering, non-porous materials) provide room integrity, facilitate surface and room decontamination, and serve to contain any large volumes of contaminated liquids that may be present (e.g., animal wastes, large-scale process fluids).
6	Laboratory must have slip-resistant floors.	It is required to prevent slips and falls and mitigate the associated the risk of exposure to infectious material via a splash, spill, parenteral inoculation, or scratch. A seamless, rolled, or resilient tile flooring (e.g., vinyl) is recommended.
7	Dedicated storage space is necessary to store the PPE and to separate it from personal clothing to prevent contamination.	Hooks, lockers, shelves, or spaces within dedicated change areas are examples of dedicated storage space for PPE.
8	Dedicated clothing change areas at the entry and exit to the containment zone provide the space necessary to don and doff dedicated PPE.	The clothing change areas may be an anteroom, part of an anteroom, or, in some cases, a designated area at the entry and exit of the containment zone that is separated into a “clean” change area and “dirty”

		change area by a line demarcating the “clean” and “dirty” areas. Provide clothing change areas at the entry to laboratory zone separating personal and laboratory clothing dedicated to laboratory (i.e., “clean” change area separated from "dirty" change area).
9	There must be an anteroom.	The presence of an anteroom at the point of personnel entry to and exit from a containment zone creates a buffer space to maintain inward directional airflow created through the negative air pressure differentials and prevents the migration of potentially contaminated air to an area of lower contamination or lower containment (i.e., outside the containment barrier or outside the containment zone).
10	Windows if can be opened must be protected with fly screen.	Basic pest control and security measures on windows that can open protect against the entry of small-sized animals and insects.
11	Laboratory must have a cleanable and resistant-surface materials and finishes.	It will provide protection against the stresses associated with activities performed inside the containment zone, which may include repeated chemical decontamination, movements, and activities causing impacts and scratches. Non-absorbent materials may include stainless steel, epoxy resin surfaces or chemical-resistant plastic laminate for benchtops, and urethane or vinyl for stools and chairs.
12	Inward directional airflow is established through a negative air pressure differentials (6–12 air changes per hour).	Ensures that air flows from areas of lower containment or low risk of contamination to areas of higher containment. IDA forces air to flow from the “clean” to “dirty” area of laboratory. A monitoring device, which visually demonstrate inward directional airflow, must be installed to verify that the HVAC system is working properly and that the inward directional airflow is being maintained.
13	Handwashing sinks preferably with “hands-free” capability must be located near the point of exit from the laboratory or in anteroom.	A dedicated handwashing sink must be located near the exit point from the containment zone. Handwashing sinks with “hands-free” capability like the infrared sensors, foot pedals/pumps, elbow-controlled taps will reduce contamination of the sink area and the potential for recontaminating washed hands.
14	Emergency eyewash and/or emergency shower facilities may be required based on risk assessment and must be located strategically at an area that is easily accessible.	Emergency eyewash and shower equipment provide on-the-spot treatment to flush out, dilute, and remove any hazardous materials, including infectious material, that have contaminated the eyes, face, or body.

		A shower is usually located at the exit from the laboratory (i.e., between "dirty" and "clean" change anterooms).
15	There must be an emergency backup power supply to support lighting, HVAC systems, BSCs, security systems, and other essential equipment.	Emergency power can be provided through the building generator or a UPS system. The continued operation of equipment critical for the containment (BSC and HVAC) and security (electronic controlled access systems and closed circuit television) of infectious material during power outages is vital to maintain containment integrity and to safeguard the security of the zone.
16	Biological safety cabinet class II is used as a primary containment device to contain infectious aerosols.	BSC provides effective personnel, environment, and product protection when working with open vessels of infectious material. The protective air curtain created at the front of the BSC is fragile and can be easily disrupted by air currents or drafts created by traffic or HVAC systems in close proximity. BSC must be located away from high traffic areas, doors, open windows, and air supply/exhaust diffusers to protect the BSC air curtain and consequently protect personnel from exposure and the release of pathogens and toxins. BSCs and other primary containment devices must be situated at the rear end or at the designated hot/dirty zone of the laboratory.
17	There must be an area within the laboratory or at a central decontamination facility within the institution for decontamination of waste prior disposal using an autoclave.	The decontamination of waste and other contaminated material inside the containment zone or their safe and secure transport to a decontamination area (on site or off site) prevent the release of pathogens from the containment zone.
18	Laboratory must be equipped with a communication system between laboratory area and the immediate outside support area.	This communication system is for electronic transfer of information and data from laboratory area to outside laboratory perimeter prevent the risk of exposure to contaminated paper and documents used within the facility. It is also essential as contingency in emergency. Communication system includes but not limited to facsimile, scanner, computer, telephone, two-way radio and/or intercom).

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Section 2: Risk Assessment and Mitigation

Introduction

From the perspective of laboratory biological safety, the hazards are the biological agents whose pathogenic characteristics give them the potential to cause harm to humans or animals should they be exposed to these agents. The harm caused by exposure to biological agents can vary in nature and can range from an infection or injury to a disease or outbreak in larger populations.

Biological hazards alone in the laboratory do not pose a risk to laboratorians. The true risk associated with a biological agent cannot be determined by only identifying its pathogenic characteristics. We must consider other hazards, such as the: (A) types of procedure(s) that will be performed on the biological agent, (B) the environment in which these procedures will take place, (C) the equipment to be used, and (D) the skill or competency of the performing laboratorian.

Risk is the combination of the *likelihood* that a hazard will cause harm and the *severity, impact, or consequence* of that harm that may arise from exposure to that hazard. The control of biological risks is informed by performing a robust risk assessment. Risk assessment therefore is a systematic process of: (A) gathering information and (B) evaluating the likelihood and severity of exposure to or release of workplace hazard(s) and (C) determining the appropriate risk control measures to reduce the risk to an acceptable level and monitoring the risk after the mitigation has been placed.

It is highly recommended to start by conducting a local risk assessment for each process step, that is, from sample collection, sample reception, clinical testing, polymerase chain reaction (PCR) to virus isolation (only when and where applicable).

Objectives

1. To describe the process in which the risk(s) arising from working with a hazard(s) are evaluated, and the resulting information is used to determine whether control measures can be applied to reduce or preferably eliminate those risks to acceptable levels.
2. To provide technical guidance to all laboratorians working on SARS-CoV-2, which is the causative agent for COVID-19.
3. To guide the laboratorian on the use of the WHO risk assessment template.

Scope and responsibility

The scope of this document is to provide technical guidance to all laboratorians who work in a biological laboratory (special intention for COVID-19 laboratories) and laboratorians who actively handle or manage other biological agents and toxins, as well as other valuable laboratory material.

The quality of a laboratory's risk assessment's results is entirely dependent on the quality of its input data. In other words, a risk assessment requires the collection and input of accurate information. Personnel assigned to contribute to a risk assessment must be thoroughly familiar with the laboratory's work activities and its biological agent's profile, procedures, equipment, and personnel as it relates to their contribution to the risk assessment. All information that feeds into the risk assessment process must be collected or assessed by those in the laboratory and who are involved in managing biorisks. This is critically dependent on the expertise of laboratory managers, principal investigators, laboratory staff, safety officers, and security personnel, among others.

It is highly recommended to start by conducting a local risk assessment for each process step, that is, from sample collection, sample reception, clinical testing, PCR to virus isolation (only when and where applicable). Specific hazards will be identified for each process step, such as aerosol exposure during sample processing, eye splash during sample processing, infectious culture material spill, and leaking sample receptors. Each process step has its own assessed grade of risk. For each identified risk, appropriate risk control measures shall be selected and implemented to mitigate the residual risks to an acceptable level. Particular consideration shall be given to risks related to human factors.

All manipulations of potentially infectious materials, including those that may cause splashes, droplets, or aerosols of infectious materials (e.g., loading and unloading of sealed centrifuge cups, grinding, blending, vigorous shaking or mixing, sonic disruption, and opening of containers of infectious materials whose internal pressure may be different from the ambient pressure) shall be performed in appropriately maintained and validated biosafety cabinet or primary containment devices by a competently trained and proficient laboratory personnel.

We will provide here a risk assessment template. This is intended to facilitate the process of risk assessment. This is very useful for routine laboratory procedures, including non-propagative diagnostic work and PCR analysis. Non-culture based diagnostic laboratory work and PCR analysis on clinical specimens from patients who are suspected or confirmed to be infected with the virus responsible for COVID-19 shall be conducted adopting practices and procedures described for conventional diagnostic and research laboratories.

Local and international guidance

Risk assessments must be repeatable and comparable, hence they shall always be conducted in a standardized and systematic way. For this reason, many organizations offer risk assessment templates, checklists, or questionnaires that provide stepwise approaches to identify, evaluate, and determine levels of risks associated with the hazards present before using this information to identify appropriate control measures. The various steps of the new risk assessment process collectively form a risk assessment framework (figure 1).

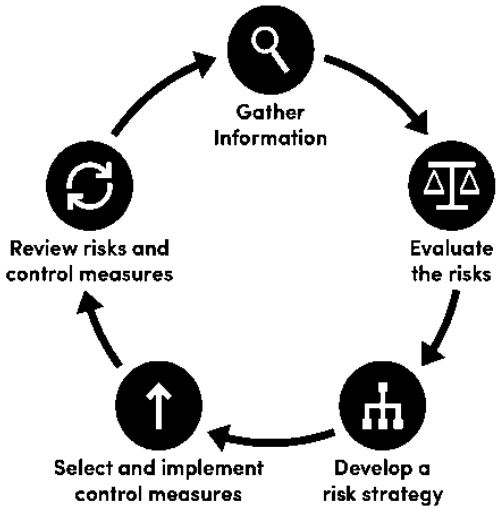

Figure 1. The risk assessment framework. Source: WHO. (in press). *Laboratory biosafety manual: 4th edition*. Geneva: World Health Organization.

The following sections describe in more detail the activities in each step of the risk assessment framework. They provide an overview of the most important components of risk assessments and the key considerations for conducting them. Emphasis on COVID-19 will be presented where applicable and deemed necessary.

The risk assessment framework

1. Gather information (hazard identification)

   Collecting and considering a wide range of information in order to accurately evaluate the risks and appropriately select the control measures needed to reduce risks to acceptable levels in the laboratory. This information goes beyond identifying the hazards – the biological agents being used – and considers the procedural and contextual situations that contribute to the overall risk.

   Providing a brief overview of the laboratory work and summarizing the laboratory activities to be conducted that are included in the scope of this risk assessment.

   Here are specific questions on gathering information for the COVID-19 handling laboratory, describing in detail:

   - The biological agents and other potential hazards (e.g., transmission, infectious dose, treatment/preventive measures, and pathogenicity).

- The laboratory procedures to be used (e.g., culturing, centrifugation, work with sharps, waste handling, and frequency of performing the laboratory activity).
- The types of equipment to be used (PPE, centrifuges, autoclaves, and BSCs).
- The type and condition of the facility where work is conducted.
- Relevant human factors (e.g., competency, training, experience, and attitude of personnel).
- Any other factors that may affect laboratory operations (e.g., legal, cultural, and socioeconomic).

2. Evaluate and prioritize the risks (characterization, evaluation, and prioritization)

Once the information is gathered, all available information on the circumstances of the work to be performed, it is necessary to use that information to identify and evaluate any risks that exist, keeping in mind:

A. To determine the *likelihood of an exposure* and/or release of a biological agent occurring.
B. The *severity of the consequences* of such an event.

With that in mind, we establish how the likelihood and consequence contribute to the inherent risk of the work to be performed, so that you can decide, based on the gathered information of the risk assessment, whether these risks are acceptable or not. This decision must be justified and documented comprehensively (see table 2).

Risk=likelihood × consequence		Likelihood of exposure/release		
		Unlikely	Possible	Likely
Consequence of exposure/release	Severe	Medium	High	Very high
	Moderate	Low	Medium	High
	Negligible	Very low	Low	Medium
Laboratory activity/procedure		**Initial risk (very low, low, medium, high, very high**	**Is the initial risk acceptable? (yes/no)**	**Priority (high/medium/low)**
Select the overall *initial* risk		Very low	Low	Medium	High	Very high

Shall work proceed without additional risk control measures?	Yes No

Table 2. Risk assessment template.

Here are some specific topics/questions on evaluating the risks for the COVID-19 handling laboratory, asking how exposure and/or release could occur:

- What potential situations are there in which exposure or release could occur?
- What is the likelihood of an exposure/release occurring? (unlikely, possible, or likely)
- What is the severity of the consequences of an exposure/release? (negligible, moderate, or severe)

Next is to prioritize the implementation of risk control measures. You identify the initial (inherent) risk of the laboratory activities before additional risk control measures have been put in place. Taking note of the following:

- When assigning priority, other factors may need to be considered, for example, urgency, feasibility/sustainability of risk control measures, delivery and installation time and training availability.
- To estimate the overall risk, take into consideration the risk ratings for the individual laboratory activities/procedures, separately or collectively as appropriate for the laboratory.

Determine the likelihood and consequence of exposure

- Note: determination will vary from facility to facility and group to group.

Consequence: based mostly on the agent

- May cause COVID-19, a novel, mild to severe.
- Currently no treatment or vaccine available.
- Moderate to major.

Likelihood: based mostly in the procedures:

- Different procedures performed by different personnel in different facilities may result in different levels of likelihood.
- Determination must be made based on the site-specific discussion.

3. Develop a risk control strategy

There are a number of different strategies that may be used to reduce and control risks. More often than not, more than one risk control strategy may need to be applied in order to reduce the risks effectively.

A good risk control strategy shall:

- Provide overall direction of the nature of the control measures that may be required to reduce unacceptable risks, without stipulating necessarily the types of control measures that can be used to achieve it.
- Be achievable using the available resources in the context of the local conditions.
- Help to minimize any resistance to the work being performed (i.e., addresses the risk perceptions of relevant stakeholders) and attract allies (e.g., approvals from national/international regulatory authorities),
- Aligns with the overall goals, objectives, and mission of the organization and facilitates success (i.e., improves public health and/or health security).

Here is an overview of some of the most common strategies employed for risk control and example of the control measures (see table 3).

Strategy	Example
Elimination	Eliminate the hazard: • Use an inactivated biological agent. • Use a harmless surrogate.
Reduction and substitution	Reduce the level of risk: • Substitute with an attenuated or less infectious biological agent. • Reduce the volume/titer being used. • Change the procedure for one that is less hazardous, for example, polymerase chain reaction rather than culture.
Isolation	Isolate the hazard: • Elimination and reduction might not be possible, particularly in a clinical setting; therefore, isolate the biological agent(s) (e.g., in a primary containment device).
Protection	Protect personnel/environment: • Use engineering controls (e.g., directional airflow). • Use personal protective equipment.

	• Vaccinate personnel.
Compliance	Have administrative controls and effective biosafety program management in place such as: • Good manufacturing practices and procedures observed by personnel. • Good communication of hazards, risks, and controls. • Appropriate training. • Clear standard operating procedures. • An established safety culture.

Table 3. Strategies for risk reduction.

4. Select and implement control measures (mitigation)

Once a risk control strategy has been developed, risk control measures must be selected and then implemented in order to fulfill the risk control strategy. This is mitigation.

In some cases, the nature of the control measures required will be predetermined, prescribed by a set of minimum standards for risk control (e.g., by internationally accepted best practices and national/international regulations).

Highlights

The majority of clinical and diagnostic laboratory work will require only the prescribed *core requirements* to effectively control risks.

It is important to note that while a hierarchy of control measures from CDC has been defined by many countries (see figure 2), it cannot be assumed that one control measure is always preferable to another (e.g., engineering controls vs PPE).

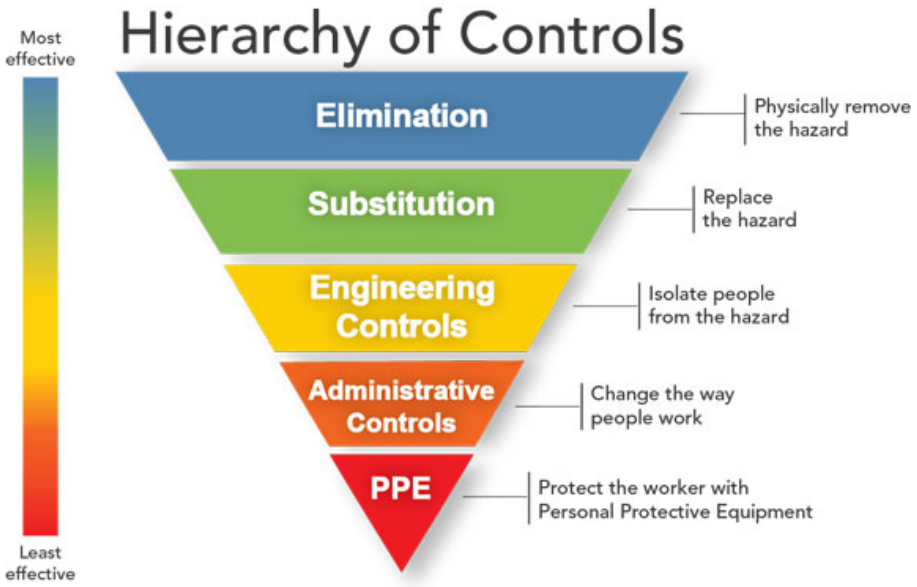

Figure 2. Hierarchy of controls. Source: CDC – National Institute for Occupational Safety and Health.

Where *heightened control measures* are applied, it is important to recalculate the level of residual risk after a control measure is selected and estimate whether this has effectively brought the residual risk to within the tolerance level. This requires a re-evaluation of the residual risk, guided by questions such as:

- Has the possibility of an exposure/release become less likely to happen?
- Have the consequences become less severe?
- Have the likelihood and consequences been reduced such that the residual risk is within the predetermined risk tolerance?
- If no, are additional controls available?
- Shall work proceed, with or without which controls?
- Who has the authority to accept the residual risk and approve the work to go ahead?
- How shall the selected control measures and subsequent approval for work to proceed be documented?

Develop risk mitigation strategies

- Identify required safety practices to address the identified risks
  - includes personal protective equipment, engineering controls, additional training, standard operating procedures, assessment strategies for staff proficiencies, drills, and so on.
- Determine whether *additional risk mitigation* strategies are necessary
  - for instance, consider upgrades and recertification of equipment and implementation of new training programs.
- Communicate risks and mitigation strategies to staff

- provide a mechanism of communication within the laboratory and a known place of record for all assessments and mitigation strategies.

• Validate risk mitigation strategies
- review risk assessment and mitigation strategies after implementation to ensure that measures were effective.

Figure 3 summarizes the level of risk and associates the risks with the types of control measures likely to be required. It highlights the following:

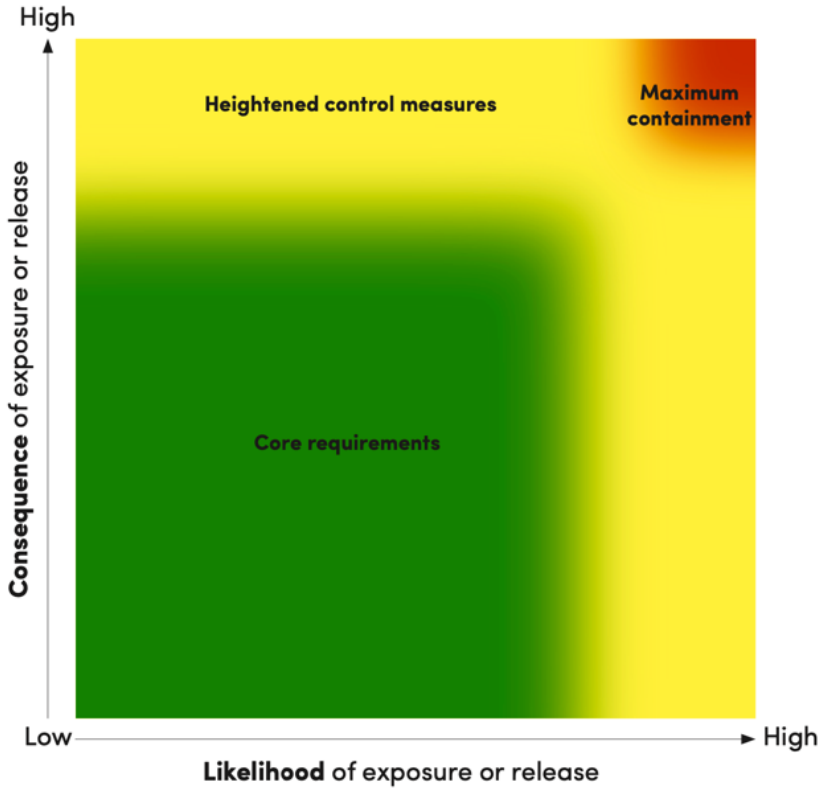

Figure 3. Risk reduction measures needed based on the likelihood and consequence level of exposure or release. Source: WHO. (in press). *Laboratory biosafety manual: 4th edition*. Geneva: World Health Organization.

Finally, once control measures have been selected, approved, and acquired, information about their purpose, function, and use must be communicated to all applicable personnel if they are to be implemented correctly and be effective. *Communication* is a vital part of biosafety and risk assessment. Without communication, it is unlikely that the control measures will reduce residual. All those working in the laboratory are responsible for following the correct practices and procedures of any risk reduction strategy that applies to them and for providing feedback on their effectiveness.

To achieve the appropriate level of awareness, training, and competency for implementation of control measures and safe laboratory operation requires, at a minimum, communication of the hazards (biological agents) present, communication of the risks associated with the procedures being performed, and communication of exactly how the control measures used can most effectively reduce those risks.

Annex 2 presents a sample lab work activity with computations for the likelihood and consequence and the total risk level prior and after mitigation. The 5 × 5 table is also presented for reference.

5. Review risks and control measures (performance monitoring)

Once performed, risk assessments must be reviewed routinely and revised when necessary, taking into consideration new information about the biological agent, changes in laboratory activities or equipment, and new control measures that may need to be applied. Suitable procedures must be put in place to ensure implementation and reliability of the controls and to ensure that they are sustainable.

A risk assessment must therefore be performed and reviewed periodically, hence performance monitoring, at a frequency that corresponds to the level of risk of the laboratory work. Typically, an annual review is adequate; however, some situations may prompt a more frequent review, such as a biosafety incident, or feedback from the laboratory personnel on the effectiveness and ease of use of the control measures that have been implemented.

Activities or events that affect the risk and will therefore trigger a risk reassessment include:

- Changes in biological agents or new information available on current biological agents.
- Changes in personnel.
- Changes in procedures and practices.
- Changes in laboratory equipment.
- Changes in international, national, or regional regulations or guidelines.
- Changes in national or regional disease status (endemicity of disease or eradication) and introduction of new technology.
- Laboratory relocation or renovation.
- An incident, accident, laboratory-associated infection, or any event where a potential for harm is identified.
- Identification and/or implementation of corrective and/or preventive action and user feedback.
- Periodic review.

Whenever a reassessment is warranted, the next step is to return to beginning of the risk assessment process where new information will be gathered relating to the change, risks will be re-evaluated, and it will be determined whether new control measures need to be implemented.

Bear in mind that this ongoing cycle of risk assessment continues to apply throughout the duration of the laboratory work.

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Annexes

Annex 1

Suggested policy on use of PPE

1. Goal:
   - To protect laboratorians, physicians, nurses, affiliates, and visitors from the hazards present in the laboratory.
   - To establish minimum PPE requirements based on best practices.
2. No unescorted visitors will be allowed to enter the laboratory at all times. Laboratory visitors must be under the direct supervision of a lab officer or staff. The host, however, must assume full responsibility for the visitor and ensure that pertinent safety training and appropriate PPE is provided and used.

3. Hosts must ensure that all visitors remove all PPE appropriately before exiting the laboratory.
4. The following PPE requirements apply to all individuals visiting diagnostic and teaching laboratories:
   - Full-length pants or equivalent must be worn at all times.
   - Closed toe footwear that covers the top of the foot must be worn at all times.
   - Long sleeves or laboratory coat covering both arms.
5. All other individuals entering laboratory space must follow the PPE requirements described above for the visitor, as well as the following:
   - Laboratory coats must be worn over personal clothing at all times.
   - Long hair and facial hair must be secured or tied back.
   - Flame-resistant laboratory coats must be worn when working with pyrophoric chemicals, water-reactive chemicals, and high volumes of flammable chemicals.
   - Disposable gloves that are protective against the hazardous or potentially hazardous materials being used must be worn. Gloves must be replaced when soiled, contaminated, or damaged.
   - Eye protection must be available and used when danger of splashing of hazardous or potentially hazardous materials could occur.
6. Depending on the work being done in a laboratory, additional and/or more specific PPE may be required.

Annex 2

Risk management process showing the activity, table references for likelihood and consequence (severity) rating and final risk level after mitigation.

Sample activities	Hazard	Severity	Existing control
Inoculating and incubating live HIV	Biohazard. Destroys the T-helper lymphocytes, crucial to the immune system. Potential source for sabotage.	4 High	1. Project evaluation by IBC. 2. BSL-3 practices by quality competent worker. 3. Specific PPE requirement (double gloves, no sharps and so on).
Extraction of *Escherichia coli* culture using high-speed centrifuge.	Equipment hazard. High-speed centrifuge hazard. Fatal centrifugal force caused by rotor imbalance.	5 Very high	1. Equipment safety feature (stop when imbalance is detected). 2. Proper safety training. 3. Regular preventive maintenance services. 4. Retiring expired rotors that exceed its shelf-life.

Table A1. Risk management: assessment – severity(consequence). BSL-3, biosafety level 3; IBC, Institutional Biosafety Committee; PPE, personal protective equipment.

Score	Impact to human physical being	Impact to community	Impact to environment
1. Negligible (insignificant)	Not likely to cause harm or injury.	Does not lead to disease in human or animal.	No impact to environment.
2. Minor (slight)	Injury of ill-health requiring first aid treatment only; discomfort is temporary and reversible.	May infect lab worker but is not serious.	No impact to environment.
3. Moderate	Injury require medical treatment. For example, deep cut, sprain and so on.	May infect lab worker but not infectious or may spread but prophylaxis is available. For example, normal flu.	May have impact that take weeks to reverse. For example, disease in fish farm.
4. Major (high)	Serious injury or long-term life-threatening occupational disease (e.g., cancer, acute poisoning and so on).	High risk of spreading to community but effective prophylaxis or treatment is available	Great impact to environment and may take years to reverse
5. Catastrophic (very high)	Fatal disease or injury.	Readily infect lab worker and transmissible to human, plant or animal. No effective treatment is available. For example, Ebola.	Irreversible. For example, Genetically modified crop release.

Table A2. Five-level scoring (how to quantify severity (consequence).

Level	Event frequency (likelihood)
1. Remote (very unlikely)	Never happen in a lifetime due to robust existing control or is not a risk.
2. Unlikely	May happen but had not occurred because of existing controls.
3. Possible	You or your colleague ever had such an accident in your lifetime.
4. Likely (frequent)	At least once a month or at constant interval .
5. Very likely (very frequent)	It's a daily problem.

Table A3. Risk management: assessment – likelihood.

Activities	Hazard	Severity	Existing control	Likelihood	Final risk level

Inoculating and incubating live HIV	Biohazard. Destroys the T-helper lymphocytes, crucial to the immune system. Potential source for sabotage.	4 High	1. Project evaluation by IBC. 2. BSL-3 practices by quality competent worker. 3. Specific PPE requirement (double gloves, no sharps and so on).	2 Unlikely.	8
Extraction of *Escherichia coli* culture using high speed centrifuge	Equipment hazard. High-speed centrifuge hazard. Fatal centrifugal force caused by rotor imbalance.	5 Very high.	1. Equipment safety feature (stop when imbalance is detected). 2. Proper safety training. 3. Regular preventive maintenance services. 4. Retiring expired rotors that exceed its shelf-life.	1 Remote.	5

Table A4. Final computation after mitigation showing final risk level. BSL-3, biosafety level 3; IBC, Institutional Biosafety Committee; PPE, personal protective equipment.

Section 3: PPE Selection and Donning

Introduction

Personal protective equipment refers to protective clothing, helmets, gloves, face shields, goggles, facemasks, and/or respirators or other equipment designed to protect the wearer from injury or the spread of infection or illness.

When used in healthcare settings, PPEs can either protect the healthcare worker or the patient. When used properly, PPE acts as a barrier between the infectious materials and any surface of the body where the said contaminant may gain entry and cause an infection or disease. It offers considerable protection from exposure to biological fluids and secretions. The use of PPE is vital also for protecting high-risk and vulnerable patients from exposure and can also minimize the extent of infection from patients with confirmed diseases/infections.

It should be emphasized however that the use of PPE has limitations. In the hierarchy of mitigation control measures, the use of PPE is considered as the last line of protection. This is because the risk posed by identified hazards is not removed; it only protects the wearer, it is uncomfortable to wear, and the likelihood of failure is high especially if the wrong PPE is used or the wearer is not trained in the use of the PPE. As such, it is a prudent practice to not rely on PPE alone when addressing the risks posed by biological materials.

Based on the current available evidence, the WHO-recommended PPE for the care of patients with COVID-19 are contact and droplet precautions, with the exception of aerosol-generating procedures, which require contact and airborne. It is emphasized that PPE is only a part of a larger infection prevention and control scheme and should be implemented as part of a multimodal strategy of management of patients with COVID-19.

Objectives

- To provide guidance on the different types, usage and specifications of PPE.
- To determine the different types of PPE depending on the activities involved through risk assessment.
- To provide guidance on the sequence of donning and doffing of PPE.

Definition of terms

Doffing: the process of removing the PPE after use.

Donning: the process of putting on the PPE.

Fit testing: the process of ensuring the suitability of use of a respirator to a specific individual.

Personal protective equipment (PPE): protective garments worn to protect the wearer from identified hazards.

Risk assessment: a robust process of assessing a scenario/situation based on risks posed by biological agents.

Seal check: a routine check to ensure fit performed prior to the use of respirators.

Scope

This covers the different types of PPE, their selection, donning, and doffing.

Responsibility

The proper selection of PPE is dependent on the risk assessment to be performed by persons involved in the laboratory testing process. Each PPE must be selected and used based on the risks identified. Guidance of each institution's Infection Prevention and Control Committee may be required as necessary.

Types of PPE

For the COVID-19 response and activities related to it, typical PPE worn by healthcare workers are as follows:

PPE	Descriptions/specifications
Respirators	Gives respiratory protection that prevents the inhalation of hazardous or infectious materials during the conduct of hazardous activities. It offers protection from aerosol-generating activities as well as droplet protection via the nose and mouth. Must conform to the US National Institute for Occupational Safety and Health (NIOHS) N95 standards or its equivalent. Refer to annex 1 for comparable standards to N95 from other countries. These should be disposable, without valves and with an adjustable strap. Those with soft edges and nose clip that adjust to the contours of the face is recommended. Respirators with higher filtering efficiency (e.g., N100 and P100) can be used but assess the breathing capacity of the wearer, duration of use, and ventilation of the room. Respirators must be fit-tested for each individual healthcare worker before use. Fit testing must also be done at least annually. Refer to annex 2 for the Occupational Safety and Health Administration recommendations for fit-testing.

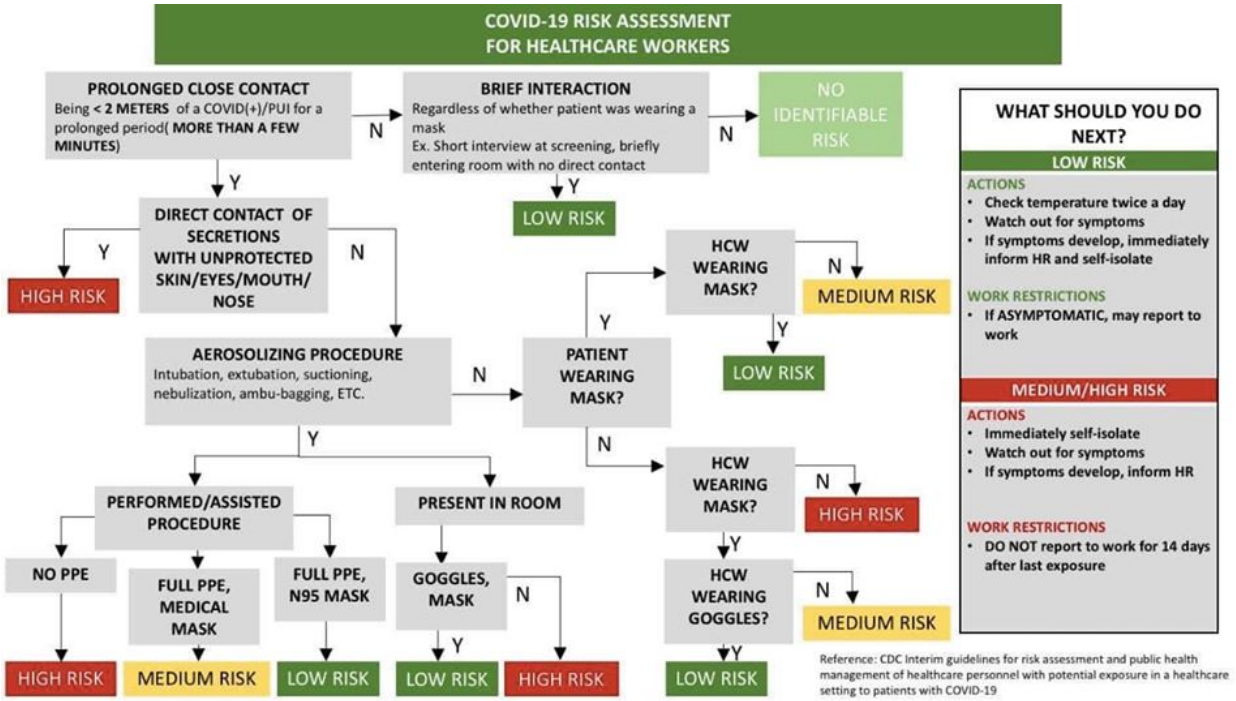
	It is recommended that a respirator seal check be done prior to the performance of activities. Refer to annex 3 for the seal check procedure. Do not confuse fit-testing with seal check. They are not interchangeable. Healthcare workers must be properly evaluated by a physician prior to the use of respirators.
Gown	Protects from splashes and contamination of clothing. Must be disposable, back-opening, solid front, long sleeved with elastic cuffs, made of polyethylene or similar material and fluid resistant. Must conform to ASTM F1671 standards or equivalent. It is recommended to use gowns with light coloration to easily detect contamination.
Coverall	Protects from splashes and contamination of clothing. Can be used as a substitute for gowns. Must be disposable, made of polyethylene or similar material, fluid resistant, non-woven, low-tinting, two-way zipper with zipper flap, elastic waist and ankle with knitted cuffs. Must conform to ASTM F1671 standards or equivalent. It is recommended to use coveralls with light coloration to easily detect contamination.
Gloves	Protects from contamination due to direct contact. Must be disposable, nitrile, powder-free, non-sterile, ambidextrous, rolled bead cuff, finger textured, length at least 24 cm. Must conform to EN374 standard or equivalent.
Face shield	Protects user from splashes and direct impact. Full face shield, anti-fog, adjustable size, with comfort stretch band and head foam. Must conform to EN 166 standard or equivalent.
Goggles	Provide protection from splashes and direct impact to the eyes. Must be light, soft, flexible, with adjustable strap, made of hard-coated polycarbonate lens or similar material, anti-fog. Must conform to EN 166 standard or equivalent. Less preferred than a face shield, because it protects the eyes only unlike a face shield that offer full face coverage.
Head cover	Protects hair from contamination due to direct contact. Disposable, polypropylene or similar material, spun-bond, non-woven, double-stitched, snug fit, fluid resistant. Must conform to ISO 4007:2018 standard or equivalent.
Shoe cover	Protects footwear from contamination due to direct contact.

	Disposable, polypropylene or similar material, non-woven, slip resistant, elastic top closure type and universal size. Must conform to Class 100 FS 209E standard or equivalent.
Surgical (medical) mask	Protects from droplet contamination. Does not offer sufficient protection from aerosol-generating activities. Must be disposable, ear loop type, 3-ply. Must conform to EN 14683 standards or equivalent.
Apron	Provide splash protection. Typically worn over gowns if gowns used are not fluid resistant or excessive slashing is expected. Must be water-proof, with sleeves, disposable, polyethylene or similar material with barrier laminate, no seams, no pockets, with tie closure. Must conform to EN467 standard or similar.
Powered air-purifying respirator	This device is an air-purifying respirator that uses a blower to force air through filter cartridges or canisters and into the breathing zone of the wearer. This process creates an air flow inside either a tight-fitting face piece or loose-fitting hood or helmet, providing a higher assigned protection than typical respirators. This PPE is recommended for laboratory healthcare workers with a failed respiratory fit test. Recommended assigned protection factor of at least 25. Test-fit not required.

Selection of PPE

There is no one-size-fit all approach for the use of PPE. The selection of PPE should depend on site-specific risk assessment and existing mandatory recommendations and guidelines. All activities and procedure being performed by a facility must be thoroughly assessed to determine the extent of risks and on how these risks can be mitigated by a particular type or set of PPE. Because activities involving the handling of COVID-19 samples suspected to contain the biological agent SARS-CoV-2 are diverse, it must be understood that each specific set of activities will involve different sets of PPE. In the risk assessment to determine the appropriate PPE, it is important to emphasize on the established mode of transmission for COVID-19, which is through droplets and direct and indirect contact.

The following matrix can be used for your risk assessments involving PPE.



The following are recommended minimum PPE to be worn by laboratory workers depending on the procedure to be performed. Take note that a site-specific risk assessment is still required to validate if these recommended PPE are addressing the risks involved.

Activity	Recommended PPE
Sample collection (swabbing, phlebotomy, etc.) from probable, suspected, and confirmed COVID-19 cases	Shoe cover, gown/coverall, head cover, N95 respirator, face shield, double gloves, and scrub suit.
Receiving of samples for laboratory testing	Shoe cover, gown, head cover, surgical mask, gloves, and scrub suit.
RNA extraction from nasopharyngeal swab/oropharyngeal swab samples	Shoe cover, gown, head cover, surgical mask, double gloves, and scrub suit.
Sample inspection and lysis	Shoe cover, gown/coverall, head cover, N95 respirator, face shield, double gloves, and scrub suit.
Master mix preparation and template adding	Shoe cover, gown, head cover, surgical mask, gloves, and scrub suit.
Amplification (PCR)	Shoe cover, gown, head cover, surgical mask, gloves, and scrub suit.
Loading of samples in rapid PCR system for SARS-CoV-2 (e.g., GeneXpert)	Shoe cover, gown/coverall, head cover, N95 respirator, face shield, double gloves, and scrub suit.
Running of samples in rapid PCR system for SARS-CoV-2 (e.g., GeneXpert)	Shoe cover, gown, surgical mask, face shield, double gloves, and scrub suit.

Routine clinical laboratory testing of patient samples (biological fluids and secretions) including rapid antibody-based tests	Shoe cover, gown/coverall, surgical mask, face shield, double gloves, and scrub suit.
Specimen transport within the facility	Surgical mask, gloves, and scrub suit.
Specimen transport to another facility prior to sample inactivation	Gown, surgical mask, double gloves, scrub suit
Specimen transport to another facility after sample inactivation	surgical mask, gloves, and scrub suit.
Mortuary services, autopsy, and gross pathology	Shoe cover, coverall, N95 respirator, face shield, double gloves, and scrub suit.
Spill management and clean-up of laboratories dedicated solely for COVID-19 testing	Shoe cover, gown/coverall, head cover, N95 respirator, face shield, double gloves, heavy duty gloves, and scrub suit.
Spill management and clean-up of general laboratory areas	Shoe cover, gown/coverall, surgical mask, face shield, double gloves, heavy duty gloves, and scrub suit.
Encoding of results within the COVID-19 testing laboratory	Surgical mask and scrub suit.
Repair, calibration, and preventive maintenance of laboratory equipment in laboratories dedicated solely for COVID-19 testing	Shoe cover, gown/coverall, head cover, N95 respirator, face shield, double gloves, and scrub suit.
Repair, calibration, and preventive maintenance of laboratory equipment in general laboratory areas	Shoe cover, gown/coverall, surgical mask, face shield, double gloves, and scrub suit.

General recommendations

1. Adhere to applicable best practices when using PPE:
    a. All laboratory personnel must change their street clothes to scrub suits within a designated area in the facility prior to donning their PPE.
    b. Scrub suits must be laundered within the facility and should not be brought outside the facility prior to decontamination.
    c. Jewelry and other personal effects must be removed prior to the donning of PPE.
    d. PPE stocks must be verified prior to each shift.
    e. Use of engineering controls such as plastic and glass barriers must be used together with other mitigation control measures when using PPE.
    f. PPE must be checked prior to donning.
    g. A buddy is recommended when donning and doffing PPE.
    h. Designate specific healthcare personnel dealing with patients with COVID-19 and samples to minimize and rationalize the use of PPE.

2. PPE should be discarded when:
    a. Exiting biocontainment areas.
    b. After every shift and midshift.
    c. When exposed to aerosol-generating procedures.

d. If visibly soiled or contaminated with blood, respiratory secretions, and other bodily fluids.

3. Extended use and reuse of PPE must be thoroughly evaluated through a risk assessment. Several guidelines and recommendations exist for extended use and reuse, but these must be validated by the facility. Checks must be in place prior to extended use and reuse to ensure that the PPE is still addressing risks. In the risk assessment for PPE reuse, consider product specifications of the PPE regarding suitability for reuse, durability of material, and suitability for decontamination among others. Examples of extended use and reuse are as follows:
   a. Use of respirators beyond the manufacturer's designated shelf-life for healthcare delivery.
   b. Extended use of the same respirator for repeated patient interactions if located together in the same dedicated room or ward.
   c. Use of face shields to minimize surface contamination of respirators.

4. Alternative PPE should only be considered in cases of serious shortage or in areas where PPE may not be available. These should be treated as last-resort temporary measures. Alternative PPE must be assessed if suitable for use during procedures involving COVID-19 samples.
   a. Cloth masks and other non-medical grade PPE should not be used within formal healthcare settings such as hospitals and clinical laboratories.
   b. Reusable cotton gowns are an option for non-aerosol generating procedures. These gowns must be thoroughly decontaminated and laundered in-house prior to reuse.

5. If PPE is reused, there must be a validated procedure to ensure that the PPE is thoroughly decontaminated and fit for reuse.
   a. Goggles and face shields can be cleaned with soap and water and disinfected using 70% alcohol or a solution of 0.1% sodium hypochlorite.
   b. When manufacturer guidance for the reuse of PPE is available, this must be strictly followed.
   c. In the absence of manufacturer guidance, a robust risk assessment must be conducted coupled with validation checks for reused PPE.

6. Hand hygiene, cough etiquette, and physical distancing must be observed at all times.

Donning of PPE

Donning refers to the proper sequence of putting on your set of PPE. The sequence may vary depending on the facility and the results of site-specific risk assessment. The sequence of donning is primarily driven by the logical sequence needed for a particular PPE set. For example, if an N95 respirator and face shield are to be used together, it would be logical to don first the respirator before the face shield.

Each facility should ideally have a designated area for the donning and doffing of PPE. It is a good practice to put cabinets with designated areas for each PPE type to facilitate the donning. A buddy system is also ideal as well as the placement of a mirror in the donning area to facilitate visual inspection of the PPEs. Posting of visual aids will also reinforce adherence to donning and doffing protocols. Once a PPE is donned, it should remain in place and used correctly for the duration of the shift.

The following is the recommended sequence for donning:

Step 1	Identify the needed PPE depending on the procedure to be performed as determined by a site-specific risk assessment.
Step 2	Remove your street clothes and put on your scrub suit.
Step 3	Remove all jewelry, personal effects, and gadgets.
Step 4	Put on a shoe cover.
Step 5	Put on your laboratory gown. Ensure that the gown is covering your upper torso down to your knees. Ensure that the sleeves cover your arms up to your wrist. Ask assistance for your gown to be tied lightly in the back.
Step 6	Put on your respirator or surgical mask. Ensure the fit of the respirator or mask to the contours of your face. Align and press the metal band to your nose bridge. If using a respirator, perform a seal check (see annex 3).
Step 7	Put on your head cover.
Step 8	Put on your face shield. Adjust the strap of your face shield so that it is fitting snugly on your head.
Step 9	Wear your gloves. Make sure that your gloves extend up to the wrist.
Step 10	Face the mirror and visually inspect your PPE. Ask your buddy to visually inspect also your PPE and do the same to him or her.
Step 11	You may now enter the area.

If using double gloves, the following donning sequence is recommended:

Step 1	Identify the needed PPE depending on the procedure to be performed as determined by a site-specific risk assessment.
Step 2	Remove your street clothes and put on your scrub suit.
Step 3	Remove all jewelry, personal effects, and gadgets.
Step 4	Put on a shoe cover.
Step 5	Put on your laboratory coat or coverall.
Step 6	Put on your respirator or surgical mask. Ensure the fit of the respirator or mask to the contours of your face. Align and press the metal band to your nose bridge. If using a respirator, perform a seal check (see annex 3).
Step 7	Put on your head cover.
Step 8	Wear your first layer of gloves. Make sure that your gloves extend up to the wrist. Gloves may be secured to your lab coat in the wrist area using a tape if desired.
Step 9	Put on your laboratory gown. Ensure that the gown is covering your upper torso down to your knees. Ensure that the sleeves cover your arms up to your wrist. Ask assistance for your gown to be tied lightly in the back.

Step 10	Put on your face shield. Adjust the strap of your face shield so that it is fitting snugly on your head.
Step 11	Put on the second layer of gloves. Ideally, the second layer of gloves should have a different color from the first layer to easily detect rips and tears. Make sure that your gloves extend up to the wrist.
Step 12	Face the mirror and visually inspect your PPE. Ask your buddy to visually inspect also your PPE and do the same to him or her.
Step 13	You may now enter the area.

Any of the steps may be omitted depending on the prescribed set of PPE for a procedure and result of the site-specific risk assessment but the logical sequence of donning must be maintained.

Doffing of PPEs

The proper doffing of PPE must be observed to avoid laboratory-acquired infections. Remember that doffing carries risks because the PPE has been exposed already to biological materials. Your facility must perform a site-specific risk assessment to determine the proper sequence of doffing. As a general rule in doffing, the most contaminated PPE is doffed first. It is ideal for a facility to have designated area for doffing. This area must have access to biohazard disposal binds for used PPE. A buddy system is recommended to ensure that proper doffing is observed. Use of visual aids can help reinforce proper doffing.

The following is the recommended sequence for doffing. This must be validated by your facility through a site-specific risk assessment.

Step 1	Remove the gloves by grasping a glove in your non-dominant hand to peel it off. Hold the peeled glove using the gloved dominant hand. Then, insert a finger from your ungloved hand to the inner side of your gloved hand and pull outward. Discard the gloves in a biohazard bin.
Step 2	Untie the disposable gown and pull toward the front of your body by grasping the inner layer of the gown. Do not touch the front and outer portions of the gown. Pull carefully away from your body while slowly folding the gown in a bundle and discard in a biohazard bin.
Step 3	Remove your shoe cover and discard in a biohazard bin then perform hand hygiene.
Step 4	Remove your face shield by grasping at the back and dispose in a biohazard bin. If the face shield is reusable, immerse it to a container containing an appropriate disinfectant.
Step 5	Remove your head cover by grasping the inner side and discard in a biohazard bin.
Step 6	Grasp the straps of your respirator or surgical mask and pull away from your face. Discard in a biohazard bin. Do not touch the other portion or your respirator or surgical mask.
Step 7	Perform hand hygiene.

When wearing double gloves, the following doffing sequence is recommended:

Step 1	Remove the gloves by grasping a glove in your non-dominant hand to peel it off. Hold the peeled glove using the gloved dominant hand. Then, insert a finger from your ungloved hand to the inner side of your gloved hand and pull outwards. Discard the gloves in a biohazard bin. Leave inner gloves on.
Step 2	Untie the disposable gown and pull toward the front of your body by grasping the inner layer of the gown. Do not touch the front and outer portions of the gown. Pull carefully away from your body while slowly folding the gown into a bundle and discard in a biohazard bin.
Step 3	If inner gloves are taped, remove the tapes.
Step 4	Unbutton/unzip your lab gown/coverall and remove by pulling away from your body. Do not touch the front side of your lab gown. Place in a segregation area for decontamination and laundering.
Step 5	Remove your shoe cover and discard in a biohazard bin.
Step 6	Remove your inner gloves by grasping a glove in your non-dominant hand to peel it off. Hold the peeled glove using the gloved dominant hand. Then, insert a finger from your ungloved hand to the inner side of your gloved hand and pull outwards. Discard the gloves in a biohazard bin.
Step 7	Remove your head cover by grasping the inner side and discard in a biohazard bin.
Step 8	Remove your face shield by grasping at the back and dispose in a biohazard bin. If the face shield is reusable, immerse to a container containing an appropriate disinfectant.
Step 9	Grasp the straps of your respirator or surgical mask and pull away from your face. Discard in a biohazard bin. Do not touch the other portion or your respirator or surgical mask.
Step 10	Perform hand hygiene.

Any of the steps may be omitted depending on the prescribed set of PPE for a procedure and result of the site-specific risk assessment but the logical sequence of doffing must still be followed from the most contaminated to the least contaminated.

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Annexes

Annex 1

Country	Performance Standard	Acceptable product classifications	Standards/Guidance Documents	Protection Factor ≥ 10
Australia	AS/NZS 1716:2012	P3 P2	AS/NZS 1715:2009	YES
Brazil	ABNT/NBR 13698:2011	PFF3 PFF2	Fundacentro CDU 614.894	YES
China	GB 2626-2006	KN 100 KP100 KN95 KP95	GB/T 18664—2002	YES
Europe	EN 149-2001	FFP3 FFP2	EN 529:2005	YES
Japan	JMHLW-2000	DS/DL3 DS/DL2	JIS T8150: 2006	YES
Korea	KMOEL-2017-64	Special 1st	KOSHA GUIDE H-82-2015	YES
Mexico	NOM-116-2009	N100, P100, R100 N99, P99, R99 N95, P95, R95	NOM-116	YES
US NIOSH Requirements	**NIOSH approved 42 CFR 84**	**N100, P100, R100 N99, P99, R99 N95, P95, R95**	**OSHA 29CFR1910.134**	**YES**

Annex 2

Fit testing

A respirator cannot protect you if it does not fit your face. It is that simple. Certain respirators, known as tight-fitting respirators, must form a tight seal with your face or neck to work properly. If your respirator does not fit your face properly, contaminated air can leak into your respirator face piece and you could breathe in hazardous substances. So before you wear a tight-fitting respirator at work, your employer must be sure that your respirator fits you. Your employer does this by performing a fit test on you while you wear the same make, model, and size of respirator that you will be using on the job. That way, you know that your respirator fits you properly and can protect you, as long as you use it correctly.

In addition, before you use a respirator or are fit-tested, your employer must ensure that you are medically able to wear it.

So what is a fit test? A “fit test” tests the seal between the respirator’s face piece and your face. It takes about 15–20 minutes to complete and is performed at least annually. After

passing a fit test with a respirator, you must use the exact same make, model, style, and size respirator on the job.

A fit test should not be confused with a user seal check. A user seal check is a quick check performed by the wearer each time the respirator is put on. It determines if the respirator is properly seated to the face or needs to be readjusted.

There are two types of fit tests: qualitative and quantitative.

A. Qualitative fit testing is a pass/fail test method that uses your sense of taste or smell, or your reaction to an irritant in order to detect leakage into the respirator face piece. Qualitative fit testing does not measure the actual amount of leakage. Whether the respirator passes or fails the test is based simply on you detecting leakage of the test substance into your face piece. There are four qualitative fit test methods accepted by OSHA:

a. Isoamyl acetate, which smells like bananas.
b. Saccharin, which leaves a sweet taste in your mouth.
c. Bitrex, which leaves a bitter taste in your mouth.
d. Irritant smoke, which can cause coughing.

Qualitative fit testing is normally used for half-mask respirators – those that just cover your mouth and nose. Half-mask respirators can be filtering face piece respirators – often called "N95s"—as well as elastomeric respirators.

B. Quantitative fit testing uses a machine to measure the actual amount of leakage into the face piece and does not rely upon your sense of taste, smell, or irritation in order to detect leakage. The respirators used during this type of fit testing will have a probe attached to the face piece that will be connected to the machine by a hose. There are three quantitative fit test methods accepted by OSHA:

a. Generated aerosol.
b. Ambient aerosol.
c. Controlled negative pressure.

Quantitative fit testing can be used for any type of tight-fitting respirator.

Many workers need to wear prescription glasses or personal protective equipment, such as safety goggles or earmuffs, while performing a job. If you fall into this category, then you must wear these items during the fit test to be sure they do not interfere with the respirator's fit.

You must be fit tested before you use a respirator in the workplace, and you must be retested at least every 12 months to make sure that the respirator you use still fits you. You must be fit tested with the specific make, model, style, and size of respirator that you will be using.

Not everyone can get a good fit with one specific respirator. If the respirator fails the fit test, then another make, model, style, or size must be tried until one is found that

fits you properly. Therefore, your employer needs to provide you with a reasonable selection of sizes and models to choose from. When you have completed the fit testing process, it is very important that you know which make, model, style, and size respirator fits your face properly and when and where you will need to wear it for protection.

Also, the fit of your respirator must be retested whenever you have a change in your physical condition that could affect the fit of your respirator. Such changes could include:

- Large weight gains or loss.
- Major dental work (such as new dentures).
- Facial surgery that may have changed the shape of your face.
- Significant scarring in the area of the seal.

Any of these changes could affect the ability of your respirator to properly seal to your face, which could allow contaminated air to leak into your respirator face piece.

If you find that the fit of your respirator becomes unacceptable, you must be allowed to select a different type of respirator and be retested. The selection may include a new make, model, style, or size of respirator.

Facial hair, like a beard or mustache, can affect your respirator's ability to protect you. Anything that comes between your face and the respirator's seal or gets into the respirator's valves can allow contaminated air to leak into the respirator face piece and you will not be protected. For example, if you have long hair, make sure it does not get between the respirator seal and your face because this can allow contaminated air to leak into the respirator.

Fit testing can be done by your employer or an outside party or a past employer. Your current employer is permitted to accept fit testing you have received from an outside party (such as a former employer) within the last 12 months, as long as you use the same respirator make, model, style, and size at your new worksite. This is known as "fit testing portability."

While recent fit testing can follow you from job to job, it is still your current employer's responsibility to ensure that the fit testing and recordkeeping requirements of respiratory protection standard have been met before you use a respirator for protection against hazardous exposures at work.

Sometimes workers own their own respirators and bring them to a job where respiratory protection is required. If your employer allows you to use your own personal respirator for protection, then your employer must still ensure that:

- Your respirator is appropriate for the hazards you face.
- Your respirator is properly cleaned, maintained, and stored.
- The proper schedule for replacing cartridges and filters is followed.

Remember, if you do not know if a respirator is needed for the task you will be doing, or if you are unsure about how to properly use a respirator or which filter or cartridge to use, talk to your supervisor before entering the hazardous area.

Annex 3

Seal check

The individual who uses a tight-fitting respirator is to perform a user seal check to ensure that an adequate seal is achieved each time the respirator is put on. Either the positive and negative pressure checks listed in this appendix, or the respirator manufacturer's recommended user seal check method shall be used. User seal checks are not substitutes for qualitative or quantitative fit tests.

I. Face piece positive and/or negative pressure checks for respirators without exhalation valves
   A. Positive pressure check. Once the N95 respirator is donned, cover your hands over the face piece and exhale gently into the face piece; the face piece should bulge slightly and no air leaks should be detected between your face and the face piece; if air leakage is detected, reposition the respirator on your face and/or readjust the tension of the straps and repeat the seal check.
   B. Negative pressure check. Close off the inlet opening of the canister or cartridge(s) by covering with the palm of the hand(s) or by replacing the filter seal(s), inhale gently so that the face piece collapses slightly and hold the breath for 10 seconds. The design of the inlet opening of some cartridges cannot be effectively covered with the palm of the hand. The test can be performed by covering the inlet opening of the cartridge with a thin latex or nitrile glove. If the face piece remains in its slightly collapsed condition and no inward leakage of air is detected, the tightness of the respirator is considered satisfactory.

II. Face piece positive and/or negative pressure checks for respirators with exhalation valves
   A. Positive pressure check. Close off the exhalation valve and exhale gently into the face piece. The face fit is considered satisfactory if a slight positive pressure can be built up inside the face piece without any evidence of outward leakage of air at the seal. For most respirators, this method of leak testing requires the wearer to first remove the exhalation valve cover before closing off the exhalation valve and then carefully replacing it after the test.
   B. Negative pressure check. Close off the inlet opening of the canister or cartridge(s) by covering with the palm of the hand(s) or by replacing the filter seal(s), inhale gently so that the face piece collapses slightly, and hold the breath

for 10 seconds. The design of the inlet opening of some cartridges cannot be effectively covered with the palm of the hand. The test can be performed by covering the inlet opening of the cartridge with a thin latex or nitrile glove. If the face piece remains in its slightly collapsed condition and no inward leakage of air is detected, the tightness of the respirator is considered satisfactory.

III. Manufacturer's recommended user seal check procedures

The respirator manufacturer's recommended procedures for performing a user seal check may be used instead of the positive and/or negative pressure check procedures provided that the employer demonstrates that the manufacturer's procedures are equally effective.

Section 4: Quality Management System for COVID-19 Testing

Introduction

Implementing a quality management system (QMS) to ensure that laboratories provide accurate and reliable results and reduce the risk of errors is important. However, in resource-constrained settings, many laboratories may not be accredited to international standards. Introducing a new test, particularly under outbreak conditions, may therefore come with a high risk of errors, and this step describes the key critical elements that laboratories should put in place to rapidly identify and minimize the risk of laboratory errors. In the absence of quality assurance (QA), inaccurate test results can lead to the wrong treatment and management decisions and lapses in surveillance of disease epidemics.

Objectives

- To ensure quality throughout the different phases of testing (pre-preanalytical, preanalytical, analytical, postanalytical, and post-postanalytical stages).
- To detect, evaluate, and correct errors due to test system failure, environmental conditions, or operator performance before patient results are reported.

Definition of terms

Documentation: laboratory forms and registers should be standardized and staff trained to fill out all documentations consistently and fully.

External quality assurance: aims to analyze the accuracy of the entire testing process from receipt of sample and testing of sample to reporting of results.

Key performance indicator (KPI): refers to collection and analysis of data at each step of the testing cascade that can serve as indicator for correct performance of the whole testing process.

Quality assurance (QA): refers to the total process implemented by a laboratory that aims to ensure that the final results reported are as accurate and reliable as possible.

Quality control (QC): refers to procedures used in each assay to assure a test run is valid and results are reliable.

Quality indicator monitoring: refers to collection and analysis of that can serve as indicator for correct performance of the total testing process.

Quality management system (QMS): a set of key quality elements that need to be integrated within the day-to-day operations in order to meet the quality objectives.

Standard operating procedures (SOPs): cover all procedures, from managing incoming specimens to authorizing and issuing test reports and shall be available to staff who shall be trained on their use.

Total turnaround time (TAT): is the time between specimen collection and result reporting to the clinician.

Scope

This QMS focuses on QA for the molecular testing for SARS-CoV-2, which has better sensitivity and specificity compared to serological and rapid antibody tests.

Note: PCR for COVID-19 is the recommended method to reliably identify COVID-19 cases.

Responsibility

All laboratory personnel in the clinical and research laboratories are responsible for these procedures as well as the documentation of the activities.

Procedures

1. QA of molecular assays

   In the diagnosis of suspected case by RT-PCR, several undesirable conditions may occur:

   A. False positive: a specimen that does not contain SARS-CoV-2 tests positive for the virus.
   B. False negative: a specimen containing a sufficient quantity of SARS-CoV-2 tests negative for the virus.
   C. Specimen not containing sufficient SARS-CoV-2 negative for the virus, a result that may not be consistent with highly suspected results from radiography.

   These three discrepant scenarios can mislead diagnosis and clinical management. QA shall help improve the accuracy and reliability of SARS-CoV-2 molecular assays, especially in the face of this highly contagious virus; hence, robust strict implementation of quality assurance shall be followed in each clinical laboratory.

   Rapid optimization of testing kit quality and standard operating procedures are top priorities for solving the issue of false negatives. Thorough performance verification of assays is required. Choosing guanidine hydrochloride buffer instead of thermal inactivation if inactivation is required will exhibit a smaller impact on RT-PCR results.

   It is advised that laboratorians standardize the procedure of nucleic acid extraction and testing to avoid false negatives.

Positive quality control materials, such as synthetic SARS-CoV-2 RNA or stocks from positive specimens, shall be used in the detection of clinical specimen, and laboratories shall participate in external quality assessment to improve testing proficiency.

RT-PCR and serological assays can be combined in the progressive and recovery stages to reduce false negatives.

2. QA of serological assays

Serological antibody testing enables analysis of the dynamics of infections with SARS-CoV-2; more importantly, it has lower operational requirements and can reduce the risk of laboratory professionals' exposure due to respiratory sampling.

Despite the advantages of serological assays, the testing performance of antibody assays shall also be considered. Some situations can lead to false positives and false negative reactions. Cross-reactivity with other subtypes of coronaviruses may be a threat. Antibody assays are susceptible to the influence of endogenous interferents, including rheumatoid factors, heterophilic antibodies, and complements as well as exogenous factors, such as specimen hemolysis, yielding false positive results. Enhancing the specificity of the antigen peptide of the reagents to reduce the cross-reactivity with other viruses and diluting the specimens and changing the enzyme-labelled antibodies can reduce the incidence of false positives.

In addition, a combination of antibody assays, clinical symptoms, and molecular results shall be used for diagnosis to minimize false positives. False negative results also require attention. There are specific testing windows for serological antibody detection: IgA and IgM usually last a short time, and IgG may be produced in the later period, so sampling at unsuitable stages may lead to false negatives. Differences in individual immune response and antibody production also may lead to false negative results. Therefore, at least two serology results at different time point combined with negative RT-PCR result are helpful to rule out false negatives.

Testing strategy is also important; the combined IgM–IgG test was reported to have better practicality and sensitivity that tests for only IgM or IgG.

3. Components of QMS unique to molecular testing

3.1. Continuing education and knowledge:

- Laboratory personnel needs to have overall knowledge and depth of understanding of the test to be performed.
- Establish an education program to ensure that laboratory personnel understand all aspects of testing.
- Competency and continuing education specific to biosafety and molecular biology educational information established through different sources.

3.2. Purchasing, inventory management, and equipment

- Inventory control and planning is essential.
- Ensure adequate supply of materials.
- Identify expiration dates based on usage to minimize wastes.
- Check calibration of equipment: thermocyclers, freezers, pipettors, and signal detection.
- Clean regularly all equipment that could potentially become contamination with RNA, DNA, or amplified nucleic acid products and cleaning documented.

3.3. Process control unique to molecular testing

- Molecular assay controls and failures
  - Assay controls are typically run at the same time as the samples in a batch and are processed in the same manner as patient samples.
  - The SOP shall include details of the controls to use, the control tolerance limits, and the corrective actions necessary when controls exceed predefined specifications.
  - Quantitative assays require positive controls at the limit of detection to ensure that assay sensitivity is within predetermined limits and maintained.
  - Periodic assessment of standards is also required to ensure that reported values are accurate.
  - For assays in which interfering substances may lead to false negative, an internal control (e.g., housekeeping gene) is necessary to ensure that the reaction is not inhibited.
  - Extraction control is also processed to ensure that reagents are not contaminated.
- Reagent prequalification
  - Assays and reagents need to be evaluated with each shipment and lot and equivalent performance established with the assays and reagents currently in use.
  - Test a set of samples and controls using the new lot/shipment side by side with the current lot.
  - Acceptance criteria for the new lot should be predefined in the SOP.
- Water quality
  - Additional purification steps may be required for assays that involve isolation of RNA.
  - For testing that involves an amplification step, such as PCR grade water that is guaranteed free from potentially amplifiable contaminating substances should be used.

3.4. Document retention

- Policies and procedures should be established regarding archiving, storage, retention, and disposal of specimen and records.
- Extended retention times of molecular genetic test reports and specimens is justified.

- The Centers for Disease Control – Morbidity and Mortality Weekly Report recommends that laboratories maintain test reports for at least 25 years after the date of initial report and retain samples as long as possible after the completion of testing and reporting results.
- Test results may be stored electronically.
- The procedure should take into consideration the different types of samples and original source, possible archiving options with pathology samples such as paraffin blocks.
- Specimens may be retained for validation studies and troubleshooting.
- Primary specimens: specimens may be consumed in toto by the nucleic acid isolation procedure; surplus may be retained until all testing is complete on the patient and then discarded following autoclaving.
- Nucleic acid samples: RNA/DNA isolated from patient specimens maybe retained until a report is generated and for a predefined period appropriate for the type of testing; sample maybe discarded after a predefined period, except in situations in which the patient consented to further use of his sample for research purposes (informed consent).

4. Quality control performed for COVID-19 testing

4.1. Prior to the start of testing, a validation or verification (consisting of known positive and negative samples) should be performed to ensure the test performs as intended. Under emergency conditions, the validation and verification studies may be limited as discussed in the previous steps.

4.2. Laboratories may take advantage of the WHO recommendation of confirming the first 5 positive specimens and 10 negative specimens (collected from patients that fit the case definition) by referring them to one of the WHO reference laboratories providing confirmatory testing for COVID-19 (refer to WHO Reference laboratories providing confirmatory testing for COVID-19).

4.3. QC must be included in each run and should cover each critical steps of the PCR analysis as shown in figure 4.

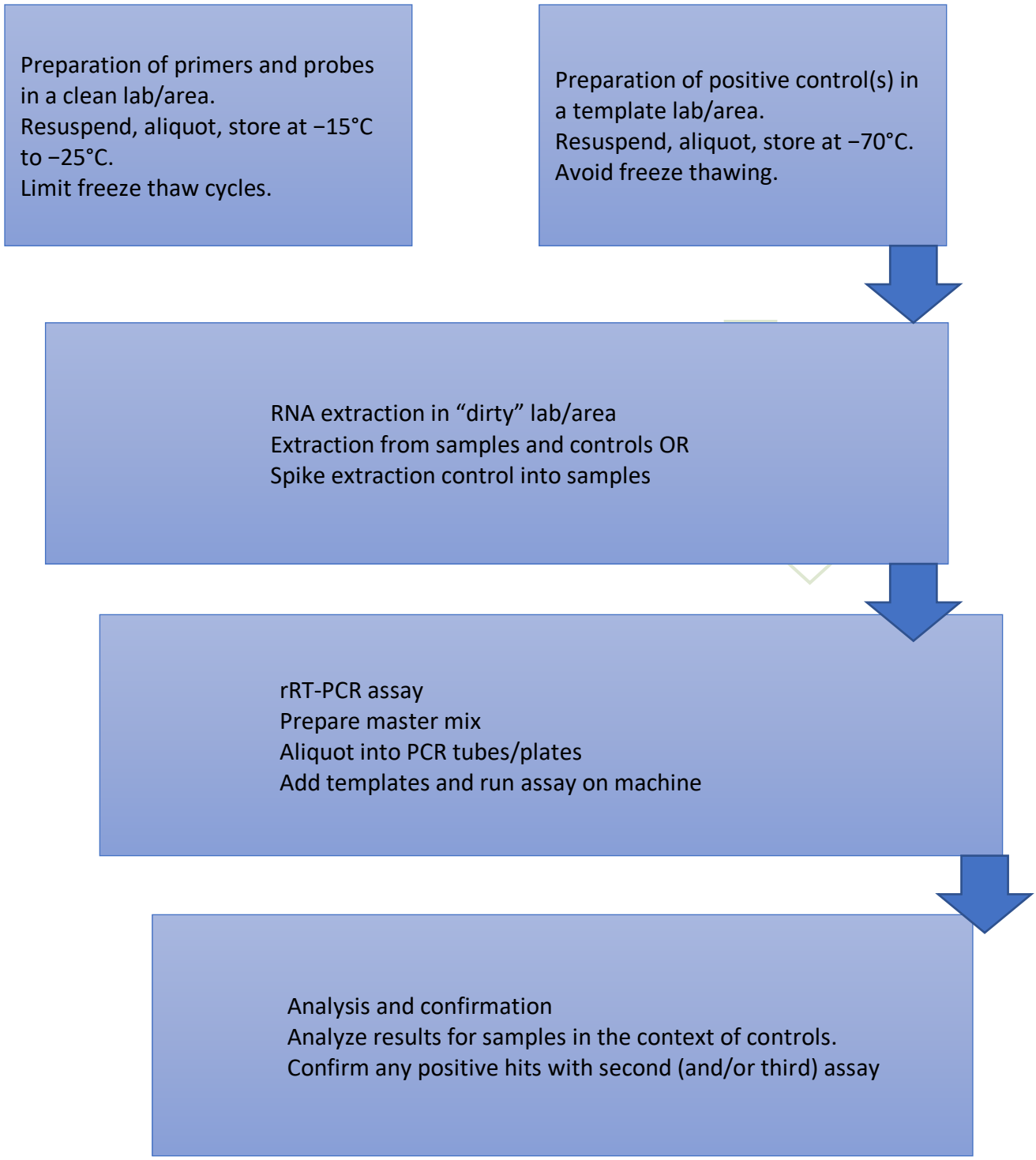

Figure 4. COVID-19 PCR workflow: key steps in sample/QC processing.

4.4. The QCs include:

- Extraction negative control: indicates whether contamination was introduced from the extraction phase.
- Extraction positive control: provides an indication of the quality

of the extracted template and whether the PCR was in anyway inhibited.

- No template control: indicates whether contamination was introduced in from the PCR phase. Also indicates whether PCR reagents have been compromised and to determine threshold.
- Positive template control(s): synthetic SARS-CoV-2 RNA/DNA (either gene fragment or whole genome): indication of limit of detection and robustness of the assay.

4.5. Commercial QCs are preferred but absence of commercial controls, laboratories can use the following:

- Negative control: water/universal transport media/viral transport media.
- Positive control: a patient sample with a known (and preferably low 25–30) Ct value (virus concentration) for human gene target, for example, RNAse P or non-human, non-SARS-CoV-2 extraction control, for example, Equine Arteritis virus.

A failure of any one of these controls (for instance, the positive control turns out to be negative) invalidates the test result and the assay must be repeated either from stored or newly collected sample after investigating and fixing the cause of the failure (e.g., contamination or degradation of the sample, or expired reagents). In case patient results were already issued, they should be recalled immediately (giving an explanation of the reason) and the patient retested urgently.

4.6. New lot QC, or lot- to- lot verification**:** newly received lot (or batch) of test kits or test components is tested using a panel of samples to confirm its performance is acceptable relative to the existing lot in use.

5. Quality issues specific to molecular testing

- Sample degradation: RNA is labile with a tendency to degrade; exposure to elevated temperatures can compromise RNA integrity; detrimental effect on assays and results.
- Extraction failures: technical failures during the extraction process may result in failure to obtain DNA/RNA; sample may have been compromised when it was obtained from the patient or while it was transported to the laboratory.
- Amplification failures: inhibitory factors present in the sample, not eliminated during sample extraction, result in inhibition of the amplification reaction, giving false negative results.
- Unusual results: results that do not fit with the clinical presentation of the patients.
- Control failures: controls with known genotypes are run; any control failure invalidates the results of the entire assay.
- Contaminated no target or reagent blanks: all assays should include a

reagent blank (no target sample is added to the reaction); if the reagent blank produces a positive result, the entire assay is invalidated; the presence of a product in the reagent blank can be due to contaminating DNA or primer dimers in PCR-based assays.

6. Key performance indicators (KPIs)

KPIs include the following and should be analyzed and reported on a regular basis at least monthly:

- Number of specimens tested, by specimen type.
- Number (%) of positive, negative, and invalid test results.
- Specimen rejection rate.
- Number (%) of failed IQC results.
- EQA/PT performance (pass/fail or % score).
- Turnaround time (TAT)
    - Laboratories should monitor the following: % results reported within target TAT, average and range of TAT. In addition to monitoring total TAT, the laboratory should also measure time from specimen collection to receipt at laboratory, time from receipt at laboratory to result reporting (within laboratory TAT), in order to identify and address bottlenecks at various stages of the diagnostic process.

7. EQA for COVID-19 testing

7.1. EQA allows the comparison of a laboratory's testing performance to a peer group of laboratories, national reference, or WHO reference laboratories. There are three different methods for EQA programs:

- Proficiency testing (PT): an external provider sends blinded, well characterized panel at intervals (usually quarterly) that will be treated like patient sample during testing, to a set of laboratories, and the results are analyzed, compared, and feedback reports generated.
- Rechecking or retesting: samples tested by one laboratory are retested by another laboratory (interlaboratory comparison). WHO recommends that the specimens of the first five (5) positive cases and the first 10 negative cases that meet the COVID-19 case definition for testing should be shipped for confirmation to the national reference or international referral laboratory for COVID-19 (WHO, 2020b, 2020c). After that, the laboratory can test for SARS-CoV-2 independently but should still collaborate with national reference laboratories or WHO referral laboratories for troubleshooting. Rechecking can be employed in the absence of a PT program.
- On-site evaluation: usually done in addition to PT or rechecking, and particularly when it is difficult to conduct traditional PT or to use the rechecking/retesting method. An evaluator (e.g., staff from national reference laboratory) will visit the laboratory to check if the laboratory is meeting quality requirements, retest and verify few test results, and provide

advice to correct any faulty procedures.

Due to COVID-19 crisis, air transportation is limited, and it may not be feasible to get PT or conduct on-site evaluation. Therefore, countries are strongly advised to use the rechecking/retesting method as an option for EQA program (send samples to the national reference or WHO reference laboratories (WHO, 2020a) and to consider remote mentoring/supervision of laboratories by the national reference laboratory using Zoom or other web conferencing systems.

A. Challenges to implementing QA

Challenges	Mitigation measure
Unavailability of controls	Positive control: use of a confirmed positive patient sample. Negative control: use water/universal transport. media/viral transport media
Most methods are under development hence no validation data	Use methods with Emergency Listing (EUL) by WHO. Check https://www.who.int/diagnostics_laboratory/EUL/en/ and third party evaluated methods and perform method verification to the extent possible.
Unavailability of EQA schemes	Develop interlaboratory comparison and also send positive samples to national reference and/or WHO reference. laboratories

B. COVID-19 specimen acceptance criteria

- For upper respiratory tract specimens: one (1) nasopharyngeal swab and one (1) oropharyngeal swab in one (1) tube with VTM/UTM. Swabs should be dacron tipped with plastic shaft.
- For lower respiratory tract specimens (sputum, BAL): sterile container (i.e., conical tube), BAL specimen at least 2–3 mL.
- Primary tube labeled legibly with at least two unique patient identifiers: name and birthdate or name and PIN/hospital number.
- Primary tube with swab and VTM secured tight and no leaks.
- Primary tube contained inside secondary container (i.e., conical tube, ziplock-sealed bag or equivalent container).
- Secondary container (conical tube) placed inside ziplock-sealed clear bag or equivalent clear container.
- Corresponding forms: fully accomplished case investigation form with legible identifiers, contact details, and date and time of collection.
- All specimens must be delivered to the laboratory as soon as possible, package specimens with coolers/packed ice, maintained cold chain at 2–8°C, received within 3 days of collection. For transport times longer than 3 days, pack specimens in dry ice and maintain cold chain at −70°C.

C. COVID-19 specimen rejection criteria

- Dry swabs: swabs not submitted in approved liquid media or non-sterile containers.
- Wrong swabs used (cotton swabs, calcium alginate swabs, media with gel and wood shaft are not acceptable).
- Leaking primary containers.
- Specimens without two patient identifiers or illegible patient identifiers.
- Samples with no required documents (case investigation form) or documents with incomplete data.
- Specimens received at room temperature longer than prescribed time duration after collection.
- Unfrozen specimens received at the laboratory >72 hours after collection. Cold chain must be maintained, and freeze-thawing of specimens is not acceptable.
-

Special considerations:

- Minor discrepancies in patient identifiers (i.e., Ma. Instead of Maria, missing middle initial) provided that second identifier is present.
- For samples other than swabs and lower respiratory tract specimens, please contact the laboratory.

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PART 2: PREANALYTICAL STAGE

Section 1: Proper Collection, Handling, and Storage of Specimens for SARS-CoV-2 Test

Introduction

All specimens collected by the laboratory professionals shall be regarded as potentially infectious. The laboratory professionals who collect, handle, transport, and test clinical specimens from patients and individuals at risk for COVID-19 shall strictly adhere to precautionary measures and biosafety practices recommended by the Department of Health.

Proper collection of specimens is a critical step for appropriate laboratory diagnosis of COVID-19 and all infectious diseases. A successful diagnostic test begins at sample collection because improper sampling can result in an inadequate specimen, rendering a diagnostic test inconclusive or incorrect. A specimen that is not collected correctly may lead to false negative test results.

Specimen collection has to be performed by laboratory personnel who have completed training and demonstrated competency.

Objectives

This document provides guidance for proper and safe collection of specimen for COVID-19 testing in the clinical and research laboratories in the Philippines.

It aims to minimize risks for laboratory staff handling specimens from patients with possible or laboratory-confirmed COVID-19.

Definition of terms

Antibodies: they are proteins that help fight off infections and usually provide protection against getting that disease again (immunity). Antibodies are disease specific.

Coronavirus disease 2019: an infectious disease caused by a newly discovered strain of coronavirus (SARS-CoV-2).

COVID-19: the abbreviated name of coronavirus disease of 2019 given by the WHO in a press release on February 11, 2020.

NAAT: stands for nucleic acid amplification test that is highly sensitive in detecting the presence of viral particles such as SARS-CoV-2. Polymerase chain reaction (PCR) is an example of NAAT technique.

NIOSH-approved N95 respirator: a particulate-filtering facepiece that filters at least 95% of airborne particles approved by the National Institute for Occupational Safety and Health, a US Federal agency responsible for conducting research and making recommendations for the prevention of work-related disease and injury. NIOSH-approved respirators have approval label on or within the packaging of the respirator.

RT-PCR: stands for reverse transcription polymerase chain reaction. A laboratory testing procedure that uses reverse transcriptase to generate complementary deoxyribonucleic acid (DNA) template from ribonucleic acid (RNA) found in patient sample. Complimentary DNA (cDNA) are then amplified by DNA polymerase enzyme and detected traditionally by gel electrophoresis. DOH and WHO recognizes RT-PCR as a sensitive test in detecting SARS-CoV-2 from patient's sample.

SARS-CoV-2: severe acute respiratory syndrome coronavirus 2 is the virus causing the coronavirus disease (COVID-19).

VTM/UTM: viral transport medium commercially manufactured by Copan (https://www.copanusa.com/sample-collection-transport-processing/utm-viral-transport/) as universal transport medium, while Becton Dickinson label their product as universal viral transport system.

Scope

This practical guidance applies to all laboratory professionals in the clinical and research laboratories in the Philippines. This guidance is adopted from Department of Health-Research Institute for Tropical Medicine (DOH-RITM), WHO and Centers for Disease Control and Prevention (CDC). Advice offered here relates to laboratory procedures conducted in clinical diagnostic and research laboratories. It does not cover virus isolation, propagation, research work, or work involving animals infected with SARS-CoV-2.

Responsibility

The laboratory professionals are responsible for these procedures as well as the documentation of the activities.

Procedures

1. Request form information

The request form captures the following:

- Patient information shall include:
  - Complete name.
  - Date of birth.
  - Sex.

  - Residential address.
  - Unique identification number.
  - Other useful information (e.g., patient hospital number, surveillance identification number, name of hospital, hospital address, room number, physician's name and contact information, name and address for report recipient).
- Date and time of sample collection.
- Anatomical site and location of specimen collection.
- Tests requested.
- Clinical symptoms and relevant patient history (including vaccination and antimicrobial therapies received, epidemiological information, risk factors, and history of travel).

2. Collection requirements

The SARS-CoV-2 testing specimens shall be collected by qualified medical technologists who have received and passed biosafety training and are equipped with the corresponding laboratory skills.

2.1. The personal protective equipment (PPE) shall consist of:

PPE	Description
Eye protection/goggles	Good seal with skin of the face, flexible PVC frame to easily fit with all face contours with even pressure, enclose eyes, and the surrounding areas, accommodate wearers with prescription glasses, clear plastic lens with fog and scratch resistant treatments, adjustable band.
Face shield	Made of clear plastic and providing good visibility to both the wearer and the patient. Adjustable band to attach firmly around the head and fit snuggly against the forehead, fog-resistant (preferable). Completely cover the sides and the length of the face. May be reusable (made of robust material that can be cleaned and disinfected) or disposable.
Surgical mask	Good breathability, internal and external faces should be clearly identified.
Long sleeved laboratory gown	Splash proof, thick material.

Double-layered latex glove	Outer layer is changed when exposed to single patient or patient's sample before touching another patient.
Waterproof boot covers	Fluid resistant, disposable.
Coverall	Fluid resistant, with zipper and flap.
Apron (optional)	Disposable, impermeable, or fluid-resistant plastic material.
Hair cap	Disposable.
Properly fit-tested grade N95 respirator or higher as NIOSH approved.	Good breathability with design that does not collapse against the mouth (e.g., duckbill, cup-shaped).

Table 1. Personal protective equipment (PPE) and description for use in specimen collection.

2.2. Materials needed:

- Case investigation form (CIF).
- Universal transport medium (UTM)/virus transport media (VTM).
- Nasopharyngeal swab, sterile dacron/rayon swab/flocked-tip swab with pliable plastic shaft.
- Oropharyngeal swab, sterile dacron/rayon swab/flocked-tip swab with pliable plastic shaft.
- Sterile tongue depressor.
- Test tube rack.
- Resealable plastic bags (ziplock).
- Laboratory sealing film (parafilm).
- Masking tape.
- Permanent tube marker.
- PPE (as in section 2.1).
- Refrigerator or Thermo box with 4–6 frozen ice packs.

3. Infection control guidelines

- Always wear PPE during collection.
- When completed, dispose of all PPE and other contaminated materials in the appropriate trash bin.
- Wash hands thoroughly with soap and water or alcohol-based hand gel before and after the procedure.
- Refer to biosafety guidelines in pre-preanalytical phase.

4. Virus collection media
   - Take out only the number of VTM needed from the freezer (−20°C)/refrigerator freezer or UTM (room temperature) where they are stored.
   - Frozen VTM shall be thawed just before use. If the collection site is far from the refrigerator, have a thermo box with 4–6 frozen ice packs on hand to maintain a refrigerated temperature during collection.
   - Check VTM/UTM for turbidity. The medium shall be clear and pinkish. Tap the tube to mix contents.

	Universal transport medium (UTM)	Virus transport medium (VTM)
No specimen	Store at 2–25°C	Store at −20°C
With NPS/OPS	Store at 2–8°C	Store at 2–8°C
Use if color of the media is	Light orange	Salmon pink
Do not use if color of the media is	Red	Yellow or any change in color

Table 2. UTM and VTM storage and color descriptions.

5. Directions for use of swabs
   - Check for the integrity of the swab.
   - Swabs shall not be used if:
     - There is evidence of damage or contamination.
     - The expiration date has passed.
     - The swab package is damaged.
     - The swab package has been opened.
     - There are other signs of deterioration.
   - Do not use excessive force when collecting swab samples from patients as this may result in accidental breakage of the swab shaft.
   - Swabs are for single use only.

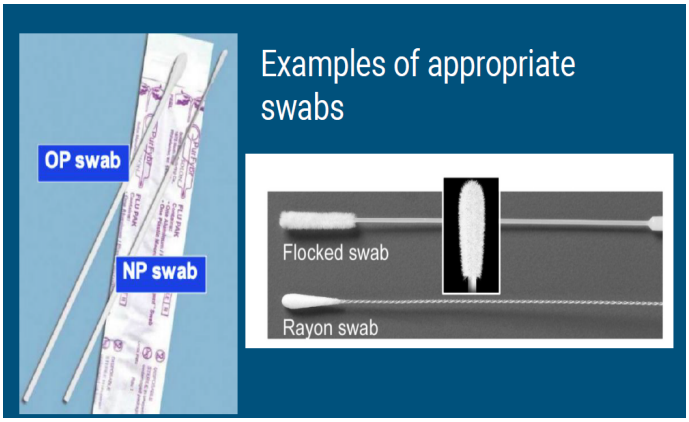

Figure 1. Examples of appropriate swabs for nasopharyngeal/oropharyngeal.

- Correct handling of swab.

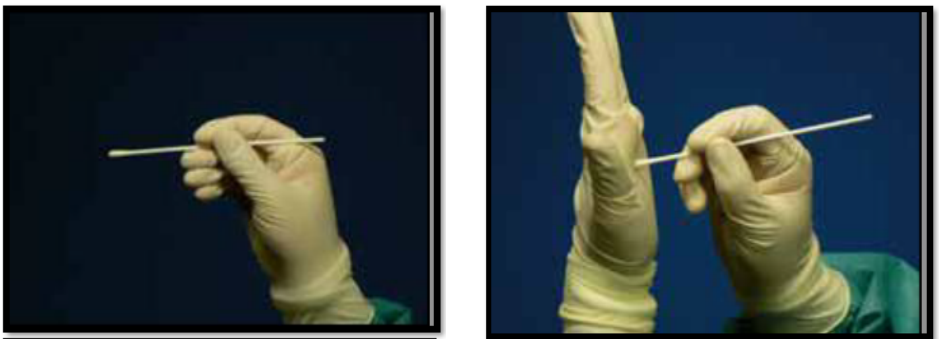

Figure 2. Swabs are held correctly during specimen collection.

6. Timing of collection

Collect specimen as soon as possible (within 14 days after the onset of symptoms). Sample collected greater than 14 days after onset has much lower chances for successful isolation of the virus.

For serological testing of whole blood, serum, or plasma IgM or IgG antibodies against SAR-CoV-2, the first specimen should be collected within acute phase (within 7 days) after the onset of disease. The second specimen is collected during 3–4 weeks after the onset of the illness.

7. Specimen collection

Label the VTM tube with the patient's full name and date of collection. The information on the label must be legible and shall match the information on the CIF. Label must remain attached under all conditions of storage and transport.

8. Categories of specimen collected

Specimen source	Samples to be collected	Tests done	Remarks
Upper respiratory tract	• Nasopharyngeal swab (NPS). • Oropharyngeal swab (OPS). • Nasal midturbinate (NMT) also called deep nasal swab. • Anterior nares specimen. • Nasopharyngeal wash/aspirate (not	Molecular tests such as RT-PCR or NAAT.	NPS and OPS are most commonly collected using VTM/UTM transported at 4°C. Use of Amies and other bacterial

	recommended due to aerosolization). • Nasal wash/aspirate in ambulatory patients (not recommended due to aerosolization).		transport medium is not acceptable. Appropriate swabs are dacron or rayon. Do not use wooden or cotton swabs. Ensure specimen container is tightly sealed and labeled properly with name, age, sex, date, and time of collection.
Lower respiratory tract	• Bronchoalveolar lavage. • Tracheal aspirate. • Pleural fluid. • Lung biopsy. • Sputum for patients with more severe respiratory disease.	Molecular tests such as RT-PCR or NAAT.	High risk for aerosolization; personnel are advised to adhere strictly to airborne infection prevention and control measures.
Blood	• 5 mL of blood in evacuated EDTA tube. • Serum from 5 mL blood non-anticoagulated evacuated tube.	NAAT/RT-PCR. IgM and IgG antibodies against SARS-CoV-2.	Testing done on whole blood or plasma. First specimen collected in acute phase (within 7 days) after the onset of disease. Second specimen collected during 3–4 weeks after the onset of the illness. Antibody tests should not be used as stand-alone test to diagnose COVID-19. To see if

			currently infected, there is a need for viral test using rRT-PCR/NAAT testing.

Table 3. Specimen source, samples to be collected, and test for the corresponding specimen (see annex 2).

9. Methods of specimen collection

The specimen collection guidelines follow standard recommended procedures of WHO, Centers for Disease Control and Prevention, and Department of Health (DOH)-Research Institute for Tropical Medicine (RITM).

- For initial diagnostic testing for COVID-19, it is recommended collecting an upper respiratory nasopharyngeal swab (NPS).
- Collection of oropharyngeal swabs (OPS) is a lower priority and if collected shall be combined in the same tube as the NPS. Collection of only OPS is acceptable if other appropriate swab types are not available.
- Collection of sputum shall only be done for those patients with productive coughs. Induction of sputum is not recommended.
- Specimens shall be collected as soon as possible once a suspect COVID-19 case is identified, regardless of the time of symptom onset.
- Maintain proper infection control when collecting specimens. Use of PPE is strictly recommended.
- Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit viral detection in PCR testing.
- Place swabs immediately into sterile tubes containing 2–3 mL of viral transport media.

9.1. Respiratory specimens

- Assemble supplies: (refer to Materials needed)
  - Check expiry date and visually inspect UTM/VTM kits.
- Wash hands. Put on appropriate PPE to protect yourself in case the patient coughs or sneezes while you are collecting the specimen.
- Explain procedure to the patient.
- If the patient has a lot of mucous in his or her nose, this can interfere with the collection of cells. Ask the patient to blow their nose to clear nasal passage of excessive mucus or use a tissue to gently clean out visible nasal mucus before a swab is taken. Have the patient dispose of the used tissue in infectious waste receptacle. The virus is not found in the mucous discharge.

- Make sure that the patient is seated in a comfortable position.

9.1.1. Upper respiratory tract specimens

A. Nasopharyngeal swab

1. Patient must be seated upright, with the head in a straight position (not extended upwards/not looking up because the swab will be directed superiorly towards the anterior cranial base, which can be dangerous).
2. Using a flexible polyester or rayon tip swab, measure from the base of the nostril toward the auditory pit. Divide the length into half in order to know into what extent will be inserted into the nostril to ensure that it reaches the posterior pharynx.

Figure 3. Approximate positioning of the posterior pharynx.

3. With the gloved hand, have the patient seated, tilt the head slightly backward. Remove visual obstructions such as excess mucus or loose nose hair. Then, insert the swab into the nostril parallel to the palate. Stop when slight resistance is met.

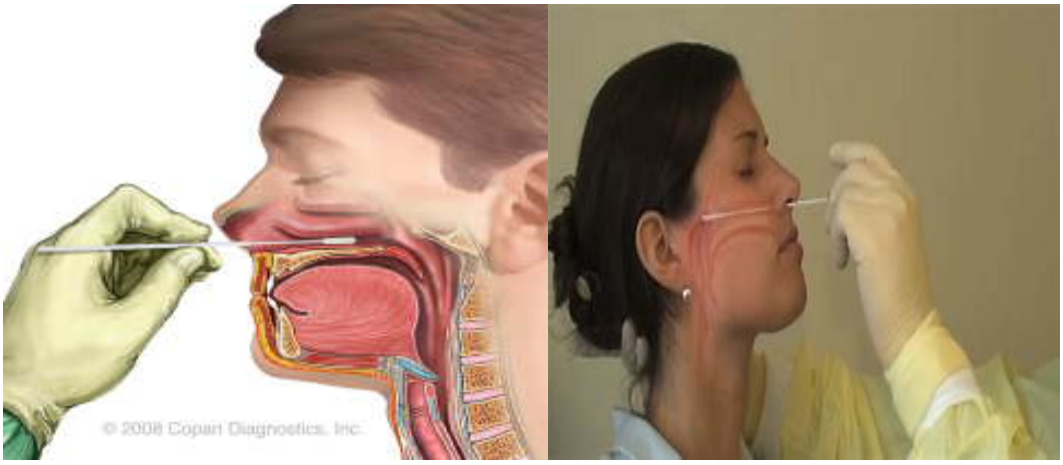

Figure 4. Proper patient position for swab insertion.

4. Rotate swab applying a little force to take large quantities of mucus. Repeat procedure in the other nostril using the same swab.
5. Place the NPS immediately in the UTM/VTM tube to avoid drying of the swab**.** Break off top of swab (it will snap off) that sticks out of the tube and close the tube tightly.
6. Remove PPE and wash hands.
7. Ensure the specimen is labeled and transported to the laboratory with completed requisition.

B. Oropharyngeal (throat) swab collection

1. Remove the swab from the package, holding it at the end of the stick.
2. Position yourself in front of the person you will be swabbing.
3. Tilt the head back slightly, open the mouth wide to expose the tonsils and back of the throat.
4. Use the tongue depressor to keep the tongue from interfering with specimen collection.
5. Insert swab into mouth and rub the swab over both tonsillar pillars and posterior oropharynx back and forth, avoid touching the tongue or roof of the mouth.
6. Withdraw the swab without touching the teeth or gums.
7. While holding the swab, remove the cap of the sterile viral transport media tube and place the swab in the tube.
8. Snap/cut off the applicator stick, then close the cap tightly.

9. Place the closed specimen container inside the biohazard specimen transport bag.

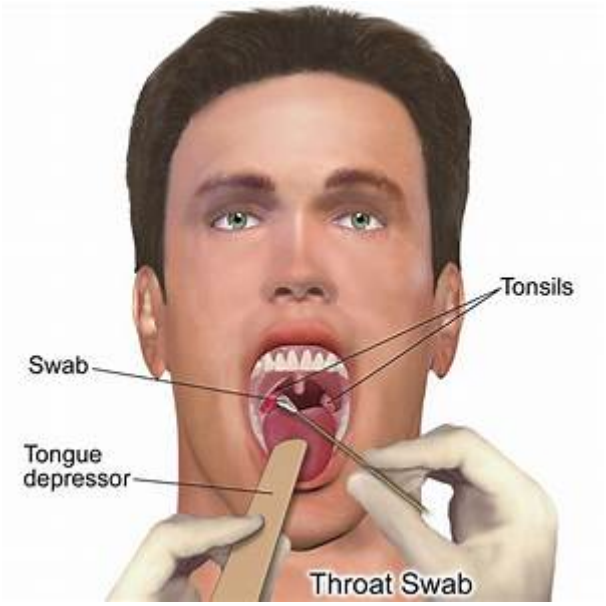

Figure 5. Oropharyngeal (throat) swab.

9.1.2. Other upper respiratory tract specimens:

C. Nasal midturbinate (NMT) swab, also called deep nasal swab

1. Tilt patient's head back 70°.
2. Gently rotate the flocked tapered swab while inserting it less than 1 inch (about 2 cm) into nostril (until resistance is met at turbinates).
3. Rotate the swab several times against nasal wall and gently withdraw the swab.
4. Repeat collection in other nostril using the same swab.

D. Anterior nares specimen

1. Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris).

2. Firmly sample the nasal membrane by rotating the swab and leaving in place for 10–15 seconds.
3. Sample both nostrils with same swab.
4. Nasopharyngeal wash/aspirate or nasal wash/aspirate (not recommended due to aerosolization but may be collected by doctors or nurses in the intensive care unit and submitted to the laboratory for analysis).

9.1.3. Lower respiratory tract specimens

- For patients who develop a productive cough, sputum shall be collected and tested for SARS-CoV-2. The induction of sputum is not recommended. For patients for whom it is clinically indicated (e.g., those receiving invasive mechanical ventilation), a lower respiratory tract aspirate or bronchoalveolar lavage sample shall be collected and tested as a lower respiratory tract specimen.
- Specimens shall be collected as soon as possible once a suspect COVID-19 case is identified, regardless of the time of symptom onset. Maintain proper infection control when collecting specimens.

A. Bronchoalveolar lavage, tracheal aspirate, pleural fluid, and lung biopsy

1. Collect 2–3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Note: due to the increased technical skill and equipment needs, collection of specimens other than sputum from the lower respiratory tract may be limited to patients presenting with more severe disease, including people admitted to the hospital and/or fatal cases.

B. Sputum

- Educate the patient about the difference between sputum and oral secretions (saliva).
- Have the patient rinse his or her mouth with water.
- Patient shall cough deeply and expectorate coughed sputum.

- Collect the sputum in a 50 mL screw-capped sterile plastic tube containing 3 mL of sampling solution. If the sputum is not collected in the sampling solution, 2–3 mL of the sampling solution can be added into the tube before testing, or add sputum digestive reagents to equal volume of sputum (see annex 3).

9.2. Blood specimen

Whole blood

1. Extract 5 mL blood using EDTA-containing vacuum tube.
2. Nucleic acid extraction shall be performed on whole blood or plasma.
3. For plasma separation, the whole blood shall be centrifuged at 1500–2000 rpm for 10 minutes.
4. A minimum of 200 µL plasma is collected in a sterile plastic tube with screw cap.

Serum or plasma

1. Collect 5–10 mL whole blood in lithium heparin, EDTA or serum separator vacuum tube. For infants, collect a minimum of 1 mL whole blood.
2. Serum separator tube requires upright standing at least 30 minutes before centrifugation.
3. Centrifuge at 1000–1300 relative centrifugal force for 10 minutes.
4. Aspirate a minimum of 200 µL serum or plasma and place it in a separate sterile tube or cryovial.

10. Sample receipt, inspection, and encoding

All swab specimens are submitted in UTM/VTM collection tube. Visual inspection of the sample is done to check sample integrity. Incorrect or leaky samples are returned or disposed of in accordance with internal safety regulations and risk assessments.

10.1. Sample receipt

- At the reception area of the laboratory, the laboratory personnel to receive the specimen shall inspect the outer box for leaks and shall disinfect the box by spraying its entire surface with disinfectant (either sodium hypochlorite or Lysol disinfectant solution). If there is a sign of leakage, disinfect the transport box in biohazard bag and send notification to the sender.
- Collect the corresponding CIF with the list of samples and check the entries for complete information. Notify sender for missing information. Samples that have been collected beyond 48 hours prior shall be rejected.

- Note the date and time of receipt of samples.

10.2. Sample inspection
- Samples are opened from packaging, inspected and swabs removed.
- Actual specimen inspection shall be done inside a certified class II biosafety cabinet following protocols of laboratory safety and in complete PPE.

10.3. Sample encoding
- Sample codes are assigned for samples that pass inspection. Sample codes and patient information are manually encoded.

11. Handling and storage of specimens for SARS-CoV-2 testing

Ideally, specimens for virus detection shall reach the laboratory as soon as possible after collection. The following specimen storage and handling recommendations shall be followed:

1. Specimens are temporarily stored at 2–8°C and shipped on cold packs promptly or within 5 days of collection.
2. In case of expected delay of specimens reaching the laboratory, use of viral transport medium is strongly recommended.
3. If further delay (over 5 days) in testing is expected, serum or plasma samples can be stored at −20°C or ideally at −70°C and shipped with dry ice. Freezing is not required if courier pick up for shipping will occur within 24 hours.
4. Serum or plasma aliquots that are frozen must remain frozen until thawed for testing. Repeated thaw and refreezing of samples is unacceptable.
5. Freezing of whole blood is not recommended as it will result to hemolysis that is contraindicated for testing.
6. A list of recommended storage from DOH as adopted from WHO is presented in annex 1.

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Annexes

Specimens to be collected from symptomatic patients and asymptomatic contacts

Specimen type	Collection materials	Transport to laboratory	Storage till testing	Comment
Nasopharyngeal and oropharyngeal swab	Dacron or polyester flocked swabs*	4 °C	≤5 days: 4 °C >5 days: -70 °C	The nasopharyngeal and oropharyngeal swabs should be placed in the same tube to increase the viral load.
Bronchoalveolar lavage	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	There may be some dilution of pathogen, but still a worthwhile specimen
Tracheal aspirate, nasopharyngeal aspirate or nasal wash	sterile container *	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	
Sputum	sterile container	4 °C	≤48 hours: 4 °C >48 hours: –70 °C	Ensure the material is from the lower respiratory tract
Tissue from biopsy or autopsy including from lung	sterile container with saline	4 °C	≤24 hours: 4 °C >24 hours: –70 °C	
Serum (2 samples acute and convalescent possibly 2-4 weeks after acute phase)	Serum separator tubes (adults: collect 3-5 ml whole blood)	4 °C	≤5 days: 4 °C >5 days: –70 °C	Collect paired samples: • acute – first week of illness • convalescent – 2 to 3 weeks later
Whole blood	collection tube	4 °C	≤5 days: 4 °C >5 days: –70 °C	For antigen detection particularly in the first week of illness
Urine	urine collection container	4 °C	≤5 days: 4 °C >5 days: –70 °C	

*For transport of samples for viral detection, use VTM (viral transport medium) containing antifungal and antibiotic supplements. For bacterial or fungal culture, transport dry or in a very small amount of sterile water. Avoid repeated freezing and thawing of specimens.

Annex 1. Specimen to be collected from symptomatic patients and asymptomatic contacts. Source: DM 2020-0034.

Specimens to be collected (WHO Interim Guidance 19 March 2020 (WHO–COVID-19-laboratory-2020.5-eng; https://apps.who.int/iris/handle/10665/331329)

Table 1. Specimens to be collected from symptomatic patients and contacts

	Test	Type of sample	Timing
Patient	NAAT	Lower respiratory tract - sputum - aspirate - lavage Upper respiratory tract - nasopharyngeal and - oropharyngeal swabs - nasopharyngeal wash/nasopharyngeal aspirate. Consider stools, whole blood, urine, and if diseased, material from autopsy.	Collect on presentation. Possibly repeated sampling to monitor clearance. Further research needed to determine effectiveness and reliability of repeated sampling.
Patient	Serology	Serum for serological testing once validated and available.	Paired samples are necessary for confirmation with the initial sample collected in the first week of illness and the second ideally collected 2-4 weeks later (optimal timing for convalescent sample needs to be established).
Contact in health-care centre associated outbreaks or other settings where contacts have symptoms, or where asymptomatic contacts have had high-intensity contact with a COVID-19 case.	NAAT	Nasopharyngeal and oropharyngeal swabs.	Within incubation period of last documented contact.
	Serology	Serum for serological testing once validated and available.	Baseline serum taken as early as possible within incubation period of contact and convalescent serum taken 2-4 weeks after last contact (optimal timing for convalescent sample needs to be established).

Annex 2. Specimens to be collected from symptomatic patients and contacts.

Specimens to be collected (WHO Interim Guidance 19 March 2020 (WHO–COVID-19-laboratory-2020.5-eng https://apps.who.int/iris/handle/10665/331329)

Formula of storage fluid for sputum digestive reagents:

Dithiothreitol	0.1 g
Sodium chloride	0.78 g

Phosphorus chloride	0.02 g
Disodium hydrogen phosphate	0.112 g
Potassium dihydrogen phosphate	0.02 g
Water	7.5 mL
pH 7.4 ± 0.2 (25°C)	

Annex 3. Formula of storage fluid for sputum digestive reagents.

Dilute the storage solution to 100 mL with deionized water before use. Sputum can also be treated with a phosphate buffer containing 1 g/L of protease K in an equal volume of sputum.

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Section 2: Packaging, Shipping, and Transport of Clinical Specimens for COVID-19 Testing

Introduction

International and national procedures have been established for the safe transport of biological materials by all modes of transportation (air, sea, and land).

The international regulations for the transport of infectious substances by any mode of transport are based on the recommendations made by the United Nations Committee of Experts on the Transport of Dangerous Goods and are presented in the form of *Model Regulations on the Transportation of Dangerous Goods*. Many countries adopt the UN Model Regulations in their entirety to stand as their national legislation, while some countries apply variations. The *Technical Instructions for the Safe Transport of Dangerous Goods by Air*, published by the International Civil Aviation Organization (ICAO), are legally binding international regulations for shipments by air. The International Air Transport Association (IATA) publishes *Dangerous Goods Regulations* that incorporate the ICAO provisions and may add further restrictions. These rules apply on all international flights. For national flights (i.e., flights within one country), national civil aviation authorities apply national legislation. Some airlines will not carry dangerous goods at all, while others will carry only a very limited range of goods.

This guidance adopted from WHO Guidance on Regulations on the Transport of Infectious Substances was modified according to the local application. However, the basic requirements of specimen packaging and transport still needs the specific requirements of referral laboratories for the preservation and viability of specific specimen for diagnosis.

The laboratory professionals must be aware on how to ensure their shipments comply with IATA regulations and ensure their test samples are not rejected by testing laboratories.

Objectives

- To provide guidance and direction to ensure proper and safe packaging, shipping, and transport of clinical specimens for COVID-19 testing in the clinical and research laboratories.
- To minimize risks for laboratory staff handling specimens during packaging, shipping, and transport of clinical specimens from patients with possible or laboratory-confirmed COVID-19.
- To ensure that the integrity of the specimens is preserved for accurate analysis by the receiving laboratory.
- To protect the public and the environment from the potential harmful effects due to improper handling of laboratory specimens.

Definition of terms

Dangerous goods: articles or substances that are capable of posing a risk to health, safety, property, or the environment and that are classified in the IATA Dangerous Good Regulations.

Diagnostic/clinical specimens: defined as any human or animal material including, but not limited to, excreta, blood and its components, tissue, and tissue fluids collected for the purposes of diagnosis.

IATA Dangerous Good Regulations for Division 6.2 – Infectious Substances: the International Air Transport Association (IATA) is a trade association of the world's airlines. One of IATA's regulations pertains to dangerous goods.

Infectious substance: a substance containing a viable microorganism, such as bacterium, virus, rickettsia, parasite, or fungus, that is known or reasonably believed to cause disease in humans or animals.

Outer container: a sturdy, leak-proof container (e.g., a box, styrofoam box, and chiller box), that is used to contain the secondary container. This protects the sample from outside influences such as physical damage and water while in transit.

Primary container or receptacle: a labeled primary watertight, leak-proof receptacle containing the specimen. The receptacle is wrapped in enough absorbent material to absorb all fluid in case of breakage.

Referral laboratory: laboratory that receives specimens from another facility for testing.

Referring laboratory: a laboratory that sends the clinical specimen to a referral laboratory for testing.

Secondary receptacle/container: a second durable, watertight, leak-proof receptacle to enclose and protect the primary receptacles. Several wrapped primary receptacles may be placed in one secondary receptacle. Sufficient additional absorbent material must be used to cushion multiple primary receptacles.

Shipper: individual or agency trained and has the authorization to transport specimen.

Scope

This practical guidance applies to all laboratory professionals in the Clinical and Research Laboratories who will perform packaging, transport, and shipping of clinical specimen from COVID-19 patients to designated referral laboratories in the Philippines and also for shipment to international referral laboratories.

Responsibility

The laboratory professionals are responsible for these procedures as well as the documentation of the activities.

Sender shall pack the sample for referral using the triple packing system.

Courier shall be responsible in transporting specimen to reach its destination.

Sample receiver shall be responsible in inspecting and recording the condition and integrity of received packaging and sample.

Procedures

The World Health Organization (WHO) has assigned COVID-19 or SARS-CoV-2 virus clinical (non-propagated) samples to category B and cultures/isolates to category A.

Pack and ship suspected and confirmed SARS-CoV-2 patient specimens as UN 3373 Biological Substance, Category B, in accordance with the current edition of the Dangerous Goods Regulations. Personnel must be trained to pack and ship according to the regulations and in a manner that corresponds to their function-specific responsibilities.

1. Documentation of sample movement

It is important to track the movement of all clinical samples from the point of collection from the patient, packaging, transport and shipment, storage, for testing and disposal.

The time and date of collection shall be recorded on the case investigation form.

2. Packaging

2.1. Local triple packaging system

- The clinical sample shall be packaged according to the triple packaging system (annex 1):
  - Primary receptacle: do not use glass containers; secure with parafilm; wrap with absorbent material; place inside an air-tight, leak-proof plastic ziplock bag.
  - Secondary container (e.g., plastic bottle): durable; leak proof.
  - Outer container (e.g., sturdy box): labeled with all the necessary signs and markings.
- All specimens shall be prepacked to prevent breakage and spillage.

- Specimen containers shall be sealed with parafilm and placed in ziplock bags.
- Place enough absorbent material to absorb the entire contents of the secondary container (containing primary container) and separate the primary containers (containing specimen). The absorbent will soak up any liquid if a breakage occurs.
- Specimens shall be transported with cold packs or other refrigerant blocks that are self-contained, not actual wet ice. This prevents leaking and the appearance of a spill.
- When large numbers of specimens are being shipped, these shall be organized in a sequential manner in boxes with separate compartments for each specimen.

Standard good practice recommends *against* the following:

- Do not place any dry ice in the "primary container" or "secondary container," foam envelopes, ziplock bags, cryovial boxes, or hermetically sealed containers.
- Do not place primary containers sideways or upside down in ziplock bags.
- Do not place any paperwork in the secondary containers or ziplock bags, so as not to damage the paperwork.
- Do not use biohazard/autoclave bags to prepack materials due to the inadequate seal of these bags.

2.2. Labelings and markings

If samples are to be shipped, the IATA Dangerous Goods Regulations describe the markings and the labels on packages for air transport.

2.3. Documentation

- Documentation required by a transporter shall be accessible without opening the package.
- Packages shall be accompanied by the necessary documentation, including customs and/or quarantine permits.

3. Transport and shipping

Transport of clinical samples is often needed from collection sites to the testing laboratories. It may be from another room, another laboratory or another facility. It may even be another city, regions, or even other countries for further testing.

The transport of infectious substances maybe subjected to various national and/or international regulations depending on the origin, destination, and/or the mode of transport being used.

The aim is always to reduce the likelihood of an exposure to and/or a release of the infectious substance in order to protect the laboratory professionals and/or the surrounding environment.

3.1. Specifics for transport of samples to the laboratory:

- Ensure that personnel who transport specimens are trained in safe handling practices and spill decontamination procedures.
- Ensure that laboratory personnel know and follow the requirements in the national or international regulations for the transport of dangerous goods (infectious substances) as applicable.
- Deliver all specimens by hand whenever possible. Do not use pneumatic tube systems to transport specimens.
- State the full name, date of birth of the suspected case clearly on the accompanying request form. Notify the laboratory as soon as possible that the specimen is being transported.
- Transport of specimens within national borders shall comply with applicable national regulations. International transport Regulations. Novel coronavirus specimens shall follow the UN Model Regulations, and any other applicable regulations depending on the mode of transport being used.

3.2. Transfer of clinical samples:

- Transfer within the laboratory
  Moving infectious clinical samples within the laboratory shall be undertaken following GMP to prevent incidents of cross-contamination and inadvertent spillage. Additional measures to consider include the following:
  - Use sealed containers such as screw-capped tubes.
  - Use deep-sided and leak-proof trays or boxes made of smooth impervious material (plastic or metal) that can be effectively cleaned and disinfected.
  - If using racks or vials, trolleys can be used for more stable transport.
- Transfer within a building
  - Plan, organize, and carry out the transfer in a way that minimizes transit through communal areas and public thoroughfares.
  - Transfer containers shall be suitably labeled to identify their contents and surfaces decontaminated before leaving the laboratory. Biohazard symbols shall be used on containers as a heightened control measure.
- Transfer between buildings on the same site
  - Containers and layers of outer packaging following the triple packaging shall be followed to minimize the risks of leakage while transferring infectious material between buildings.
  - Packaging shall be labeled in a way that the sender, recipient, and contents of the package are clearly identifiable. It shall include biohazard symbols where appropriate.

- Laboratory personnel involved in the transfer shall be provided with suitable awareness training on the risks present during the transfer process and hot to safely reduce them.
- Spill kits shall be readily available and appropriate personnel trained in their use.
- Recipients shall be notified in advance of the transfer occurring.

- Offsite transport of infectious substances

People at risk during off-site transportation are not only those involved in the transport, but also the public whose path might be crossed in transit. Therefore, ensuring infectious substances are safety contained and handled may be of interest to local, national, and/or international authorities.

3.3. Means of specimen transport

The means of transport depend on the destination of the specimens to be referred.
The means of transport include:

- Transport by land
  - Delivery by person
    There are specimens that are hand carried from one laboratory to another by a trained health facility staff.
  - Delivery by courier
    The courier companies that are transporting laboratory specimens are required to have undergone training and shall be duly accredited/authorized in order to maintain the viability of samples and to implement the safety transport guidelines.
  - Delivery by bus, taxi, motorbike, tricycle, or bicycle
    The laboratory specimens shall be transported by a trained person.

- Transport by air
  - Delivery by courier or cargo forwarder with airline. It is required that appropriate trainings are provided to persons and organizations processing laboratory specimens to be transported by air.
- Transport by ship/boat
  - Transport by ship/ship cargo
    The ship/ship cargo companies that are transporting laboratory specimens are required to have undergone training and shall be duly accredited/authorized in order to maintain the viability of samples and to implement the safety transport guidelines.
  - Transport by boat
    The laboratory specimens shall be transported by a trained person.

4. Guidance for local transport of infectious substances

- Prepare all the materials needed.
- After specimen collection, disinfect the primary container, be careful not to erase the label.
- Seal the cover of the specimen container using parafilm and wrap the primary container using an absorbent material (cotton or gauze).
- Put the primary container inside a ziplock bag and seal tightly, then place it inside the secondary container and seal properly.
- Disinfect the secondary container and place it inside the outer container.
- Make sure to take into account the transport requirements of the specimen/s for transport. If the samples require cold temperature, place 4–6 ice packs, one at the bottom, all four sides and at the top.
- Seal the outer container properly and disinfect the outside of the container.
- Place the necessary labels (shipper and sender's details) and forms (CIF and Linelist) in separate ziplock bags and seal tightly. Securely tape the labels and forms outside the box.
- Coordinate with the courier on how to send out the samples.
- Once the samples are picked up by the courier, coordinate with the receiving laboratory and inform them on your pending shipment.

5. Roles and responsibilities of the sender, carrier, and receiver in specimen packaging and transport

It is the responsibility of the sender to ensure the correct designation, packaging, labeling, and documentation of all diagnostic specimens for transport including the monitoring record of status of specimens. The efficient transport and transfer of diagnostic specimens requires good coordination between the sender, the carrier, and the receiver (receiving laboratory) to ensure that the material is transported safely and arrives on time and in good condition. Such coordination depends upon well-established communication and a partnership among the three parties. All have specific responsibilities to carry out in order to achieve an effective and successful transport.

5.1. The referring agency/sender:

- Makes advance arrangements with the receiver of the specimens; calls the recipient to verify the shipping address and obtain the name and phone number of the contact person; find out when the contact person will be able to receive the shipment.
- Makes advance arrangements with the carrier/courier to ensure that the shipment will be accepted for appropriate transport and that the shipment (direct transport if possible) is undertaken by the most direct routing, avoiding arrival at weekends; shipments shall not go out on Thursday or Friday unless the recipient agrees that they will be there to receive the package during the weekend or holiday.

- Prepares necessary documentation including request forms, specimen status of specimen monitoring, permits if necessary, dispatch, and shipping documents.
- Notifies the receiver of transportation arrangements once these have been made, well in advance of expected arrival time.

5.2. The carrier/courier:

- Provides the sender with the necessary shipping documents and instructions for their completion.
- Provides advice to the sender about correct packaging.
- Assists the sender in arranging the most direct routing and then confirms the routing.
- Maintains and archives the documentation for shipment and transport.
- Monitors required holding conditions of the shipment while in transit.
- Notifies the sender of any anticipated (or actual) delays in transit.
- Notifies the sender of the completed or uncompleted delivery of the package.

5.3. The receiver:

- Arranges for the most timely and efficient collection on arrival.
- Immediately acknowledges receipt to the sender and records the date and time of receipt of specimen.
- Returns a copy of the monitoring record of specimen status to the sender.

Shipments should not be dispatched until:

- Advance arrangements have been made between the sender, carrier, and receiver.
- The receiver has confirmed that there will be no delay incurred in the delivery of the package to its destination.

6. Materials required for collection, packaging, and transport of clinical specimens

Figure 1. Requirements for clinical samples collection, packaging, and transport.

7. Procedure for specimen packaging and transport

Figure 2. Procedure for specimen packaging and transport.

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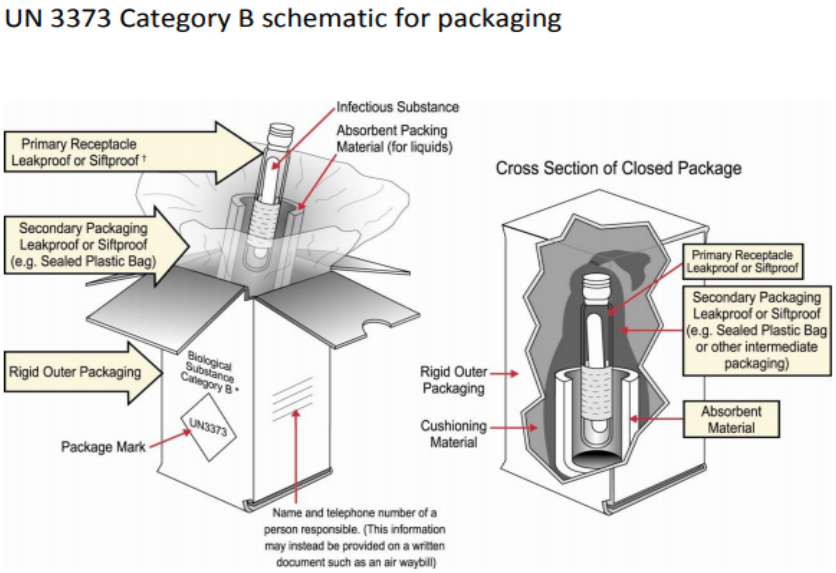
Annex

Annex 1

Triple packaging system



Annex 1. CDC schematic for packaging UN 3373 Category B.

Section 3: Referral of Clinical Specimen for SARS-CoV-2 Testing

Introduction

World Health Organization (WHO) recommended designation of referral hospitals for COVID-19 as part of the surge plans for health facilities.

In view of this, the DOH Department Order (DM) issued DOH-DM-2020-0069 reminding to send specimen to RITM for COVID-19 testing.

However, in emergency settings when the volume of tests exceeds a laboratory's capacity as in COVID-19 pandemic or when a temporary interruption in operations requires calling on another laboratory for surge capacity, referrals to another laboratory is necessary. Samples may even be sent to another laboratory with expertise and proper facilities in accordance with the DOH licensing requirements for facilities handling COVID-19 specimens.

Initially, the RITM served as the only confirmatory testing laboratory for COVID-19 in the Philippines. To prepare and ensure that our healthcare system is able to address the need to test suspected cases, DOH accelerated the testing facilities and testing capacities all over the country. Additional facilities started to conduct confirmatory testing to augment RITM capacity. To date, the government has strengthened its expanded targeted testing capacity. From a single national reference laboratory – the RITM – in March 2020, the country now has more than 100 accredited subnational and regional COVID-19 testing laboratories as of September 9, 2020. These laboratories have developed capability to conduct confirmatory test for COVID 19. More other laboratories are being prepared for accreditation to handle confirmatory tests.

Objective

The objective of this document is to ensure proper protocol in referring COVID-19 clinical specimen to designated referral laboratories within the country and out of the country for further testing.

Definition of terms

Case Investigation Form (CIF): a document that contains patient and sample information and accompanies a referred sample.

COVID-19 testing laboratory: a health facility where COVID-19 testing (SARS-CoV-2 detection) is done on specimens from COVID-19 patients.

DOH Accredited COVID Laboratories: DOH accredited private and government laboratories capable of doing confirmatory test for COVID-19 testing.

Referral Laboratory for COVID-19: laboratory accredited/designated by DOH to become reference laboratory for COVID-19 patients' testing.

Referring facility/laboratory: a facility that refers sample to referral laboratory.

Subnational and regional laboratories for COVID-19: Confirmatory tests for COVID-19 are decentralized to subnational and regional laboratories under the supervision of RITM which is the national reference laboratory.

Scope

This procedure will be used for proper referral of clinical specimens from COVID-19 patients to designated referral and DOH accredited COVID laboratories in the Philippines and also to international Reference Laboratories for further testing.

Responsibility

The laboratory professionals are responsible for these procedures as well as the documentation of the activities.

Procedures

During the COVID-19 pandemic, the DOH sent directives of referring samples to designated laboratories for SARS-CoV-2 confirmatory testing.

1. Directive guideline concerning referral of specimens

    1.1. All licensed level 2 and level 3 hospitals, whether private or government, are eligible to become COVID-19 referral hospitals/laboratories provided that they pass the accreditation process of the DOH thru the Health Facilities Regulation Bureau.

    1.2. To become licensed to run COVID-19 tests, a laboratory must pass the five-stage processes that include assessment, validation, or on-site assessment by experts, personnel training, proficiency testing or validation by RITM, and full-scale implementation.

    1.3. Healthcare facilities managing patients with COVID-19 need to coordinate with the regional epidemiology and surveillance unit (RESU) for specimen referral to RITM or any accredited COVID-19 testing laboratories. All specimen referrals shall be coordinated through the RESU.

1.4. Hospitals, disease reporting units (DRUs) and RESUs shall be familiar with biosafety protocols (outlined by RITM biosafety guideline) and the guidelines on specimen collection and transport prior to sending specimens.

1.5. In addition, all DRUs are to accomplish a complete line list of specimens referred to the Institute. This is undertaken to improve specimen tracking and minimize delays in the disposition and release of results due to missing vital laboratory information.

1.6. It is the responsibility of the referring facility or DRU to coordinate with the courier to ensure prompt delivery of specimens to RITM.

2. Role of the COVID-19 referral laboratory

2.1. Prioritize the management of COVID-19 cases.
2.2. Accept referred confirmed COVID-19 patients from other hospitals through appropriate and coordinated transfers.
2.3. Collect specimens and facilitate testing of patients for COVID-19.
2.4. Ensure adequacy of infrastructure, human resource complement and essential supplies for COVID-19 cases.

3. Process on referral of specimens for COVID-19 test

3.1. Notify the accredited COVID-19 testing facility for possible shipment of sample for COVID-19 testing.
3.2. Prepare the required documents for referral.
3.3. Laboratory referral request form for COVID-19.
3.4. CIF per patient.
3.5. Line list of specimens.
3.6. Summary list of referred specimen form for COVID-19 testing.
3.7. Fill-out all forms completely, legibly and accurately. No items should be left blank.
3.8. Accomplish properly and completely the CIF with legible identifiers, contact details, date and time of collection. The name of the contact person for the said facility including complete official contact details (name of health facility, office landline/mobile phone number, and email address) should be indicated in the CIFs and Linelist.
3.9. Separately pack the filled out forms in a sealed clean plastic bag/case and attach it firmly to the outside portion of the third outer container to avoid soaking the documents.
3.10. Prepare the sample for transport using the “Triple Packaging System” in accordance with the current edition of the IATA.

3.11. If the sample cannot be referred, maintain cold chain at 2–8°C for 3 days or −70°C for more than 3 days.
3.12. The documents (CIFs and Linelist) shall be placed in a water-resistant plastic resealable bag (e.g., ziplock) which is secured outside the specimen container.
3.13. The personnel that will prepare and transport the specimen shall be in complete recommended PPE (double gloves, scrub suit, laboratory gown (disposable), closed shoes, and fit-tested N95 mask).

4. General steps in handling, packing, and transport of specimen for referral

Step 1. Determine the referral laboratory network system and their requirements
1.1. National Reference Laboratories.
1.2. Regional (subnational/referral).
1.3. Others (research laboratories or further testing out of country).
Step 2. Classify specimens to be transported. Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological Substance Category B."
Step 3. Pack the specimen using triple packaging system with proper labeling in each receptacle.
Step 4. Document and label the outer container box.
Step 5. Plan the transport.
Step 6. Transport only properly packed specimens.
Step 7. Document received specimens.

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PART 3: ANALYTICAL STAGE

Section 1: Diagnostic Testing for COVID-19

Introduction

Diagnostic testing for COVID-19 is critical to tracking the virus, understanding epidemiology, informing case management, and suppressing transmission.

This document transcribes the strategic use of diagnostic testing in tracking the virus.

WHO has published laboratory testing guidance for COVID-19 in suspected human cases.

Commercially available COVID-19 tests currently fall into two major categories:

1. The first category includes molecular assays for detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viral RNA using polymerase chain reaction (PCR)-based techniques or nucleic acid hybridization-related strategies.
2. The second category includes serological and immunological assays that largely rely on detecting antibodies produced by individuals as a result of exposure to the virus or on detection of antigenic proteins in infected individuals.

It is important to understand that these two categories of tests have overlapping purposes in management of the COVID-19 pandemic. Testing for SARS-CoV-2 viral RNA identifies SARS-CoV-2 infected individuals during the acute phase of infection. Serological testing subsequently identifies individuals who have developed antibodies to the virus and could be potential convalescent plasma donors. It also furthers the ability to conduct contact tracing and monitor the immune status of individuals and groups over time.

The SARS-CoV-2 epidemic is spreading worldwide. Accurate diagnostic assays can offer a robust way for the timely identification of infected individuals, which is a key to preventing transmission of SARS-CoV-2. RT-PCR is widely employed in the molecular diagnosis of SARS-CoV-2 infection in laboratory, and serological antibody assays are supplementary methods. Both methods should be designed according to the characteristics of SARS-CoV-2. Gene regions including ORF1ab, N, and S genes are commonly targeted regions for RT-PCR; and RBD, S1, S and N proteins or synthetic antigens can be used to detect antibodies in serological assays.

Objective

To provide guidance and procedure for COVID-19 testing for the laboratories in the Philippines.

Definition of terms

COVID-19 expanded testing: defined as testing all individuals who are at-risk of contracting COVID-19 infection.

Directional/laminar airflow: a system of circulating filtered air in parallel-flowing planes used in a biological safety cabinet that reduces the risk of airborne contamination.

High efficiency particulate air (HEPA) filter: a special kind of air filter used to remove 99.97% of particles that have a size greater than or equal to 0.3 μm.

Inactivation: a procedure to render a select agent or regulated nucleic acid as non-viable while retaining characteristic(s) of interest for future use.

Limit of detection/limit of quantitation: the smallest concentration of a select agent that can be reliably detected or measured by an analytical procedure.

Non-infectious nucleic acid: nucleic acids not capable of producing infectious forms of a select agent virus (e.g., regulated positive sense RNA virus genomes).

Polymerase chain reaction (PCR): a method widely used to rapidly make millions to billions of copies of a specific DNA sample, allowing scientist to take a very small sample of DNA and amplify it to a large enough amount to study in detail.

Scope

This document covers COVID-19 diagnostic testing used by the professional laboratory personnel in the clinical and research laboratories.
This is specific to clinical diagnostic laboratory practice in the Philippines. Advice offered here relates to laboratory procedures conducted in clinical diagnostic laboratories.

Responsibility

All laboratory personnel in the clinical and research laboratories are responsible for these procedures as well as the documentation of the activities.

Procedures

1. Testing algorithm (see annex 1)

   The testing algorithms released by DOH through Department Memo are the following:

   1.1. DOH-DM-2020-0180 Revised Interim Guidelines on Expanded Testing for COVID-19.

1.2. DOH-DM-258 is a more updated guideline.
1.3. DOH-DM-2020-0220 Interim Guideline on the Return-to-Work.

2. Principles and procedures for testing SARS-CoV-2

2.1. Important reminders on laboratory facility, instruments, and kits

- Laboratory design is a key element of quality assurance to molecular assay in the clinical laboratories. Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. Unidirectional workflow is required across these rooms.
  a. 1st area (preparation area): prepare testing reagent (clean room).
  b. 2nd area (specimen processing): process the specimen and controls (inactivation/extraction room).
  c. 3rd area (amplification area): where PCR is conducted.

  Additional area is dedicated to:
  - Checking and sorting of samples.
  - Template room.

- Even with closed systems, sample preparation has to be performed in a Class II biosafety cabinet (BSC).
- Other recommended laboratory design elements for amplification assays include separate ventilation systems for preamplification and postamplification areas, maintaining negative pressure in the postamplification area.
- The clinical laboratory should be equipped with instruments and trained personnel in strict compliance with the requirements of local and national regulations.
- After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.
- Components contained within a kit are intended to be used together. Do not mix or exchange components from different kit lots. Prior to assay, each component must be thoroughly thawed and briefly centrifuged. Avoid repeated freeze–thaw cycles.
- All pipette tips and centrifuge tubes in the assay should be sterile and DNase/RNase-free. To prevent contamination, filtered pipette tips are required and should be replaced after the addition of each reagent or sample.
- All lab workbench and supplies should be cleaned and disinfected regularly using 70% ethanol or 10% sodium hypochlorite.

2.2. Molecular assay for detection of viral nucleic acid

Considerations about the sample and the laboratory:

- Samples should be used only to analyze the minimum necessary for the etiological diagnosis and management of the patient.
- The inactivation and manipulation of the sample should be carried out in BSL2+ environment of containment with Class II BSC. Installations should have a sink with water supply, a refrigerator, and heating block.
- The samples should only be opened within BSCs (class II). If a centrifugation process is required, it should be done in equipment with closed buckets.
- Laboratory staff who treats the samples should wear all the personal protective equipment (PPE) recommended for the sampling.
- The sample should be inactivated, handled only by trained laboratory professionals and qualified for the management of potentially infectious specimens with high-risk pathogens.
- For biochemical and hematological tests, the use of equipment or closed analytical systems is highly recommended to minimize contact with the sample.
- Upon leaving the laboratory, laboratory personnel shall doff all the PPE and place them in a biological risk bag for autoclaving/decontamination processes.
- Do not attempt to perform viral isolation.

Steps involved:

1. Sample reception, sample inspection, sample encoding (preanalytical stage).
2. Aliquoting and inactivation. Samples are aliquoted and inactivated, chemically or by heating. The rest of the sample is banked.
3. RNA extraction. Sample RNA is extracted, using a viral RNA kit, an automated extractor, or other options.
4. Pre-PCR reagent preparation (master mix). Kit reagents are prepped into a master mix and dispensed into the qPCR plates/strip tubes.
5. Template addition. The extracted RNA templates are added into each well following the plate layout.
6. rRT-PCR run.
7. Results analysis. Positive and negative controls are validated. RNase P controls per sample are validated. Positive results are called based on Ct values.

2.2.1. Virus inactivation

Inactivation and aliquoting

- Samples are aliquoted and inactivated, chemically or by heating. The rest of the sample is banked.

- For molecular diagnosis, the inactivation processes should ensure a total loss of the infectiousness while conserving the integrity of the nucleic acid.
- In general, the use of highly denaturing conditions destabilizes the viral envelope, eliminates cellular nucleases, and maintains the structure of RNA for later analyses Thus the use of lysis solutions composed of guanidine salts (guanidine thiocyanate and guanidine isothiocyanate) has been proven to be efficient for the inactivation of enveloped RNA viruses. There are different commercial reagents widely used in the laboratories familiarized with molecular techniques (e.g., Tripure and Trizol) that offer a very good performance. Furthermore, most of the RNA extraction kits (viral or total) provide lysis buffers with the required denaturing characteristics. In any case, instructions of the manufacturer and standardized protocols of each laboratory should be followed, as well as the established personal protective measures.
- The viral transport medium, where the nasopharyngeal swab/oropharyngeal swab is preserved, can be inactivated and processed for molecular detection. Make sure to remove carefully the swab in a bag for infectious waste for disposal.
- Samples for amplification can also be inactivated through heat treatment at 60°C for 60 minutes. However, in order to boost biosafety, use of a denaturing solution in combination with the inactivation by heat is recommended.
- Any inactivation procedure that requires opening the sample container or has the potential for aerosol creation should take place inside a biosafety cabinet.
- Samples for RNA extraction are collected in storage buffers designed to inactivate proteins and preserve the RNA. These buffers often contain detergents, such as SDS, that should inactivate an enveloped virus. However, inactivation by two of the most common lysis buffers, Trizol and AVL buffer (used in Qiagen kits), has been evaluated for inactivation of the virus.
- This procedure is carried out with Class II safety cabinet by trained authorized laboratory personnel only, with PPE at all times. After further inspection of sample integrity, the swab is removed and, if the sample contains viral transport medium, 100 µL of each transferred to 2 mL screw cap tube prefilled with 1 mL of 5M guanidine thiocyanate L6 virus inactivation buffer. If the swab is submitted dry, it is immersed in inactivation buffer. Following transfer, both the collection tube and the inactivated viral sample are visually checked to confirm identity. Only one sample is processed at a time to avoid any risk of sample swapping. Inactivated individual viral samples are then moved to a separate area for RNA extraction.

See Annex 2 for different methods of virus inactivation.

2.2.2. RNA extraction principle

Prior to performing RT-PCR, RNA extraction must be carried out first. RNA extraction is the purification of RNA from biological samples. RNA extraction is a delicate process, as cells and the environment secrete high concentrations of enzymes that destroy nucleic acids; therefore, the process must be carried out in a careful and quick manner.
Most kits contain sterile buffers, solutions that lyse cells in order to access the RNA within the cells and a way of separating the RNA from DNA, proteins and other macronutrients found in the sample of cells.
The RNA extraction kits in general have few steps.

- First, the patient's sample is mixed with a solution that lyses the cells in order to release the genetic material.
- After inactivation of RNAse activity, denaturation of nucleoprotein complexes and removal of contaminating DNA and proteins must occur in order to purify the RNA.
- The resulting cellular debris and the extracted RNA can then proceed to the RT-PCR step.

RNA extraction basics

Isolating intact RNA requires four steps:

1. Disruption of cells or tissue.
2. Inactivation of endogenous ribonuclease (RNase) activity.
3. Denaturation of nucleoprotein complexes.
4. Removal of contaminating DNA and proteins.

The most important step is the immediate inactivation of endogenous RNases that are released from membrane-bound organelles when cells are disrupted.
RNA purification methods typically use silica membrane-based, resin-based and magnetic options for nucleic acid binding and incorporate DNase treatment to remove contaminating genomic DNA. Purified RNA is then eluted from the solid support.
RNA is notoriously susceptible to degradation, and RNases are ubiquitous. Many commercially available RNA purification methods include specific chemicals to inactivate RNases present in cell or tissue lysates and may also include RNase inhibitors to safeguard against RNA degradation throughout the procedure.
See annex 3 for different RNA extraction methods.

2.2.3 RT-PCR

RT-PCR starts with laboratory conversion of viral genomic RNA into DNA by RNA-dependent DNA polymerase (reverse transcriptase). This reaction relies on small DNA sequence primers designed to specifically recognize complementary sequences on the RNA viral genome and the reverse transcriptase to generate a short complementary DNA copy (cDNA) of the viral RNA. In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses. This is done using a fluorescent dye or a sequence-specific DNA probed labelled with a fluorescent molecule and a quencher molecule, as in the case of Taqman assays. An automated system (thermocycler) then repeats the amplification process for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or electrical signal.

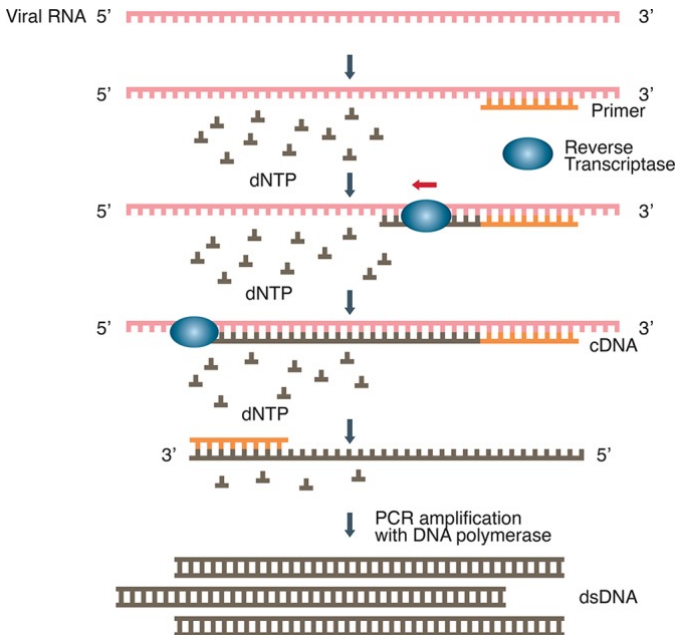

Figure 1. [13]Reverse transcription-polymerase chain reaction (RT-PCR). The RT-PCR creates a cDNA copy of a specific segment of the viral RNA, which is converted to dsDNA that is exponentially amplified.

RT-PCR has traditionally been carried out as a one-step or a two-step procedure.

- One-step real-time RT-PCR uses a single tube containing the necessary primers to run the entire RT-PCR reaction.

- Two-step real-time RT-PCR involves more than one tube to run the separate reverse transcription and amplification reactions but offers greater flexibility and higher sensitivity than the one-step procedure. It requires less starting material and allows for the ability to stock cDNA for quantification of multiple targets.

The one-step procedure is generally the preferred approach for detection of SARS-CoV-2 because it is quick to set up and involves limited sample handling and reduced bench time, decreasing chances for pipetting errors and cross-contamination between the RT and real-time PCR steps.

To date, the majority of molecular diagnostic tests have utilized the real-time RT-PCR technology targeting different SARS-CoV-2 genomic regions, including the ORF1b or ORF8 regions, and the nucleo capsid (N), spike (S) protein, RNA-dependent RNA polymerase (RdRP), or envelope I genes.

RT-PCR tests are constantly evolving with improved detection methods and more automated procedures.

Although RT-PCR is the most widely used method for detecting SARS-CoV-2 infections, it has the disadvantage of requiring expensive laboratory instrumentation, highly skilled laboratory personnel, and can take days to generate results. As a result, a number of companies and laboratories around the globe are working to further improve the efficiency and timeliness of the RT-PCR technologies and develop various other techniques.

Test	Molecular targets	Scope	Limit of blank	Reference specimens	Storage conditions
WHO					
	E gene	First-line screening	3.9 copies × reaction	Nasopharyngeal AND oropharyngeal swab or wash in ambulatory patients, lower respiratory specimens (sputum and/or endotracheal aspirate or bronchoalveolar lavage)	≤5 days: 2–8 °C
	RdRp gene	Confirmatory testing	3.6 copies × reaction		>5 days: ≤70 °C
	N gene	Additional confirmatory testing	N/A		(dry ice)
CDC					
	N1/2/3 gene	Combined assay	1.0–3.2 copies/µL	Nasopharyngeal AND oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage and nasopharyngeal wash/aspirate or nasal aspirate	≤4 days: 4 °C
	RNase P gene	Control assay	N/A		>4 days: ≤70 °C

E gene, envelop gene; N gene, nucleocapside gene; RdRp gene, RNA-dependent RNA polymerase gene; RNase P gene, human RNase P gene.

Table 1. Comparison of the (real time) reverse transcription polymerase reaction (rRT-PCR) diagnostic assay of the World Health Organization (WHO) and the Centers for Disease Control

and Prevention (CDC) for diagnosing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

See Annex 4 for different rRT-PCR methods.

2.3. Serological and immunological assays

While RT-PCR-based viral RNA detection has been widely used in diagnosis of COVID-19, it cannot be used to monitor the progress of the disease stages and cannot be applied to broad identification of past infection and immunity.

Serological testing is defined as an analysis of blood serum or plasma and has been operationally expanded to include testing of saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies.

This test plays an important role in epidemiology and vaccine development, providing an assessment of both short-term (days to weeks) and long-term (years or permanence) trajectories of antibody response, as well as antibody abundance and diversity.

IgM first becomes detectable in serum after a few days and lasts a couple of weeks upon infection and is followed by a switch to IgG. Thus, IgM can be an indicator of early stage infection, and IgG can be an indicator of current or prior infection. IgG may also be used to suggest the presence of postinfection immunity.

In recent years, the sophistication and sensitivity of immunological assays have increased not only for the detection of antibodies themselves but also for the application of antibodies (primarily monoclonal antibodies) to the detection of pathogen-derived antigens.

These tests have a huge potential for the epidemiology of COVID-19, but test results can be impacted by at least three situations: (1) a subset of subjects with a positive result from molecular genetic assays for SARS-CoV-2 infection are seronegative due to the lag in antibody production following infection, (2) the subjects may be seropositive yet negative for molecular genetic assay results reflecting clearance of an earlier, milder infection, and (3) limitation in sensitivity and specificity of the assays.

The last issue is particularly important because even a small percentage of false positive results due to low specificity (cross-reaction) may lead to misleading predictive antibody prevalence among a given population, which may have undesirable impact on the socioeconomic decisions and overall public confidence in the results.

The determination of SARS-CoV-2 exposure relies largely on the detection of either IgM or IgG antibodies that are specific for various viral antigens including, but not exclusively, the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleo capsid protein.

The methodology for these determinations includes the traditional enzyme-linked immunosorbent assay (ELISA), immunochromatographic lateral flow assay, neutralization bioassay, and specific chemosensors.

Each of these formats brings advantages (speed, multiplexing, and automation) and disadvantages (trained personnel and dedicated laboratories). Complementary to these antibody-detecting methods are the rapid antigen tests wherein antibodies are used to detect the presence of viral antigen(s) in serological samples.

Development of high-throughput serology tests is a current focus of major diagnostic companies.

Principle of serologic assays

In SARS-CoV-2 infection, RBD, S and N proteins serve as the main antigens to stimulate the immune response of the body, producing IgA, IgM and IgG antibodies. The titer of secretory IgA indicates mucosal immune responses against SARS-CoV-2. IgM indicates the acute infectious stage, while IgG represents middle and later stages of infection or previous infection.
See annex 5 for different serological methods.

2.4. Updates on diagnostics of COVID-19 as of September 9, 2020

2.4.1. Real-time reverse transcription-polymerase chain reaction (rRT-PCR) test

- To date, the most reliable test for diagnosis of COVID-19 has been the rRT-PCR test performed using nasopharyngeal swabs or lower respiratory tract specimens.
- It is recommended that both a nasopharyngeal swab and oropharyngeal swab are collected and processed together.
- Saliva has been studied as an alternative specimen but is still currently being validated by the Research Institute of Tropical Medicine (RITM).
- rRT-PCR tests are also available in cartridge-based test kits. New improve kits with short turnaround time are already available.
- Only kits validated by RITM shall be used.
- The qualitative reporting of results of SARS-CoV-2 as positive or negative is sufficient for the diagnosis. However, it may also be

supplemented by a cycle threshold (Ct) report indicating the brand of the kit and machine used.

2.4.2. Pooled testing

- Pooled testing strategies are currently being evaluated and validated.
- Pooled testing can be done among asymptomatic persons belonging to targeted populations below:
  - Communities with prevalence rate of 10% or less.
  - Surveillance of healthcare workers.
  - Workplace testing including market vendors and transport workers.
  - Border testing at ports of entry for inbound foreign travelers and returning residents.
  - Overseas deployment of overseas Filipino workers (OFWs) and returning OFWs.
  - Frontline government workers.
  - Locally stranded individuals.
- Use of a pool sample of five (5) is recommended to be used.
- Laboratories that will conduct pooled testing shall develop guidelines and procedures for the purpose of pooled testing in accordance with the recommendations of local studies on pooled testing.

2.4.3. Antigen testing

- An antigen test detects the presence of viral proteins or antigens, which is expressed only when the virus is replicating. This test is best used to identify acute or early infection.
- Rapid antigen tests are most useful during the acute phase of the disease when the viral load is high (within 5 days after onset of symptoms).
- A positive result suggests presence of virus, whereas a negative result suggest absence of the virus.
- Only properly validated, FDA-authorized COVID-19 antigen tests should be used with minimum of 80% sensitivity and 95% specificity recommendation for use.
- Use and interpretation of antigen test should only be at the direction of a qualified licensed healthcare professional.
- The processing of specimen shall be done by a properly trained healthcare worker.

- Results should be read after the recommended time has elapsed using direct visualization or the dedicated electronic reader in accordance with the recommendations of the manufacturer.
- Antigen test is currently recommended only for very specific purposes:
    - Targeted screening and diagnosis of SUSPECT and PROBABLE cases of COVID-19 meeting the clinical and/or epidemiologic criteria defined by WHO in the hospital and community settings
    - Testing of patients in the hospital setting.
- A positive antigen test in an individual with a high index of suspicion is interpreted as a confirmed case of COVID-19. A negative antigen test result shall be further confirmed with rRT-PCR test.

2.4.4. Antibody testing

- An antibody test detects the presence or absence of antibodies against the virus present in patient serum. This is NOT a confirmatory or diagnostic test. This will only determine whether a person has recent infection with SARS-CoV-2 and best used 14 days or later after onset of illness.
- Only antibody-based test kits approved by the FDA with acceptable performance of >90% sensitivity and >95% specificity may be used.
- Rapid antibody tests (rATs) using lateral flow technology shall NOT be used as a standalone test for diagnosis of COVID-19.
- rATs are NOT recommended for use in seroprevalence surveys/contact tracing, return-to-work decisions, entry-to-country/province policies.
- Laboratory-based immunoassays such as chemiluminescence assay (CLIA/ECLIA) and enzyme-linked immunosorbent assay are the preferred tests for antibody determination over rATs.

2.5. Rapid antibody testing

Laboratory procedure for COVID-19 IgM/IgG rapid diagnostic test (RDT)

The COVID-19 IgM/IgG RDT is a rapid, qualitative lateral flow immunoassay kit for the detection of human IgM and IgG against SARS-CoV-2 virus infection using finger stick (no anticoagulant) and K2EDTA-anticoagulated venous whole blood. The test results can aid in diagnosis of SARS-CoV-2 virus infection. The

diagnosis of COVID-19 must be made based on history, signs, symptoms, exposure likelihood, and other laboratory evidence in addition to the COVID-19 IgM/IgG RDT.

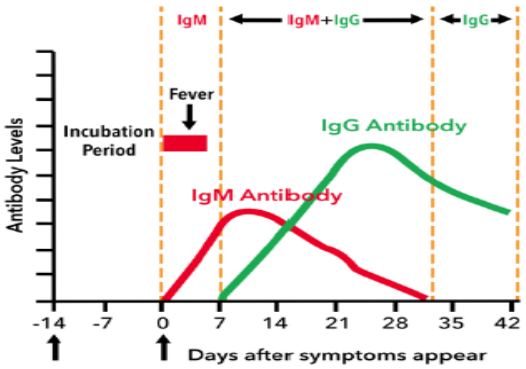

Figure 2. It is widely accepted that immunoglobulin M (IgM) provides the first line of defense during viral infections, followed by the generation of adaptive, high affinity immunoglobulin G (IgG) responses for long-term immunity and immunological memory. Therefore, testing of COVID-19 IgM and IgG antibodies is an effective method for the rapid diagnosis of COVID-19 infection. Furthermore, detection of COVID-19 IgM antibodies tends to indicate a recent exposure to COVID-19, whereas detection of COVID-19 IgG antibodies indicates a later stage of infection. Thus, this combined antibody test could also provide information on the stage of infection.

Test principle

The rapid IgM–IgG combined antibody test for COVID-19 is immunochromatography based. The test card contains one colloidal gold-labelled recombinant novel coronavirus antigen and quality control antibody colloidal gold marker, two detection lines (G and M lines), and one quality control line (C) fixed on a nitrocellulose membrane.

M is fixed with monoclonal anti-human IgM antibody for detecting the novel coronavirus IgM antibody. G is fixed with monoclonal antihuman IgG antibody for detecting the novel coronavirus IgG antibody. The quality control antibody is fixed on the C line.

When appropriate amount of test sample is added to the sample well of the test cassette, the sample will move forward along the test card via capillary action.

If the sample contains IgM antibody, the antibody will bind to the colloidal gold-labelled novel coronavirus antigen. The antibody/antigen complex will be captured by the anti-human IgM antibody immobilized on the membrane, forming a red M line and indicating a positive result for the IgM antibody.

If the sample contains IgG antibodies, the antibody will bind to the colloidal gold-labelled novel coronavirus antigen and the antibody/antigen complex will be captured by the antibody immobilized on the membrane, forming a red G line and indicating a positive result for the IgG antibody.

If neither antibody is present, a negative result is displayed.

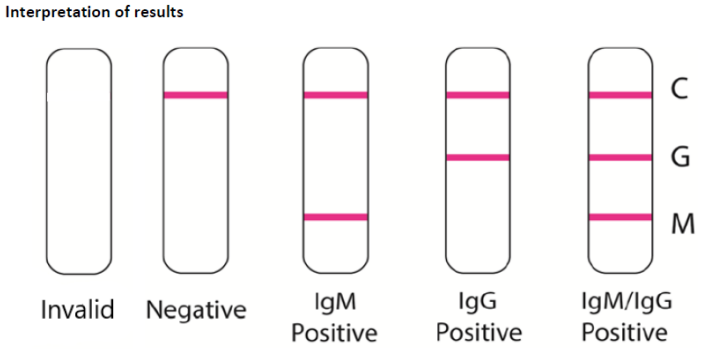

Figure 3. Interpretation of result.

A total of three detection lines are possible, with the control (C) line appearing when sample has flowed through the cassette:

1. Negative result: if only the quality control line (C) appears and the detection lines G and M are not visible, then no novel coronavirus antibody has been detected and the result is negative.
2. Positive result, M only: if both the quality control line (C) and the detection line M appears, then the novel coronavirus IgM antibody has been detected and the result is positive for the IgM antibody.
3. Positive result, G only: if both the quality control line (C) and the detection line G appears, then the novel coronavirus IgG antibody has been detected and the result is positive for the IgG antibody.
4. Positive result, G and M: if the quality control line (C) and both detection lines G and M appear, then the novel coronavirus IgG and IgM antibodies have been detected and the result is positive for both the IgG and IgM antibodies.
5. Invalid result: if the control line does not appear, the test is invalid.

Test method limitations

- This method can only be used to detect the IgG and IgM antibodies of the novel coronavirus in human blood, serum or plasma. It cannot be used with other body fluids or secretions.
- This is only for qualitative testing and the specific content of each antibody must be measured using other quantitative methodologies.
- Negative results may be caused by low concentrations of the novel coronavirus IgG/IgM antibody in the sample and therefore cannot completely rule out the possibility of infection.
- Test results can be affected by temperature and humidity.

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Annexes

Annex 1. DOH testing algorithms

DOH-DM-2020-0220 Revised Interim Guidelines on Expanded Testing for COVID-19, May 11, 2020

COVID-19 expanded testing covers the following groups:

1. Suspect cases.
2. Individuals with relevant history of travel and exposure (or contact), whether symptomatic or asymptomatic.
3. Healthcare workers with possible exposure, whether symptomatic or asymptomatic.

Due to global shortage of testing kits and limitation in local capacity for testing, there is a need to rationalize available tests and prioritize subgroup A (patients or healthcare workers with severe/critical symptoms and relevant history of travel/contact) and subgroup B (patients or healthcare workers with mild symptoms, relevant history of travel/contact and considered vulnerable). However, in view of the expansion of testing capacity and to ensure healthcare workforce safety, subgroup C (patients or healthcare workers with mild symptoms and relevant history of travel/contact) will be tested and health workers prioritized.
The real-time polymerase chain reaction (rRT-PCR) testing is the confirmatory test to diagnose COVID-19 as recommended by WHO.
Rapid antibody-based test kits shall not be used as stand-alone test to definitively diagnose or rule out COVID-19.
Only approved kits by Food and Drug Administration (FDA) as validated by Research Institute for Tropical Medicine (RITM) shall be used by the clinical laboratories in the Philippines.

The expanded use of point-of-care rapid antibody-based test kits through validation and sero-epidemiological studies shall be explored for subgroup D (patients or healthcare workers with no symptoms by relevant history of travel/contact).
Specific guidelines have been mapped out and directed for the expanded tests on the following groups:

A. Licensed medical doctors may request and administer antibody-based tests with ensuing responsibilities.
B. For healthcare workers: symptomatic and asymptomatic.
C. Symptomatic non-health care workers.
D. Asymptomatic non-health care workers.
E. For returning overseas Filipinos.
F. For surveillance of areas with suspected COVID-10 community-based transmission.

The testing algorithms as directed by DOH is shown in tables A1 and A2.

Initial Result		Action	Action after 14-day quarantine or until asymptomatic, whichever is longer
IgM	IgG		
(-)	(-)	Swab for RT-PCR • If RT-PCR (+), **COVID-19 CASE**. Treat and isolate accordingly. • If RT-PCR (-), not a COVID-19 case, but has to complete 14-day isolation. No need to repeat antibody tests. • If RT-PCR not available, isolate for 14 days or until asymptomatic, whichever is longer	Repeat antibody test if RT-PCR is not available. If IgM(-) & IgG(+), or IgM(+) & IgG(+), or IgM(-) & IgG(-), • release from quarantine If IgM(+)&IgG(-), • extend quarantine by seven-day increments and repeat testing • If persistently IgM positive but IgG negative for two consecutive retestings after the 1st 14 day period, consider potential false positives and confer with infectious diseases specialists.
(-)	(+)		No need to repeat antibody test. Release from quarantine.
(+)	(-)	**Probable COVID-19 Case**: Swab for RT-PCR • If RT-PCR (+), **COVID-19 CASE**. Treat and isolate accordingly. • If RT-PCR (-), not a COVID-19 case, but has to complete 14-day isolation. Repeat antibody test. • If RT-PCR not available, isolate for 14 days or until asymptomatic, whichever is longer	Repeat antibody test RT-PCR (-) or RT-PCR not available. If IgM(-) & IgG(+), or IgM(+) & IgG(+), or IgM(-) & IgG(-), • release from quarantine If IgM(+)&IgG(-), • extend quarantine by seven-day increments and repeat testing • If persistently IgM positive but IgG negative for two consecutive retestings after the 1st 14 day period, consider potential false positives and confer with infectious diseases specialists.
(+)	(+)		

** Rapid antibody-based test is an adjunct test and shall **not be used as a standalone test** to definitively diagnose or rule out COVID-19*

The medical practitioner shall wear **full personal protective equipment provided by the health institution, when collecting specimens both for RT-PCR and rapid antibody test.*

Table A1. Use of rapid antibody tests as adjunct test for testing COVID-19 among symptomatic patients with relevant history of travel/exposure.

Results on Day 1 Quarantine		Action
IgM	IgG	
(-)	(-)	**Repeat testing on Day 14** • If still negative, release from quarantine
(+)	(+)	**Presumed recovered case** • Release from quarantine • No need to repeat the test at the end of the quarantine period
(-)	(+)	
(+)	(-)	**Probable COVID-19 Case**: Repeat testing on Day 14 • If still IgM (+) and IgG (-), extend quarantine by 7 day increments ○ If persistently IgM (+) and IgG (-) for two consecutive retestings after the 1st 14 day period, refer to infectious diseases specialist • If IgG (+), regardless of IgM result, release from quarantine

This tool is applicable only when there is sufficient supply of rapid antibody test kits and assurance that health workers administering the tests are equipped with full personal protective equipment.

Table A2. Use of rapid antibody-based tests for testing COVID-19 among asymptomatic patients with relevant history of travel or exposure.

DOH-DM-2020-0220 Interim Guideline on the Return-to-Work

Screening of returning employees and workers:
Returning employees and workers physically reporting to their place of employment shall be screened for symptoms of COVID-19.
Symptomatic returning employees and workers:
Returning employees and workers who are symptomatic with relevant history of travel/exposure on the date of work resumption shall *not* be allowed to physically return to work. They must be tested and treated following the DOH guideline on symptomatic cases.
Testing for asymptomatic returning employees:
Testing using RT-PCR among representative samples for baseline can be conducted to look for any evidence of asymtomatic transmitters. The decision matrix is shown in table A3.

Using RT-PCR as Baseline

RT-PCR Result	Action
Positive	• Isolate, manage and refer accordingly. • All close contacts shall be isolated and tested with RT-PCR as well.
Negative	May continue working with usual precautions.

If symptoms develop, test using RT-PCR. Cost of testing for symptomatic returning employees shall be subject to PhilHealth policies. All costs not covered by PhilHealth shall be borne by the employer.

Table A3. Decision matrix for sample representative of asymptomatic employees with relevant history of travel and/or exposure.

Alternatively, testing using FDA-approved and RITM validated rapid antibody-based tests among representative samples for baseline can also be conducted up to every 14 days.

Using Rapid Antibody Test every 14 days

IgM	IgG	Action
(-)	(+)	Not actively infected. May continue working with usual precautions.
(-)	(-)	
(+)	(+)	
(+)	(-)	Isolate for 14 days. Repeat testing on day 14 of quarantine. • If results are still IgM positive and IgG negative, extend quarantine by seven-day increments and repeat testing. • If persistently IgM positive but IgG negative for two consecutive retestings after the 1st 14 day period, consider potential false positives and confer with infectious diseases specialists.

Table A4. Use of rapid antibody-based tests among representative samples for baseline.

Annex 2. Different methods of virus inactivation

A. Sansure inactivation procedure
1. Aliquot 200 µL of each sample in 1.5 mL microcentrifuge tube.
2. Prepare control by aliquoting 200 µL of nuclease-free water in a 2.5 mL microcentrifuge tube.
3. Place all aliquoted samples on the heat block and incubate at 65ºC for 12 minutes.
4. After incubation, make sure tubes are properly capped, put in a cryobox and prepare for RNA extraction.

B. QIAmp Viral Inactivation

1. Arrange the appropriate number of carrier RNA aliquots in microcentrifuge tubes stored in the −20ºC freezer.
2. Add 560 µL of Buffer AVL to each 1.5 mL microcentrifuge tube containing the carrier RNA.
3. Add 140 µL of inactivated sample to Buffer AVL-carrier RNA in the microcentrifuge tube.
4. Pulse vortex for 15 seconds.
5. Incubate at room temperature (15ºC–25ºC) for 10 minutes.
6. Wipe each tube with 70% ethyl alcohol and place in the transport cryobox.
7. Ready for RNA extraction procedure.

C. GenAmplify Viral RNA Purification Kit

1. Arrange the appropriate number of 7 µL carrier RNA aliquots in microcentrifuge tubes stored in the −29ºC freezer.
2. Add 300 µL of Buffer NVL to each 1.5 mL microcentrifuge tube containing the carrier RNA.
3. Sample lysis: add 200 µL of sample to the micro centrifuge tube containing buffer NVL-carrier RNA.
4. Mix thoroughly by 10× pipetting in and out and 10× tapping the tube with the finger. For proper lysis, the complete mix of sample and buffer NVL is essential.
5. Incubate at room temperature (15ºC–25ºC) for 10 minutes.
6. Wipe each tube with 70% ethyl alcohol and place in transport cryobox.
7. Ready for RNA extraction.

D. Magnetic-Bind RNA Extraction Kit (Magnetic Nanoparticle Technology)

Specimen preparation and inactivation:

1. For inactivating the virus, water bath to 56ºC for 30 minutes (for sputum specimen incubate at 55ºC for 15 minutes).
2. Recommended to add 2–4 times the volume of Maccura sample preservation (SPS) to 1 volume of the specimen (incubate at 37ºC for 15 minutes).
3. If sputum specimen will be used, sputum should be liquefied with phosphate buffer which contain 1g/L proteinase K.
4. For oropharyngeal swabs, put the collected oropharyngeal swabs into the disposable virus sampling tube containing virus preservation solution, discard the tail and tighten lid.

Annex 3. Different methods of RNA extraction

A. Sansure Extraction Procedure

1. Centrifuge inactivated sample at 12 000 rpm for 10 minutes.
2. Discard supernatant by pipetting.
3. Add 50 µL sample release reagent
4. Incubate for 10 minutes at room temperature.
5. Centrifuge at 1000 rpm for 1 minute.

6. Disinfect the tubes and racks. Place extracted RNA in a transport box for testing or for storage.

B. QIAamp Viral RNA Extraction Procedure

Preparation:

1. Reconstitute buffer concentrates with absolute ethanol.
2. Aliquot the reconstituted buffer in 15 mL conical tubes.
3. Prepare the spin columns.
4. Prepare and label appropriate number of 1.5 mL microcentrifuge tubes for elution.
5. RNA extraction.
6. Add 560 µL of absolute ethyl alcohol to the sample and mix by pulse vortexing.
7. Briefly centrifuge tube to remove drops from the lid.
8. Apply 630 µL of solution into QIAamp mini column. Close cap and centrifuge at 6000 × *g* for 1 minute. Discard collection tube containing filtrate and replace with a new one. Repeat this step once.
9. Apply 500 µL of Buffer AW1 into QIAamp mini column. Close the cap and centrifuge at 6000 × *g* for 1 minute. Place the QIAamp mini column in a clean collection tube and discard the tube containing the filtrate.
10. Apply 500 µL of Buffer AW2 into QIAamp mini column. Close the cap and centrifuge at 2000 × *g* for 3 minutes. Place the QIAamp mini column in a clean collection tube and discard the tube containing the filtrate.
11. Centrifuge at 6000 × *g* for 1 minute to completely remove residual buffer. Discard collection tube and place QIAamp mini column into a clean 1.5 mL microcentrifuge tube.
12. Apply 40 µL of Buffer AVE equilibrated to room temperature. Close cap and incubate at room temperature for 1 minute. Centrifuge at 6000 × *g* for 1 minute.
13. Repeat to increase RNA yield.
14. Disinfect the tubes and racks. Place extracted RNA in a transport box and disinfect.
15. Ready for amplification or for storage.

C. GenAmplify viral RNA Purification Kit

Preparation:

1. Reconstitute buffer concentrates with absolute ethanol.
2. Aliquot in 15 mL conical tubes with enough volume of buffers to be used for the extraction process.
3. Prepare appropriate number of spin columns.
4. Prepare appropriate number of 1.5 mL microcentrifuge tubes for elution.
5. RNA extraction.
6. Briefly centrifuge the tubes with the lysates to remove the drops from the lid.
7. Add 350 µL of buffer RN1 to the sample lysate. Mix thoroughly by 10× pipetting in and out and 10× tapping the tube with the finger. Briefly centrifuge tube to remove drops from the lid.
8. Transfer up to 750 µL of the solution above to labelled spin column. Close cap and centrifuge at 6000 × *g* for 1 minute. Discard pass through and reinsert the collection tube. Repeat this step with remaining solution if mixture is more than 750 µL. W to

spin column. Close the cap and centrifuge at 6000 × *g* for 1 minute. Discard pass through and reinsert the collection tube.

9. Apply 500 µL of buffer RNW into spin column. Close the cap and centrifuge at 6000 × *g* for 1 minute. Discard pass through and reinsert the collection tube.
10. Centrifuge again at 14 000 × *g* for 1 minute to completely remove residual buffers. Discard collection tube and transfer spin column to labelled 1.5 mL microcentrifuge tube.
11. Apply 35 µL of nuclease-free water on the center of the spin column. Close cap and incubate at room temperature for 1 minute. Centrifuge at 5000 × *g* for 2 minutes.
12. Disinfect the tubes and racks. Place extracted RNA in a transport box and disinfect.

D. Viral RNA extraction by spin column technology

I. Procedural precautions

As with any test procedure, good laboratory technique is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of kit reagents or amplification mixtures. All reagents should be closely monitored for purity. Discard any reagents that may be suspect.

Note the following:

- Ensure all sample preparation and amplification and detection reagents are brought to room temperature.
- Ensure all samples are brought to room temperature.
- Set-up the disposables. Visually inspect for defects and labels as needed.

II. Sample and control protocol

- Sample material: use aqueous nucleic acid (NA) preparations.
- Negative control: always run at least one no-template control (NTC) – replace the template NA with water.
- Positive control: run a positive control – replace the template NA with provided positive control.

III. Reagent preparation

1. Reconstitute poly (A) carrier RNA by dissolving in 0.4 mL elution buffer prepare aliquots of 50 µL for running 8 × 12 purifications. Prepare aliquots of 100 µL for running 4 × 25 purifications. Store unused material at −15°C to −25°C for up to 12 months.
2. Prepare Inhibitor Removal Buffer (IRB) by adding 20 mL of absolute ethanol into Inhibitor Removal Buffer. Mix by inverting 5–10 times. Label and date bottle once ethanol has been added. Store at +15°C to +25°C. Solution is stable until expiration date on kit label.
3. Prepare Wash Buffer (WASH) by adding 40 mL of absolute ethanol to Wash Buffer. Mix by inverting 5–10 times. Label and date bottle once ethanol has been added. Store at +15 to +25°C. Expiration date is printed on the kit label.
4. Prepare binding buffer supplemented with poly(A) solution

4.1. Add the appropriate volume of the binding buffer into a 15 mL conical tube and the reconstituted carrier RNA.
4.2. Cap tube and mix by inverting 10–15 times immediately dispense the solution after mixing (DO NOT VORTEX).

IV. Sample preparation

1. To a nuclease free 1.5 mL microcentrifuge tube, add 200 µL sample and 400 µL working solution (carrier RNA supplemented binding buffer).
2. Mix the solution by inverting 10–15 times.
3. Incubate the solution for 10 minutes at +15°C to +25°C.
4. Insert one high pure filter tube into a collection tube. Pipette the entire volume (approximately 600 µL) of the sample into the upper reservoir of the filter tube.
5. Centrifuge the tube at 8000 × *g* for 15 seconds.
6. After centrifugation, remove the filter tube from the collection tube, discard the flow through liquid and the collection tube. Insert the filter tube into a *new* collection tube.
7. After reinserting the filter tube, add 500 µL inhibitor removal buffer to the upper reservoir of the filter tube assembly.
8. Centrifuge the tube at 8000 × *g* for 1 minute. Discard flow through liquid and combine the filter tube with a new collection tube.
9. After removal of inhibitors, add 450 µL wash buffer to the upper reservoir of the filter tube (first wash).
10. Centrifuge at 8000 × *g* for 1 minute.
11. After the first wash and centrifugation, remove the filter tube from the collection tube, discard the flow through liquid and the collection tube. Insert the filter tube into a *new* collection tube.
12. Add 450 µL wash buffer to the upper reservoir of the filter tube (second wash).
13. Centrifuge at 8000 × *g* for 1 minute. Leave the filter tube – collection tube in the centrifuge and spin it for 10 seconds at maximum speed (approximately 13 000 × *g*) to remove any residual was buffer.
14. Discard the collection tube and insert the filter tube into a clean, sterile 1.5 mL microcentrifuge tube.
15. To elute the viral RNA, add 50 µL elution buffer to the upper reservoir of the filter tube.
16. Centrifuge the tube assembly at 8000 × *g* for 1 minute.
17. The microcentrifuge tube now contains the eluted viral RNA.

Note: If isolates cannot be used immediately, store the eluted viral RNA at −80°C for later analysis.

E. Direct sample release technique (PCR-fluorescence probing)

I. Sample extraction:
   1. Pipet 200 µL of specimen into 1.5 mL DNase-free and RNase-free Eppendorf tube.
   2. Centrifuge at 12 000 rpm for 5 minutes.

3. Discard the supernatant fluid carefully, avoid removing the precipitation in the bottom.
4. Add 50 µL sample release reagent into each tube, vortex for 5 seconds.
5. Lysed sample can be directly added to the rRT-PCR reaction.

II. Reagent preparation:
1. Room temperature diagnostic kit.
2. Prepare the 2019-nCoV-PCR Master Mix based on the total number of specimens, 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control and mix thoroughly. The remaining reagent must be stored at −20°C immediately.
   - 26 µL of 2019nCoV-PCR Mix + 4 µL 2019-nCoV-PCR-Enzyme Mix (one sample).
3. Add 30 µL of 2019-nCoV-PCR Master Mix into each well. Cover the wells and transfer to the sample processing area.
4. Add 20 µL of the extracted RNA to the well pre-filled with reagent mix in the following order.
   - 2019-nCoV-PCR Negative control, patient sample (s) and 2019-nCoV-PCR Positive control.
5. Cover the each well, centrifuge at 2000 rpm for 10 seconds.
6. Place into Applied Biosystems ABI 7500 real-time RT-PCR system. Record the exact location of controls and each specimen.

F. Magnetic-bind RNA extraction kit (magnetic nanoparticle technology)

RNA extraction procedure via magnetic nanoparticle:
1. Mark 1.5–2 mL centrifuge tube and add 10 µL of extraction reagent *(Proteinase K).*
2. Add 200–300 µL of specimen. Vortex for 5 seconds and spin down.
3. Prepare magnetic beads mix:

Name	Reagent	Volume/test	Number of tests
Magnetic beads Mix	Extraction reagent ③	5 µL	N=n+2
	Internal Control	2 µL	

Note: the number of tests is N=n+2, where n is the number of samples to be tested and 2 indicates negative control and positive control. Based on laboratory conditions, due consideration shall be given to the losses in the mixture dispensing process.

4. Add 600 µL of extraction reagent (*Lysis buffer*) and 5 µL of extraction reagent (*Magnetic nanoparticles*). Vortex for 10 seconds and incubate for 10 minutes at ambient temperature.
5. Spin down and place tube on magnetic separator. Hold for 3 minutes and discard supernatant by slowly pipetting it out.

6. Add 800 µL of extraction reagent (*wash buffer 1*) to each tube. Vortex for 5 seconds, spin down briefly and place on magnetic separator, hold for 3 minutes. Discard supernatant by slowly pipetting it out.
7. Add 700 µL of extraction reagent (*wash buffer 1*) and 100 µL of extraction reagent (*mineral oil*). Vortex for 5 seconds, spin down briefly and place in the magnetic separator.
8. Hold for 3 minutes, two layers of supernatant are obviously visible. Insert the pipette tip to the bottom, slowly pipette out and discard the bottom layer, vortex for 30 seconds and place the tube on the magnetic separator.
9. Hold for 3 minutes, pipette out and discard residual supernatant.
10. Add 35 µL of extraction reagent (*elution buffer*). Vortex to resuspend brown sediments. Incubate at 60°C for 10 minutes.
11. Spin down briefly. Place the tube in magnetic separator and hold for 3 minutes; the supernatant can be used for subsequent test or transferred to a clean Eppendorf tube and stored at −20°C or below.

G. Superparamagnetic microspheres extraction kit

Assay procedure

A. Lysis
   1. Add 100 µL proteinase K in 15 mL centrifuge tube, add 1 mL of sample. Add 800 µL Lysis buffer.
   2. Vortex and heat centrifuge tube for 10 minutes at 55°C (every 5 minutes, vortex for 10 seconds).

B. Mixing
   1. Add 0.5 mL isopropanol, vortex for 30 seconds, then add 100 µL Beaverbeads.
   2. Vortex and leave for 10 minutes at room temperature.
   3. Put on the magnetic separator and stay for 20 seconds, discard supernatant.

C. Cleaning
   1. Add 3 mL washing buffer. Vortex for 1 minute, place into magnetic separator until solution is clear.
   2. Remove supernatant and remove from magnetic separator.
   3. Add 3 mL 75% ethanol, vortex for 1 minute, place into magnetic separator until solution is clear.
   4. Remove supernatant and remove from magnetic separator.
   5. Add 800 µL 75% ethanol and transfer the solution into 1.5 mL centrifuge tube.
   6. Vortex 1 minute and wait for a minute at room temperature.
   7. Place the centrifuge tube into magnetic separator until solution is clear.
   8. Remove solution from the tube lid and bottom.

D. Drying
   1. Keep the centrifuge tube on the magnetic separator.
   2. Leave for 10 minutes at room temperature.

3. Note: if there is liquid residue in the reaction tube during drying process, use a small range pipettor to remove liquid.

E. Elution

1. Add 50 µL preheated 55ºC elution buffer, vortex for 1 minute.
2. Heat at 55ºC for 5 minutes.
3. Place centrifuge tube in a magnetic separator until solution is clear.
4. Remove supernatant to a new 1.5 mL centrifuge tube.
5. The circulating DNA/RNA is obtained after purification, it can be stored −20ºC.

Annex 4. Different rRT-PCR methods

1. Sansure Biotech

Preparation of reagents

1. Take out each component from the diagnostic kit and place them at room temperature. Allow the reagents to equilibrate at room temperature, then vortex each of them respectively for later use.
2. Prepare the 2019-nCoV-PCR Master Mix (26 µL 2019-nCoV-PCR Mix + 4 µL 2019-nCoV-PCR-Enzyme Mix) based on the total number of specimens, 2019-nCoV-PCR-Positive Control and 2019-nCoV-PCR-Negative Control and mix thoroughly. The remaining reagent must be stored at −20°C immediately.

	1 sample	10 samples	24 samples	48 samples
2019-nCoV-PCR Mix (µL)	26	260	624	1248
2019-nCoV-PCR-Enzyme Mix (µL)	4	40	96	192
Note: the above configuration is for reference only.				

Table A5. Master mix preparation.

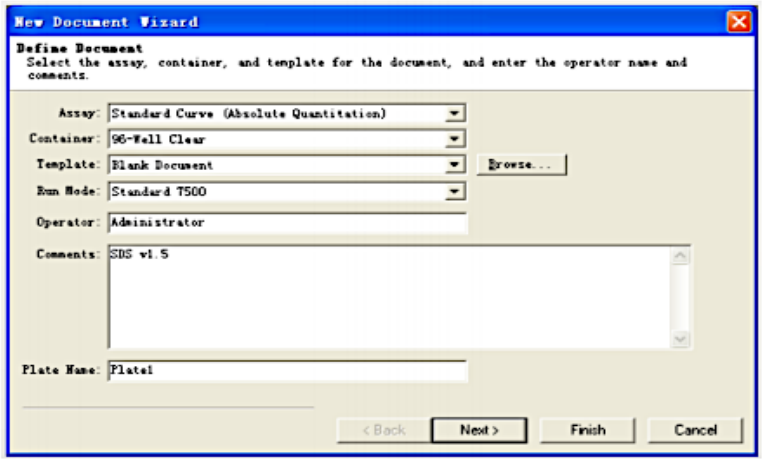
3. Add 30 µL of 2019-nCoV-PCR Master Mix into each well. Cover the wells and transfer to the sample processing area. Add 20 µL of the extracted RNA to the well prefilled with reagent mix in the following order: 2019-nCoV-PCR-Negative Control, patient specimen(s), and 2019-nCoV-PCR-Positive Control. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into Applied Biosystems ABI 7500 real-time RT-PCR system and record the exact location of controls and each specimen.

Running a PCR amplification on ABI 7500 using 7500 software V.1.5:

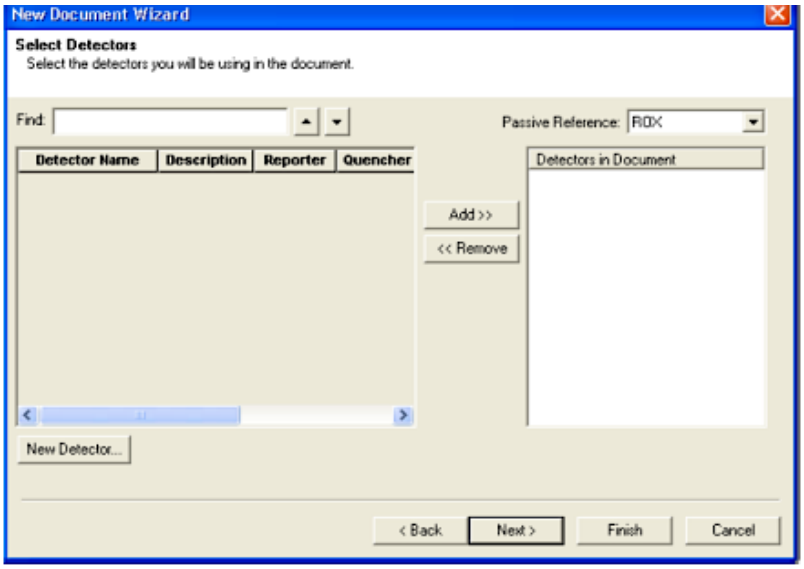
1. Start ABI 7500 real time PCR system: turn on the computer connected to the system first, then turn on ABI 7500 real time PCR system.
2. Load the instrument: push the tray door to open it, load the prepared plate containing samples and controls into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder. Close the tray door.

Set up the experiment run:

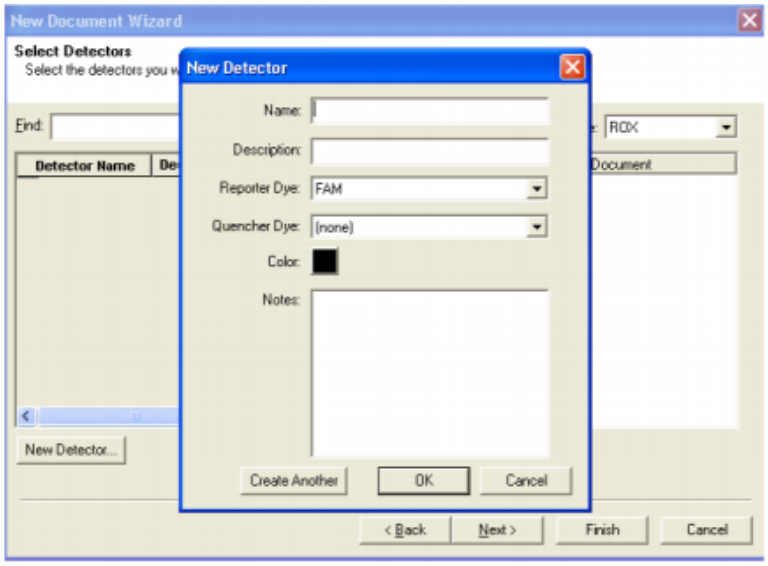
1. Double-click ABI 7500 icon (7500 software V.1.5) on the desktop. A new window should appear, select Create New Document from the menu.



2. Click Next and a new screen will appear.



3. Click New Detector and a new screen will appear.

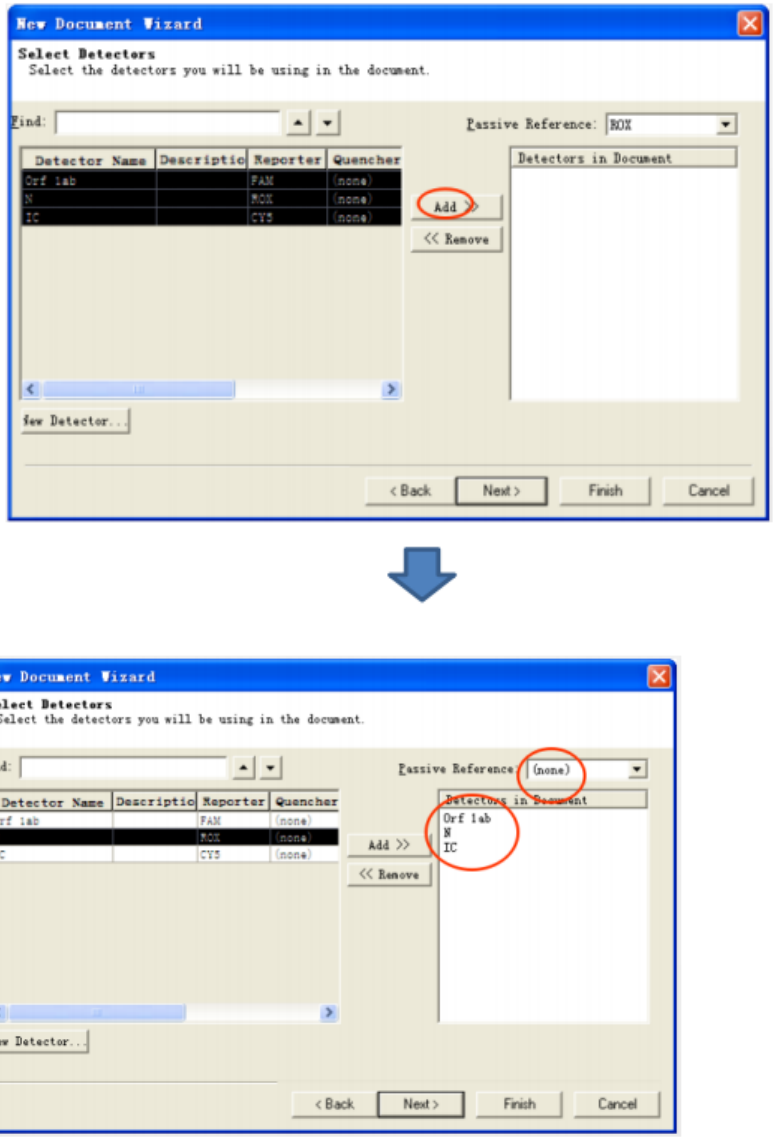


4. Start by creating the ORF lab detector. Include the following:
   a. Name: ORF1ab.
   b. Description: leave blank.
   c. Reporter Dye: FAM.
   d. Quencher Dye: (none).
   e. Color: to change the color of the detector indicator to the following:
      1. Click on the color square to reveal the color chart.
      2. Select a color by clicking on one of the squares.
      3. After selecting a color click OK to return to the New Detector screen.
   f. Click the OK button of the New Detector screen to return to the screen shown above.

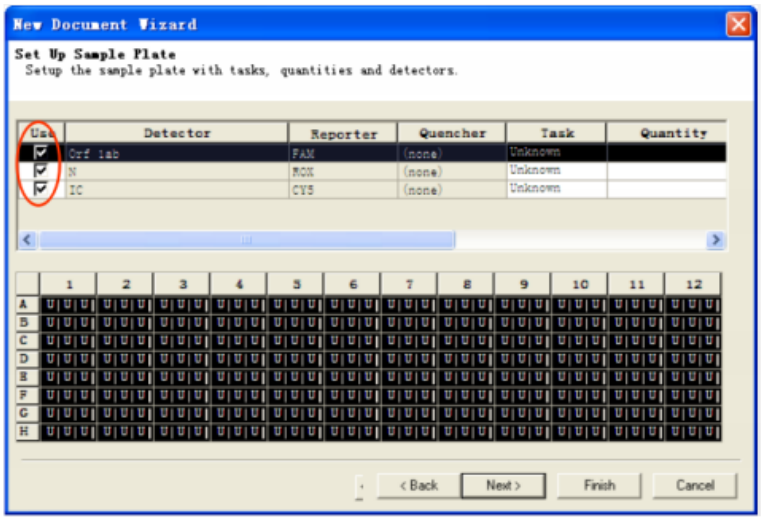
5. Repeat the step for each target in the panel.

Name	Reporter dye	Quencher dye
ORF1ab	FAM	(none)
N	ROX	(none)
IC	CY5	(none)

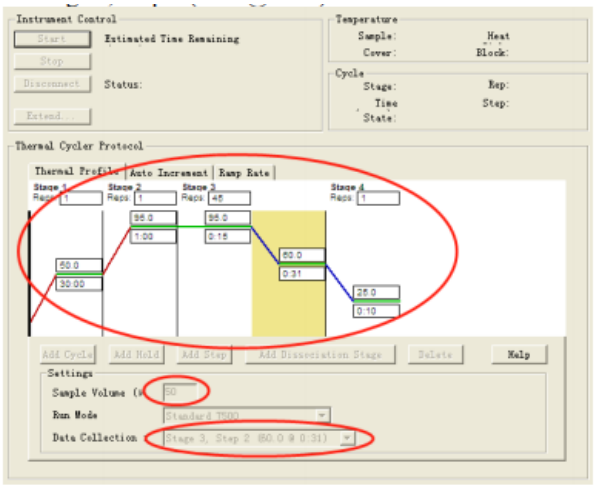
6. After each Detector is added, the Detector Name, Description, Reporter and Quencher fields will become populated in the Select Detectors screen. Before proceeding, the newly created detectors must be added to the document. To add the new detectors to the document, click Add. Detector names will appear on the right hand side of the Select Detectors window. Once all detectors have been added, select (none) for Passive Reference at the top right hand drop down menu.



7. Click Next, select the well containing the samples and controls, and then click the Detector.



8. Click Finish.
9. Select the Instrument tab. Set the parameters as follows:
   Stage 1: 50°C for 30 min. 1 cycle.
   Stage 2: 95°C for 1 min. 1 cycle.
   Stage 3: 95°C for 15 seconds. 60°C for 31 seconds, 45 cycles.
   Stage 4: 25°C for 10 seconds. 1 cycle.
   Sample volume: 50
   Data collection at stage 3, step 2 (60.0 @ 0:31).

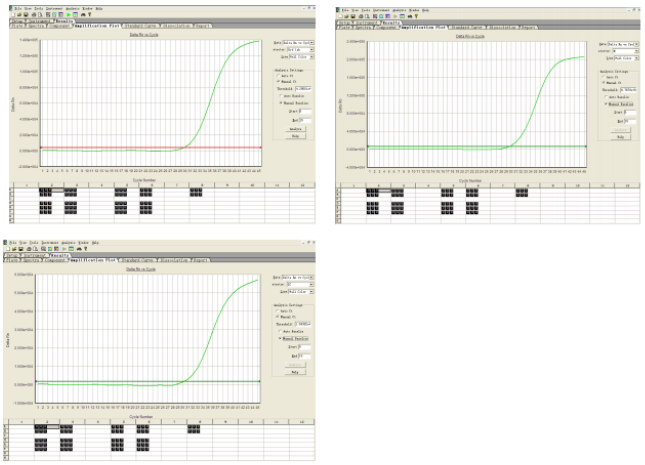


10. Save the document and then click Start to run the evaluation.

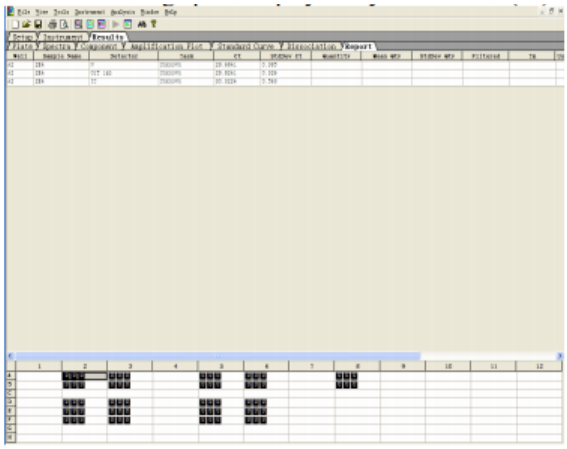
Data analysis

1. See below for step-by-step operation of ABI 7500 using 7500 software V.1.5 for data analysis.
2. After the run is completed, click Results. Click Amplification Plot tab and view and adjust the raw data.
3. In the Data window, Deta Rn vs Cycel should be selected.

4. In the Detector window, “ORFlab” “N” and “IC” should be selected.
5. The Start (cycle) window should be “3-15”. The End (cycle) window should be 5–20. Users can adjust the values according to the actual situation.
6. Adjust the threshold just above the curve from NTC (noise).
7. Lastly, be sure to click “Analyse” icon to update the analysis.



8. Click Report icon above the graph to display the cycle threshold (Ct) values.



Interpretation of results

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The Ct cut-off value of this kit is set as 40, and the end user is required to review fluorescent curves before final interpretation. All the positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples.

Positive and negative controls

The positive and negative controls for each run are interpreted further.

1) Positive and Negative Controls

The positive control and negative control for each run are interpreted as described in Table 2 below.

Table 2. Positive and Negative Control Interpretation.

2019-nCoV-PCR-Positive Control			2019-nCoV-PCR-Negative Control			Results	Actions
ORF1ab (FAM)	N (ROX)	IC (CY5)	ORF1ab (FAM)	N (ROX)	IC (CY5)		
+	+	+	-	-	-	Valid	Continue to result interpretation
Any one of them shows negative			Not considered			Invalid	rRT-PCR failed, re-run
Not considered			Any one of them shows positive				Extraction, rRT-PCR contaminated, re-run
Result of (-): Ct value >40 or Undetermined Result of (+): Ct value ≤ 40 If there is contamination for the re-run, please perform decontamination procedures.							

Table A6. Positive and negative control interpretation.

Examination and interpretation of patient specimen results.

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.
Table A7 below describes the results interpretation concerning the use of the controls provided with the test. The Ct cut-off value of this kit is set to 40, and the end user is required to review fluorescent curves before final interpretation. All positive curves should be typical S-shape amplification curve or without plateau for weakly positive samples (38≤Ct≤40).

Table 3. Interpretation of Results based on Controls.

ORF1ab (FAM)	N (ROX)	IC (CY5)	Results
+	+	Not considered	2019-nCoV Positive
+	-		
-	+		
-	-	+	2019-nCoV Negative
-	-	-	Invalid
Result of (-): Ct value >40 or Undetermined Result of (+): Ct value ≤ 40 Invalid Result: There is no typical S-shape amplification curve or Ct > 40 or No Ct detected for ORF1ab gene (FAM), N gene (ROX) and internal control (CY5), indicating that the specimen concentration is too low, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.			

Table A7. Interpretation of results based on controls.

Limitations:

- False positive and false negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.

- Mutation in the target sequence of SARS-CoV2 or change in the sequence due to virus evolution may lead to false negative results.
- Improper reagent storage may lead to false negative results.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

Troubleshooting

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Verify each component and ensure the volumes of reagent dispensed during preparation of the master mixture are correct. Repeat PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
	PCR tube not properly sealed	Ensure plates are sealed correctly.
If the fluorescent signal does not display the sigmoidal characteristic	Components degraded	Use a new batch.
	Poor quality of RNA samples carrying interferences	Repeat the test with the neat extracted RNA and 1:10 dilution of the extracted RNA.
	PCR equipment failure	Repeat the test or contact the equipment supplier

Table A8. Troubleshooting.

2. Xpert Xpress SARS-CoV-2

Principle:
The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Gene Xpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction, and amplification as well as detection of target sequences in simple or complex samples using real-time PCR assays.

Materials and equipment:
- Xpert Xpress SARS-CoV-2 cartridges.
- Disposable transfer pipettes.
- Biosafety cabinet Class II.

Storage
- Store the Xpert Xpress SARS-CoV-2 cartridges at 2–8^{o}C.

Precautions:
- Wear clean PPEs. Change gloves between handling each specimen.

- Do not open the Xpert Xpress SARS-CoV-2 cartridge lid except when adding specimen.
- Do not use a cartridge that has been dropped after removing it from the packaging.
- Do not shake the cartridge. Shaking or dropping a cartridge after opening the cartridge lid may yield non-determinate results.
- Do not place the sample ID label on the cartridge lid or the barcode label on the cartridge.
- Do not use a cartridge with a damaged barcode label.
- Do not use a cartridge that has a damaged reaction tube.
- Each single-use Xpert Xpress sARS-CoV-2 cartridge is used to process one test. Do not reuse processed cartridges.
- Do not use a cartridge if it appears wet or if the lid seat appears to have been broken.

Specimen collection, transport, and storage

- Proper specimen collection, storage, and transport are critical to the performance of this test. Inadequate specimen collection, improper specimen handling, and/or transport may yield a false result. Nasopharyngeal, nasal, and mid-turbinate swabs and nasal wash/aspirate specimens can be stored at room temperature (15–30°C) for up to 8 hours and refrigerated (2–8°C) up to 7 days until testing is performed on the GeneXpert Instrument System.

Procedure:

Important: start the test within 30 minutes of adding the sample to the cartridge.

1. Remove a cartridge from the package.
2. Check the specimen transport tube is closed.
3. Mix specimen by rapidly inverting the specimen transport tube 5 times. Settle aerosol first before opening the cap of the specimen transport tube. (Note: this should be done in BSCII because of the aerolization).
4. Open the cartridge lid.
5. Remove the transfer pipette from the wrapper.
6. Squeeze the top bulb of the transfer pipette completely and tehn place tip in the specimen transport tube.
7. Release the top bulb of the pipette to fill the pipette before removing from the tube. After filling the pipette, excess sample will be seen in the oveflow reservoir bulb of the pipette. Check that the pipette does not contain bubbles (avoid bubbles).
8. To transfer the sample to the cartridge, squeeze the top bulb of the transfer pipette completely again to empty the contents of the pipette (300 µL) into the large opening in the cartridge. Dispose the used pipette.
9. Close the cartridge lid.
10. Load the cartridge into GeneXpert Dx machine.

Starting the test:
Important notice: before starting the test, make sure that the system contains module with GeneXpert Dx software V.4.7b and that the Xpert Xpress SARS-CoV-2 Assay definition file is imported into the software.

1. Turn on the GeneXpert Dx Instrument system. Turn on the instrument first and then the computer. Log into the windows operating system. The GeneXpert software will launch automatically.
2. Log on to the system software. Click create test.
3. Type the patient ID number and patient name.
4. Scan the barcode on the Xpert Xpress SARS-CoV-2 cartridge. Using the barcode information, the software automatically fills the boxes for the following fields.
    - Reagent lot number.
    - Cartridge serial number.
    - Expiration date.
    - Selected assay.

If the barcode on the cartridge does not scan, repeat the test with new cartridge or input the barcode manually.

5. Click start test
    - Locate the module with the blinking green light, open the instrument module door and LOAD the cartridge.
    - Close the module door. The test will START as the green light blinking stop.
    - The module door automatically unlock when the test is FINISHED.
    - Remove the cartridge and dispose in appropriate sample waste container according to standard operating procedure.

Interpretation of result
The result is interpreted automatically by the GeneXpert System and is clearly shown in the VIEW RESULT window. The Xpert Xpress SARS-CoV-2 test provides test result base on the detection of two gene targets according to the algorithms listed in table A9.

Xpert Xpress SARS-CoV-2

Table 1. Xpert Xpress SARS-CoV-2 Possible Results

Result Text	N2	E	SPC
SARS-CoV-2 POSITIVE	+	+/-	+/-
SARS-CoV-2 PRESUMPTIVE POS	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

Table A9. Interpretation of result.

Annex 5. Different serological methods

1. Enzyme-linked immunosorbent assay (ELISA)

ELISA is a micro well, plate-based assay technique designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones. The test can be qualitative or quantitative, and the time to results is typically 1–5 hours. In the case of SARS-CoV-2 as shown in figure A1, the plate wells are typically coated with a viral protein. If present, antiviral antibodies in the patient samples will bind specifically, and the bound antibody–protein complex can be detected with an additional tracer antibody to produce a colorimetric or fluorescent-based readout. ELISA is speedy, has the ability to test multiple samples, and is adaptable to automation for increased throughput but can be variable in sensitivity and is suitable for point-of-care determinations.

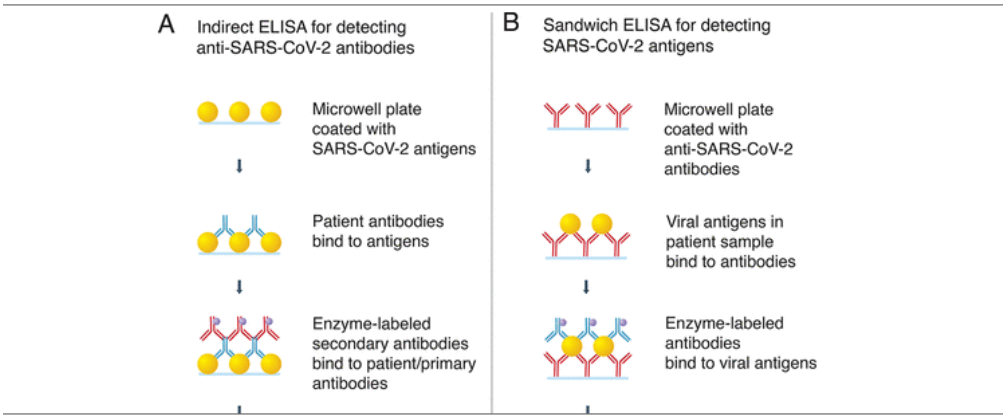


Figure A1. ELISA assays detecting antibodies (A) or antigens (B).

2. Lateral flow immunoassay

This test is typically a qualitative (positive or negative) chromatographic assay that is small, portable, and used at the point-of-care. The test is a type of RDT as the result can be obtained in 10–30 minutes. In practice, fluid samples are applied to a substrate material that allows the sample to flow past a band of immobilized viral antigen. If present, anti-CoV antibodies are collected at the band where, along with co-collected tracer antibodies, a color develops to indicate the results as shown in the figure A2. The test is inexpensive and requires no trained personnel but provides only qualitative results. When used in conjunction with symptomology, a diagnosis of infection may be feasible. Rapid antigen tests, where anti-CoV antibodies are used in place of immobilized viral antigen, allow for a more direct assessment of ongoing infection.

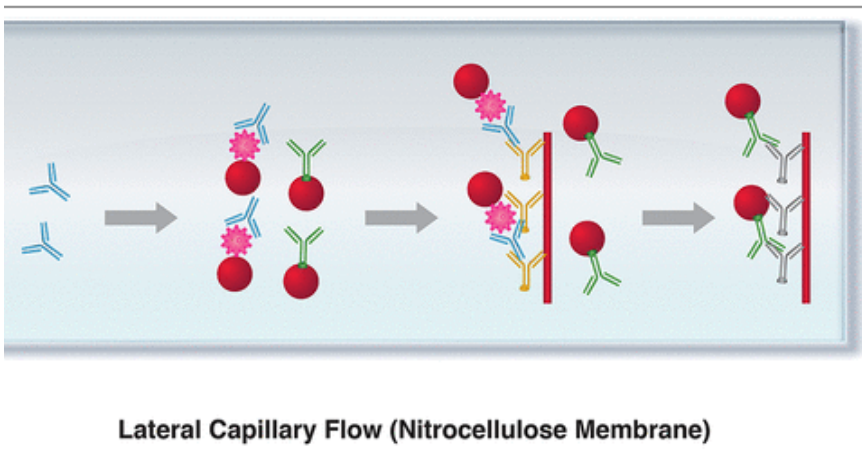


Figure A2. Lateral flow immunoassay for detection of anti-SARS-CoV-2 antibodies. Samples move via capillary flow on the nitrocellulose membrane. When anti-SARS-CoV-2 antibodies are present, they bind to the labelled antigen and continue to move until they are captured by the immobilized antihuman antibodies. The presence of the captured antibody–antigen complex is visualized as a colored test band. The labelled control antibodies comigrate until they are captured at the control band.

3. Neutralization assay

   Neutralization assays determine the ability of an antibody to inhibit virus infection of cultured cells and the resulting cytopathic effects of viral replication. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their levels can be measured by determining the threshold at which they are able to prevent viral replication in the infected cell cultures. The time to results for neutralization assays is typically 3–5 days, but recent advances have reduced this to hours. This type of testing requires cell culture facilities, and in the case of SARS coronavirus, biosafety level 3 laboratories. Despite these limitations, determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and, in the long term, for vaccine development.

4. Luminescent immunoassay

   Luminescent immunoassays comprise methods that lower the limits of detection for antibody-based reagents. Generally, they involve chemiluminescence and fluorescence.

PART 4: POSTANALYTICAL STAGE

Section 1: Encoding/Reporting of Results

Background

The timely encoding and release of results is vital in ensuring that the government has a clear epidemiological picture of COVID-19 and thus be able to enact appropriate remedies and measures. Timely release of results will also facilitate the immediate implementation of quarantine protocols and contact tracing.

Objectives

1. To provide guidance on the manner of encoding and reporting of COVID-19 test results.
2. To provide guidance on the confidentiality and data protection of COVID-19 test results.

Definition of terms

Confidentiality: the limits and restrictions to ensure privacy of personal information.
Encoding: the process of transferring the results of the tests into the test result forms.
Notification: the process of providing authorities, persons, and concerned personnel with the results of the COVID-19 testing.
Transmittal: the process of conveying information to authorities, persons, and concerned personnel with the results of the COVID-19 testing.

Encoding of results

Double-check all laboratory requests and specimen labels prior to the encoding of results. Check the completeness of entry for the following data:

- Date collected.
- Lab accession number.
- Date received.
- Patient's complete name: surname, first name, middle initial.
- Age.
- Sex.
- Date of birth.
- Referring hospital.
- Type of specimen.

The medical technologists who performed the run must check all the information for completeness and errors. This should be verified by the supervising medical technologists and forwarded to the pathologist for further review and verification prior to release.

Manner of reporting

Results of the test shall be signed by the medical technologist who performed the assay, verified by the supervising medical technologist, and approved by the pathologist prior to release. The manner of reporting depends on the kit used and the institutional policy of the testing laboratory. Generally, the following format is used.

SARS-CoV-2 (causative agent of COVID-19) viral RNA detected	Positive for SARS-CoV-2 (causative agent of COVID-19)
SARS-CoV-2 (causative agent of COVID-19) viral RNA not detected	Negative for SARS-CoV-2 (causative agent of COVID-19)
Invalid due to specimen quality	Negative for test internal control (most likely due to poor specimen quality

Notification

COVID-19 testing laboratories are mandated to report to the Department of Health (DOH) the results of COVID-19 testing done within 24 hours of identification and completion of testing using the notification system involving the Regional Epidemiology Surveillance Unit (RESU) and Epidemiological Bureau (EB). If results are positive or equivocal, this should be reported immediately. Furthermore, laboratories should ensure the updating of the 'Laboratory Results Reporting Template' form provided by the DOH.

Transmittal of results

Laboratory results shall be sent immediately by designated persons to the DOH Executive Committee, EB, Research Institute for Tropical Medicine, RESU, and Infection Control Committee head or point person of requesting hospitals and institutions. An official transmittal shall be sent immediately and signed individual results to follow.

Confidentiality

All COVID-19 test results are covered by existing laws and regulations pertaining to patient confidentiality, data privacy, and data protection. This includes but is not limited to Republic Act No. 10173 also known as "Data Privacy Act of 2012" and Republic Act 11332 also known as "Mandatory Reporting of Notifiable Diseases and Health Events of Public Health Concern Act". All laboratory personnel involved in the collection and processing of COVID-19 related data shall put in place the minimum organizational, physical, and technical security measures and standards for data protection as set by National Privacy Commission and the Department of Information and Communications Technology and shall uphold and protect the data privacy rights of every individual at all times.

References

Department of Health. (2020). Administrative order 2020-0013: revised administrative order no. 2020-0012 “Guidelines for the Inclusion of the Coronavirus Disease 2019 (COVID-19) in the List of Notifiable Diseases for Mandatory Reporting to the Department of Heath”.

Department of Health. (2020). Administrative order 2020-0014: Guidelines in securing a license to operate a COVID-19 testing laboratory in the Philippines.

Department of Health and National Privacy Commission. (2020). Joint memorandum circular 2020-0002: privacy guidelines on the processing and disclosure of COVID-19 related data for disease surveillance and response.

National Institute of Molecular Biology and Biotechnology. (2020). Standard operating procedures for SARS-CoV-2 testing version 2.

Section 2: PPE Doffing

Please refer to “Section 3: PPE Selection and Donning” in Part 1: Pre-preanalytical Stage.

Section 3: Waste Management

Introduction and background

The current public health crisis necessitates the establishment of COVID-19 testing laboratories throughout the country as part of the measures instituted for this pandemic. Laboratory professionals working in these laboratories are strictly required to wear appropriate personnel protective equipment (PPE) to cater to the numerous samples to be tested. The COVID-19 testing laboratories are expected to generate not only infectious wastes but also general wastes and pathological wastes, among others. Thus, the volume of infectious wastes and other types of wastes in the laboratory during this pandemic is expectedly high.

The Centers for Disease Control and Prevention mentioned that there is no evidence yet that suggests an additional packaging or disinfection of laboratory wastes from COVID-19 patient specimens. Thus, existing guidelines on the handling of hazardous wastes can be generally applied for this specific situation. Although the country has already established guidelines for the management of health care wastes (*Health Care Waste Management Manual, 4th edition*), the Department of Health has also released additional advisories and interim guidelines in the management of health care wastes during this pandemic.

Objectives

This document aims to collate all existing guidance from the international community, national requirements as well as the local guidelines on healthcare waste management. This will help the COVID-19 testing laboratories to ensure that laboratory workers and the community will not be inadvertently exposed to the biohazards in the handling, storage, transport, and disposal of healthcare wastes.

Definition of terms

General waste: wastes that do not pose a hazard to the environment and human health. This would include general office waste in the laboratory.

Hazardous waste/materials: wastes/materials that have substantial or potential threats to public health or the environment.

Healthcare waste: wastes produced as a result of the activities of a medical institution, medical research facility, or a laboratory.

Infectious waste: wastes suspected to contain pathogens capable of causing disease. This would include solid wastes and liquid waste generated from possible, probable, and confirmed patients

with COVID-19. All PPE used for disinfection of the work area, including non-critical medical items and environment, shall also be considered as infectious waste.

Pathological wastes: consists of tissue, organs, body parts, blood, and body fluids of humans and animals.

Scope

This guideline is intended for the COVID-19 testing laboratories established by the national government, regional offices, local government units, and private establishment.

Responsibility

Top management will administer compliance with the waste management guidelines. Biosafety committee craft guidelines and will conduct a regular review of the effectiveness of the waste management system.

Biosafety officer/head will monitor the implementation of the waste management guidelines. Pollution control officer/waste management head will lead in the implementation of the guidelines complying with the Department of Environment and Natural Resources and the local health authority requirements.

Waste management personnel is responsible for implementing the waste handling decontamination and disposal procedures.

Procedures and guidelines

Waste generation

All wastes generated from a suspect, probable, and confirmed patients with COVID-19 are considered as infectious. These wastes can be mixed with other infectious wastes in the facility. Infectious wastes should be properly segregated at the source from other types of non-infectious waste.
Waste minimization can be achieved by recycling or reusing materials not directly used in the processing of samples in the laboratory.

Segregation and collection

Proper segregation of infectious wastes from general wastes and other types of waste must be ensured and placed in appropriate containers. Waste receptacles and containers must be properly labeled with appropriate color coding as well as the type of waste that should be disposed of in each container.

Color of waste receptacle	Type of waste
Black	Non-infectious dry waste.
Green	Non-infectious wet waste.
Yellow	Infectious and pathological waste.
Yellow with a black band	Chemical waste.
Orange	Radioactive waste.
Red	Sharps and pressurized containers.

Facilities must use waste receptacles that will not require the physical opening of the covering by hands, preferably a pedal-powered receptacle. In the absence of pedal-powered receptacles or other mechanisms similar to it, a bin with swinging lids can be an alternative. In the absence of both, a receptacle with no covering is the last option. The facility needs to have a waste record keeping to keep track of the amount of waste generated. Waste receptacles must be collected when they are three-quarters full already.

Storage

Wastes from COVID-19 testing laboratories shall be stored temporarily in a designated area within the facility away from public access areas until transported to a central storage area.

Central storage of infectious wastes shall bear a biohazard symbol and must be tiled or alternative materials for easy cleaning and disinfection. Storage areas should be constructed with screens and other materials to prevent the entry of pests, insects, and other animals. The storage areas should have an appropriate lock system for biosecurity purposes.

Infectious waste can be stored in the central storage areas no exceeding 48 hours during the cool season and 24 hours only during the hot season. In the presence of refrigerated storage (3–8°C), infectious wastes can be stored for more than a week before transporting to an off-site treatment facility. Compacting of untreated infectious wastes for off-site disposal is not allowed.

Transport and disposal

Transport of infectious waste from the facility to the storage area must be done on wheeled trolleys or wheeled bins dedicated for such purpose using a pre-established route to minimize possible contact with the public during the transport. The transport of non-infectious wastes should have a separate route from infectious wastes. Regardless of the type of waste, the transport must be scheduled wherein the facility is less busy and the presence of people is minimal. The trolley used for transport must have no sharp edges that can damage containers during the process of loading and unloading. These trolleys must be disinfected every after use.

All infectious wastes in a COVID-19 testing laboratory must be autoclaved at 121°C for 30 minutes before disposal. Such an autoclave shall use biological indicators to monitor the efficacy of the autoclave process regularly. Treated infectious wastes may

be disposed of in a sanitary landfill, separate from the general waste. These treated infectious wastes must be placed in a dedicated area in a sanitary landfill.

References

Center for Disease Control. (2020). interim laboratory biosafety guidelines for handling and processing specimens associated with coronavirus disease 2019 (COVID-19).

Department of Health. (2011). *Health care waste management manual* (3rd ed.).

Department of Health. (2020). Department circular no. 2020-0191, "Circulation of the Health Care Waste Management Manual 4th Edition".

Department of Health. (2020). Department memorandum no. 2020-0170, interim guidelines on the management of health care waste in health facilities, community quarantine units, and temporary treatment and monitoring facilities with cases of coronavirus disease 2019 (COVID-19).

Department of Health. (2020). Department memorandum no. 2020-0167, interim guidelines on the proper handling and disinfection of non-critical items used in the management of COVID-19 patients in all health facilities and temporary treatment and monitoring facilities.

Department of Health. (2020). Department memorandum no. 2020-0208, interim guidelines on enhancing the infection prevention and control measures through engineering and environmental controls in all health facilities and temporary treatment and monitoring facilities during the COVID-19 pandemic.

RITM Biorisk Management Office. (2020). Interim biosafety guidelines for laboratories handling and testing SARS-COV-2 (COVID 19) specimen.

World Health Organization. (2020). Water, sanitation, hygiene, and waste management for the COVID-19 virus, Interim guidance.

Section 4: Decontamination, Disinfection, and Spills Management

Background

A basic knowledge of disinfection and sterilization is crucial for biosafety in the laboratory, *especially in time of COVID-19 pandemic*. Since heavily soiled items cannot be promptly disinfected or sterilized, it is equally important to understand the effect of organic matter on the efficacy of disinfectants. In this regard, the following general principles apply to all known classes of microbial pathogens. Specific decontamination requirements will depend on the type of experimental work and the nature of the infectious agent(s) being handled. The generic information given here can be used to develop both standardized and more specific procedures to deal with biohazard(s) involved in a particular laboratory. They should ensure sufficient contact time depending on the nature of the disinfectant, the surface, presence of organic material, and other factors.

There is much to learn about the novel coronavirus (severe acute respiratory syndrome coronavirus (SARS-CoV-2)) that causes coronavirus disease 2019 (COVID-19). Based on what is currently known about the virus and about similar coronaviruses that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), spread from person to person happens most frequently among close contacts (within 6 feet). This type of transmission occurs via respiratory droplets, but disease transmission via aerosols is currently uncertain. Transmission via droplets presents the greater risk, when the droplets settle on the surface they can present a risk of fomite transmission; surfaces also present at risk when infected hand touches that surface (e.g., high touch surfaces such as sink taps.

Current evidence suggests that SARS-CoV-2 may remain viable for hours to days on surfaces made from a variety of materials. Cleaning of visibly dirty surfaces followed by disinfection is a best practice measure for prevention of COVID-19 and other viral respiratory illnesses.

It is unknown how long the air inside a room occupied by someone with confirmed COVID-19 remains potentially infectious. Facilities will need to consider factors such as the size of the room and the ventilation system design (including flowrate (air changes per hour) and location of supply and exhaust vents) when deciding how long to close off rooms or areas used by ill persons before beginning disinfection. Taking measures to improve ventilation in an area or room where someone was ill or suspected to be ill with COVID-19 will help shorten the time. Respiratory droplets do not remain airborne; they settle on surfaces; aerosols remain airborne.

Objectives

- To provide guidance on proper way to decontaminate and disinfect areas that could possibly been infected with SARS-CoV-2 virus.
- To identify recommended disinfectants for various items and settings.
- To help limit the survival of SARS-CoV-2 in key environments.
- To provide guidance on how to properly and effectively manage spills.

Definition of terms

Antimicrobial: an agent that kills microorganisms or suppresses their growth and multiplication.

Antiseptic: a substance that inhibits the growth and development of microorganisms without necessarily killing them. Antiseptics are usually applied to body surfaces.

Biocide: a general term for any agent that kills organisms.

Calcium hypochlorite: an organic compound with formula Ca(ClO)2. It is the main active ingredient of commercial products called bleaching powder or chlorine powder.

Chemical germicide: a chemical or a mixture of chemicals used to kill microorganisms.

Chlorine: a chemical element with the symbol of Cl and atomic number 17. Chlorine-containing compounds such as calcium hypochlorite and sodium hypochlorite are used as disinfectants.

Cleaning: the removal of microbes, dirt, and impurities from surfaces. Cleaning does not kill microbes, but by removing them, it lower their numbers and the risk of spreading infection.

Decontamination: any process for removing and/or killing microorganisms. The same term is also used for removing or neutralizing hazardous chemicals and radioactive materials.

Disinfectant: a chemical or mixture of chemicals used to kill microorganisms, but not necessarily spores. Disinfectants are usually applied to inanimate surfaces or objects.

Disinfection: a physical or chemical means of killing microorganisms but not necessarily spores.

Microbicide: a chemical or mixture of chemicals that kills microorganisms. The term is often used in place of “biocide,” “chemical germicide,” or “antimicrobial.”

Sporocide: a chemical or mixture of chemicals used to kill microorganisms and spores.

Sterilization: a process that kills and/or removes all classes of microorganisms and spores.

Sodium hypochlorite: is a chemical compound with formula NaOCl or NaCl. It is most often encountered as a pale greenish-yellow dilute solution commonly known as liquid bleach or simply bleach.

Scope

It covers the total cycle related to different phases of COVID-19 testing in various settings.

Responsibility

Decontamination and disinfection is a responsibility of all health personnel involved in the entire process of COVID-19 testing.

Specific job descriptions may be given to designated personnel such as safety officers, laboratory personnel as well as to laboratory aides and responders.

Procedures and guidelines

While little is known about this novel virus, the comparable genetic characteristics between the virus responsible for COVID-19 and MERS-CoV suggest that COVID-19 may be susceptible to disinfectants with proven activity against enveloped viruses, including sodium hypochlorite (bleach; for example, 1000 parts per million (ppm) (0.1%) for general surface disinfection and 10 000 ppm (1%) for disinfection of blood spills); 62%–71% ethanol; 0.5% hydrogen peroxide; quaternary ammonium compounds; and phenolic compounds, if used according to the manufacturer's recommendations. Other biocidal agents such as 0.05%–0.2% benzalkonium chloride or 0.02% chlorhexidine digluconate can be less effective.

Particular attention should be paid not only to the selection of the disinfectant but also the contact time (e.g., 10 minutes for 10% sodium hypochlorite), dilution (i.e., concentration of the active ingredient) and expiry date after the working solution is prepared.

Human coronaviruses in general are known to persist on inanimate surfaces such as metal, glass or plastic for up to a maximum of 9 days (WHO interim guidance).

Principles of decontamination, sterilization, and disinfection

Decontamination

In order to implement a laboratory biosafety program, it is important to understand the principles of decontamination, cleaning, sterilization, and disinfection. Definitions of sterilization, disinfection, antisepsis, decontamination, and sanitization need to be reviewed to avoid misuse and confusion.

Decontamination (sometimes abbreviated as decon, dcon, or decontam) is the process of removing or neutralizing an object or substance to remove contaminants such as microorganisms or hazardous materials, including chemicals, radioactive substances, and infectious diseases. The purpose of decontamination is to prevent the spread of microorganisms and other noxious contaminants that may threaten the health of human beings or animals, or damage the environment (Wikipedia).

Decontamination in the microbiology laboratory must be carried out with great care. It may entail disinfection of work surfaces, decontamination of equipment so it is safe to handle, or may require sterilization. Regardless of the method, the purpose of decontamination is to

protect the laboratory worker, the environment, and anyone who enters the laboratory or handles laboratory products away from the laboratory. Reduction of cross-contamination in the laboratory is an added benefit.

Decontamination renders an area, device, item, or material safe to handle (i.e., safe in the context of being reasonably free from a risk of disease transmission). The primary objective is to reduce the level of microbial contamination so that infection transmission is eliminated. The decontamination process may be ordinary soap and water cleaning of an instrument, device, or area. In laboratory settings, decontamination of items, spent laboratory materials, and regulated laboratory wastes is often accomplished by a sterilization procedure such as steam autoclaving, perhaps the most cost-effective way of decontaminating a device or an item.

The presence of any organic matter necessitates longer contact time with a decontamination method if the item or area is not precleaned. For example, a steam cycle used to sterilize precleaned items is 20 minutes at 121°C. When steam sterilization is used to decontaminate items that have a high bioburden and there is no precleaning (i.e., infectious waste), the cycle is longer.

Decontamination in laboratory settings often requires longer exposure times because pathogenic microorganisms may be protected from contact with the decontaminating agents.

Methods of decontamination

- Physical cleaning.
- Water purification.
- Ultrasonic cleaning.
- Disinfection.
- Antisepsis.
- Sterilization.

While several decontamination methods are available, physical cleaning, disinfection, and sterilization are those most commonly used during COVID-19 pandemic. Thus, these methods are described here.

Cleaning

Cleaning is the removal of dirt, organic matter, and stains. Cleaning includes brushing, washing, or damp mopping with water containing a soap or detergent. Dirt, soil, and organic matter can shield microorganisms and can interfere with the killing action of decontaminants (antiseptics, chemical germicides, and disinfectants). Many germicidal products claim activity only on precleaned items. Precleaning must be carried out with care to avoid exposure to infectious agents.

Materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for precleaning and disinfection.

Sterilization

Any item, device, or solution is considered to be sterile when it is completely free of all living microorganisms and viruses. The definition is categorical and absolute (i.e., an item is either sterile or it is not). A sterilization procedure is one that kills all microorganisms, including high numbers of bacterial endospores. Sterilization can be accomplished by heat, ethylene oxide gas, hydrogen peroxide gas, plasma, ozone, and radiation (in industry). From an operational standpoint, a sterilization procedure cannot be categorically defined. Rather, the procedure is defined as a process, after which the probability of a microorganism surviving on an item subjected to treatment is less than one in one million. This is referred to as the "sterility assurance level."

Disinfection

It is generally a less lethal process than sterilization. It eliminates nearly all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial spores) on inanimate objects. Disinfection does not ensure an "overkill'' and therefore lacks the margin of safety achieved by sterilization procedures. The effectiveness of a disinfection procedure is controlled significantly by a number of factors, each one of which may have a pronounced effect on the end result. Among these are:

- The nature and number of contaminating microorganisms (especially the presence of bacterial spores).
- The amount of organic matter present (e.g., soil, feces, and blood).
- The type and condition of instruments, devices, and materials to be disinfected.
- The temperature.

Disinfection is a procedure that reduces the level of microbial contamination, but there is a broad range of activity that extends from sterility at one extreme to a minimal reduction in the number of microbial contaminants at the other. By definition, chemical disinfection and, in particular, high-level disinfection, differs from chemical sterilization by its lack of sporicidal power. This is an oversimplification of the actual situation because a few chemical germicides used as disinfectants do, in fact, kill large numbers of spores even though high concentrations and several hours of exposure may be required. Non-sporicidal disinfectants may differ in their capacity to accomplish disinfection or decontamination. Some germicides rapidly kill only the ordinary vegetative forms of bacteria such as staphylococci and streptococci, some forms of fungi, and lipid-containing viruses, whereas others are effective against such relatively resistant organisms as *Mycobacterium tuberculosis* var. bovis, non-lipid viruses, and most forms of fungi.

Spaulding classification

In 1972, Dr. Earl Spaulding proposed a system for classifying liquid chemical germicides and inanimate surfaces that has been used subsequently by Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), and opinion leaders in the USA. This system, as it applies to device surfaces, is divided into three general categories based

on the theoretical risk of infection if the surfaces are contaminated at time of use. From the laboratory perspective, these categories are:

- Critical: instruments or devices that are exposed to normally sterile areas of the body that require sterilization.
- Semicritical: instruments or devices that touch mucous membranes may be either sterilized or disinfected.
- Non-critical: instruments or devices that touch skin or come into contact with persons only indirectly can be either cleaned and then disinfected with an intermediate-level disinfectant, sanitized with a low-level disinfectant, or simply cleaned with soap and water.

In 1991, microbiologists at CDC proposed an additional category, environmental surfaces (e.g., floors, walls, and other "housekeeping surfaces") that do not make direct contact with a person's skin. Spaulding also classified chemical germicides by activity level:

High-level disinfection

This procedure kills vegetative microorganisms and inactivates viruses but not necessarily high numbers of bacterial spores. Such disinfectants are capable of sterilization when the contact time is relatively long (e.g., 6–10 hours). As high-level disinfectants, they are used for relatively short periods of time (e.g., 10–30 minutes). These chemical germicides are potent sporicides and, in the USA, are classified by the FDA as sterilant/disinfectants. They are formulated for use on medical devices, but not on environmental surfaces such as laboratory benches or floors.

Intermediate-level disinfection

This procedure kills vegetative microorganisms, including *M. tuberculosis*, all fungi, and inactivates most viruses. Chemical germicides used in this procedure often correspond to Environmental Protection Agency (EPA) approved "hospital disinfectants" that are also "tuberculocidal." They are used commonly in laboratories for disinfection of laboratory benches and as part of detergent germicides used for housekeeping purposes.

Low-level disinfection

This procedure kills most vegetative bacteria except *M. tuberculosis*, some fungi, and inactivates some viruses. The EPA approves chemical germicides used in this procedure in the USA as "hospital disinfectants" or "sanitizers."

Actions of commonly used chemical germicides

Chemical germicides

Many types of chemicals can be used as disinfectants and/or antiseptics. As there is an ever-increasing number and variety of commercial products, formulations must be carefully selected for specific needs.
The germicidal activity of many chemicals is faster and better at higher temperatures. At the same time, higher temperatures can accelerate their evaporation and also degrade them. Particular care is needed in the use and storage of such chemicals in tropical regions, where their shelf-life may be reduced because of high ambient temperatures. Many germicides can be harmful to humans or the environment. They should be selected, stored, handled, used, and disposed of with care, following manufacturers' instructions. For personal safety, gloves, aprons, and eye protection are recommended when preparing dilutions of chemical germicides. Chemical germicides are generally not required for regular cleaning of floors, walls, equipment, and furniture. However, their use may be appropriate in certain cases of outbreak control. Proper use of chemical germicides will contribute to workplace safety while reducing the risk from infectious agents. As far as possible, the number of germicidal chemicals to be used should be limited for economic reasons, inventory control, and to limit environmental pollution. Commonly used classes of chemical germicides are described further, with generic information on their applications and safety profiles. Unless otherwise indicated, the germicide concentrations are given in weight/volume (w/v).

Chlorine (sodium hypochlorite)

Chlorine, a fast-acting oxidant, is a widely available and broad-spectrum chemical germicide. It is normally sold as bleach, an aqueous solution of sodium hypochlorite (NaClO), which can be diluted with water to provide various concentrations of available chlorine.

Chlorine, especially as bleach, is highly alkaline and can be corrosive to metal. Its activity is considerably reduced by organic matter (protein). Storage of stock or working solutions of bleach in open containers, particularly at high temperatures, releases chlorine gas thus weakening their germicidal potential. The frequency with which working solutions of bleach should be changed depends on their starting strength, the type (e.g., with or without a lid) and size of their containers, the frequency and nature of use, and ambient conditions. As a general guide, solutions receiving materials with high levels of organic matter several times a day should be changed at least daily, while those with less frequent use may last for as long as a week.

A general all-purpose laboratory disinfectant should have a concentration of 1 g/L available chlorine. A stronger solution, containing 5 g/L available chlorine, is recommended for dealing with biohazardous spillage and in the presence of large amounts of organic matter. Sodium hypochlorite solutions, as domestic bleach, contain 50 g/L available chlorine and should therefore be diluted 1:50 or 1:10 to obtain final concentrations of 1 g/L and 5 g/L, respectively. Industrial solutions of bleach have a sodium hypochlorite concentration of nearly 120 g/L and must be diluted accordingly to obtain the levels indicated above.

Granules or tablets of calcium hypochlorite ($Ca(ClO)_2$) generally contain about 70% available chlorine. Solutions prepared with granules or tablets, containing 1.4 g/L and 7.0

g/L, will then contain 1.0 g/L and 5 g/L available chlorine, respectively. Bleach is not recommended as an antiseptic but may be used as a general-purpose disinfectant and for soaking contaminated metal-free materials. In emergencies, bleach can also be used to disinfect water for drinking, with a final concentration of 1–2 mg/L available chlorine.

Chlorine gas is highly toxic. Bleach must therefore be stored and used in well ventilated areas only. Also, bleach must not be mixed with acids to prevent the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment, so that indiscriminate use of chlorine-based disinfectants, in particular bleach, should be avoided.

Alcohols

Ethanol (ethyl alcohol (C_2H_5OH)) and 2-propanol (isopropyl alcohol $(CH_3)_2CHOH$) have similar disinfectant properties. They are active against vegetative bacteria, fungi, and lipid-containing viruses but not against spores. Their action on non-lipid viruses is variable. For highest effectiveness, they should be used at concentrations of approximately 70% (v/v) in water: higher or lower concentrations may not be as germicidal. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items.

Mixtures with other agents are more effective than alcohol alone, for example, 70% (v/v) alcohol with 100 g/L formaldehyde, and alcohol containing 2 g/L available chlorine. A 70% (v/v) aqueous solution of ethanol can be used on skin, work surfaces of laboratory benches, and biosafety cabinets and to soak small pieces of surgical instruments. Since ethanol can dry the skin, it is often mixed with emollients. Alcohol-based hand rubs are recommended for the decontamination of lightly soiled hands in situations where proper handwashing is inconvenient or not possible. However, it must be remembered that ethanol is ineffective against spores and may not kill all types of non-lipid viruses. Alcohols are volatile and flammable and must not be used near open flames. Working solutions should be stored in proper containers to avoid the evaporation of alcohols. Alcohols may harden rubber and dissolve certain types of glue. Proper inventory and storage of ethanol in the laboratory is very important to avoid its use for purposes other than disinfection. Bottles with alcohol-containing solutions must be clearly labelled to avoid autoclaving.

Quaternary ammonium compounds

Many types of quaternary ammonium compounds are used as mixtures and often in combination with other germicides, such as alcohols. They have good activity against some vegetative bacteria and lipid-containing viruses. Certain types (e.g., benzalkonium chloride) are used as antiseptics.

The germicidal activity of certain types of quaternary ammonium compounds is considerably reduced by organic matter, water hardness, and anionic detergents. Care is therefore needed in selecting agents for precleaning when quaternary ammonium compounds are to be used for disinfection. Potentially harmful bacteria can grow in quaternary ammonium compound solutions. Owing to low biodegradability, these compounds may also accumulate in the environment.

Hydrogen peroxide and peracids

Like chlorine, hydrogen peroxide (H_2O_2) and peracids are strong oxidants and can be potent broad-spectrum germicides. They are also safer than chlorine to humans and the environment.

Hydrogen peroxide is supplied either as a ready-to-use 3% solution or as a 30% aqueous solution to be diluted to 5–10 times its volume with sterilized water. However, such 3%–6% solutions of hydrogen peroxide alone are relatively slow and limited as germicides. Products now available have other ingredients to stabilize the hydrogen peroxide content to accelerate its germicidal action and to make it less corrosive.

Hydrogen peroxide can be used for the decontamination of work surfaces of laboratory benches and biosafety cabinets, and stronger solutions may be suitable for disinfecting heat-sensitive medical/dental devices. The use of vaporized hydrogen peroxide or peracetic acid (CH3COOOH) for the decontamination of heat-sensitive medical/surgical devices requires specialized equipment.

Hydrogen peroxide and peracids can be corrosive to metals such as aluminum, copper, brass and zinc, and can also decolorize fabrics, hair, skin, and mucous membranes. Articles treated with them must be thoroughly rinsed before contact with eyes and mucous membranes. They should always be stored away from heat and protected from light.

Approaches in local environmental decontamination

Surfaces can be decontaminated using a solution of sodium hypochlorite (NaOCl); a solution containing 1 g/L available chlorine may be suitable for general environmental sanitation, but stronger solutions (5 g/L) are recommended when dealing with high-risk situations. For environmental decontamination, formulated solutions containing 3% hydrogen peroxide (H_2O_2) make suitable substitutes for bleach solutions.

Decontamination of biological safety cabinet (BSC)

To decontaminate class I and class II cabinets, it should be performed by a BSC certified with proper training and protective equipment.

Handwashing/hand decontamination

Whenever possible, suitable gloves should be worn when handling biohazardous materials. However, this does not replace the need for regular and proper handwashing by laboratory personnel. Hands must be washed after handling biohazardous materials and animals and before leaving the laboratory.

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them, but the use of germicidal soaps is recommended in high-risk situations. Hands should be thoroughly lathered with soap, using friction, for at least 20 seconds, rinsed in clean water and dried using a clean paper or cloth towel (if available, warm air hand dryers may be used).

Foot-operated or elbow-operated faucets are recommended. Where not fitted, a paper/cloth towel should be used to turn off the faucet handles to avoid recontaminating washed hands.

As mentioned, alcohol-based hand rubs may be used to decontaminate lightly soiled hands when proper hand washing is not available.

Heat disinfection and sterilization

Heat is the most common among the physical agents used for the decontamination of pathogens.

"Dry" heat, which is totally non-corrosive, is used to process many items of laboratory ware that can withstand temperatures of 160°C or higher for 2–4 hours. Burning or incineration (see below) is also a form of dry heat. "Moist" heat is most effective when used in the form of autoclaving.

Boiling does not necessarily kill all microorganisms and/or pathogens, but it may be used as the minimum processing for disinfection where other methods (chemical disinfection or decontamination and autoclaving) are not applicable or available.

Sterilized items must be handled and stored such that they remain uncontaminated until used.

Autoclaving

Saturated steam under pressure (autoclaving) is the most effective and reliable means of sterilizing laboratory materials. For most purposes, the following cycles will ensure sterilization of correctly loaded autoclaves:

1. 3 minutes holding time at 134°C.
2. 10 minutes holding time at 126°C.
3. 15 minutes holding time at 121°C.
4. 25 minutes holding time at 115°C.

Local guidelines and recommendations

To address specific concerns in local setting, concerned government agencies, such as Department of Health (DOH) and Research Institute for Tropical Medicine, issued interim guidelines to standardize decontamination and disinfection practices in the different local scenarios. Guidance mentioned here were mostly lifted from said issuances.

General guidelines

A. The sodium hypochlorite solution at 0.5% (equivalent to 5000 ppm) using a ratio of 1:10) shall be used for disinfecting surfaces including soiled clothes, toilets, body fluid spilled on the floors, vehicles, roads, disposed personal protective equipment (PPEs) and similar healthcare wastes, and others.
B. If other options for handwashing (e.g., alcohol-based rub, soap, and water) are not available, sodium hypochlorite solution at 0.05% (equivalent to 500 ppm) using a ratio of 1:100)) may be used for hand washing. However, it must be used with caution because frequent use may lead to dermatitis, which could increase risk of infection.
C. Other types of chemical disinfectant such as ammonium chloride, phenols and hydrogen peroxide shall be used according to manufacturer's requirements.
D. All individuals dealing with the disinfection process shall wear appropriate PPE.
E. Proper hand hygiene shall be practiced before and after the disinfection activity.

Specific guidelines

A. Preparation of the 0.5% sodium hypochlorite solution (1:10 solution) for surface disinfection
    1. Using commercially available household bleach at 5% active chlorine, dilute 1 part of bleach to 9 parts of clean water.
    2. Using chlorine powder/granules/tablet at 60%–70% active chlorine, dissolve 1 tablespoon of chlorine (equivalent to 10 grams) to 2 L of clean water. Mix the solution thoroughly using a stick.

B. Preparation of the 0.05% sodium hypochlorite solution for hand washing (1:100 solution)

    Using the 0.5% solution of household bleach, add 1 part of the solution to 9 parts of clean water. For example, add 100 mL of solution to 1 L of clean water.

C. General disinfection

    Sodium hypochlorite (prepare 1:100).
    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 BCS and other equipment.

D. Space decontamination

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 1 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.

E. Environmental surface

- Environmental surfaces shall be kept visibly clean based on the established protocols of the health facility.
- Visible dirt on surfaces shall be promptly removed using detergent and water, or a disinfectant approved for use by the health facility. The approved disinfectants shall be used according to the manufacturer's instructions for cleaning and disinfecting environmental surfaces.
- Frequently touched surfaces shall be cleaned and disinfected (e.g., door knobs, bed rails, and light switches) on a more frequent schedule, as established by the health facility.
- Cleaning and disinfecting solutions shall be prepared daily or when needed.
- Mops and cloths used for cleaning and disinfecting shall be cleaned after every use and shall be allowed to dry before the next use.

F. Health records/documents

- All health workers managing suspect, probable and confirmed patients with COVID-19 shall practice hand hygiene at all times – before and after documentation. Hand hygiene supplies (i.e., soap and water or alcohol-based hand rubs) shall be available and accessible to health workers.
- All documentation shall be done at the designated area away from the patient.
- Disinfectant machines (e.g., virus disinfectant portable machine and UV light, if available, shall be used for decontamination of health records.

- All decontaminated health records shall be placed on a designated plastic container prior to forwarding to the health records office. The Infection Control Team or staff shall lead the decontamination process.

G. Electronic devices

All electronic devices shall be decontaminated as follows:

- Devices shall be disconnected and/or unplugged before decontamination.

- Visible dirt shall be removed using cleaning and disinfecting products for identified electronic devices. Manufacturer's instructions shall be followed for cleaning and/or disinfecting electronic devices.
- If no manufacturer's guidance is available, the use of alcohol-based wipes or spray containing at least 70% alcohol shall be considered for disinfection.
- Surfaces shall be dried thoroughly to avoid pooling of liquids.
- Consider placing wipeable covers to parts of the electronic devices (e.g., keyboards and monitors) for easier cleaning.

H. Disposal of used PPE

All PPE used for disinfection of non-critical medical items and environment shall be considered as infectious waste, which shall be properly treated prior to disposal in accordance with healthcare waste management policies and procedures.

Autoclaving contaminated laboratory wastes

All potentially contaminated laboratory wastes must be autoclaved at 121°C psi for 30 minutes prior to 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.

I. Storage of chlorine and prepared solutions

1. Store chlorine (liquid or powder) in air-tight non-metallic containers, away from heat, light and humidity in a ventilated area.
2. Carefully close disinfectant containers after use.
3. Never place in contact with water, acid, fuel, detergents, organic or inflammable materials (e.g., food, paper, or cigarettes)
4. Change the prepared solutions every day. Do not prepare too much solution at a time to avoid wasting.

J. Use of disinfection tents/misting chambers

1. The US CDC reported the lack of microbicidal efficacy in the use of disinfectants but also adverse health effects where these methods were utilized. *The use of misting tents for persons wearing regular clothes without PPEs may pose the following issues:*
    a. Safety of the chemical disinfectant used. Based on literature, commonly used chemical disinfectants such as hypochlorite are irritant to the skin and the mucous (eyes, nose, and throat). It may

also have adverse health effects when inhaled in an enclosed environment.

b. Efficacy of the chemical disinfectant. There are limited evidence-based studies to show that chemical used for surface disinfectants intended to eliminate the human coronavirus would have the same efficacy when applied in the ambient environment or in humans.

2. Pending additional studies on demonstrating safety and efficacy, the use of disinfection tents, misting chambers, or sanitation booths for individuals without full PPE shall not be allowed.
   a. Given the evolving nature of evidence for COVID-19, activities conducted by local government units and agencies prior to the release of this issuance shall not be held against the implementing agency.
   b. All implementing agencies are advised to repurpose the materials and staff initially indicated for these activities to efforts consistent with DOH guidelines such as for use of chemicals for surface disinfection, use of booths for temporary isolation or triage areas, or reallocation of staff for contact tracing.
   c. Individuals in full PPEs, characterized as having no external exposure, may be subjected to misting or spraying before doffing their full PPEs with careful consideration of the eyes, nose, and mouth/throat.

K. Spills management

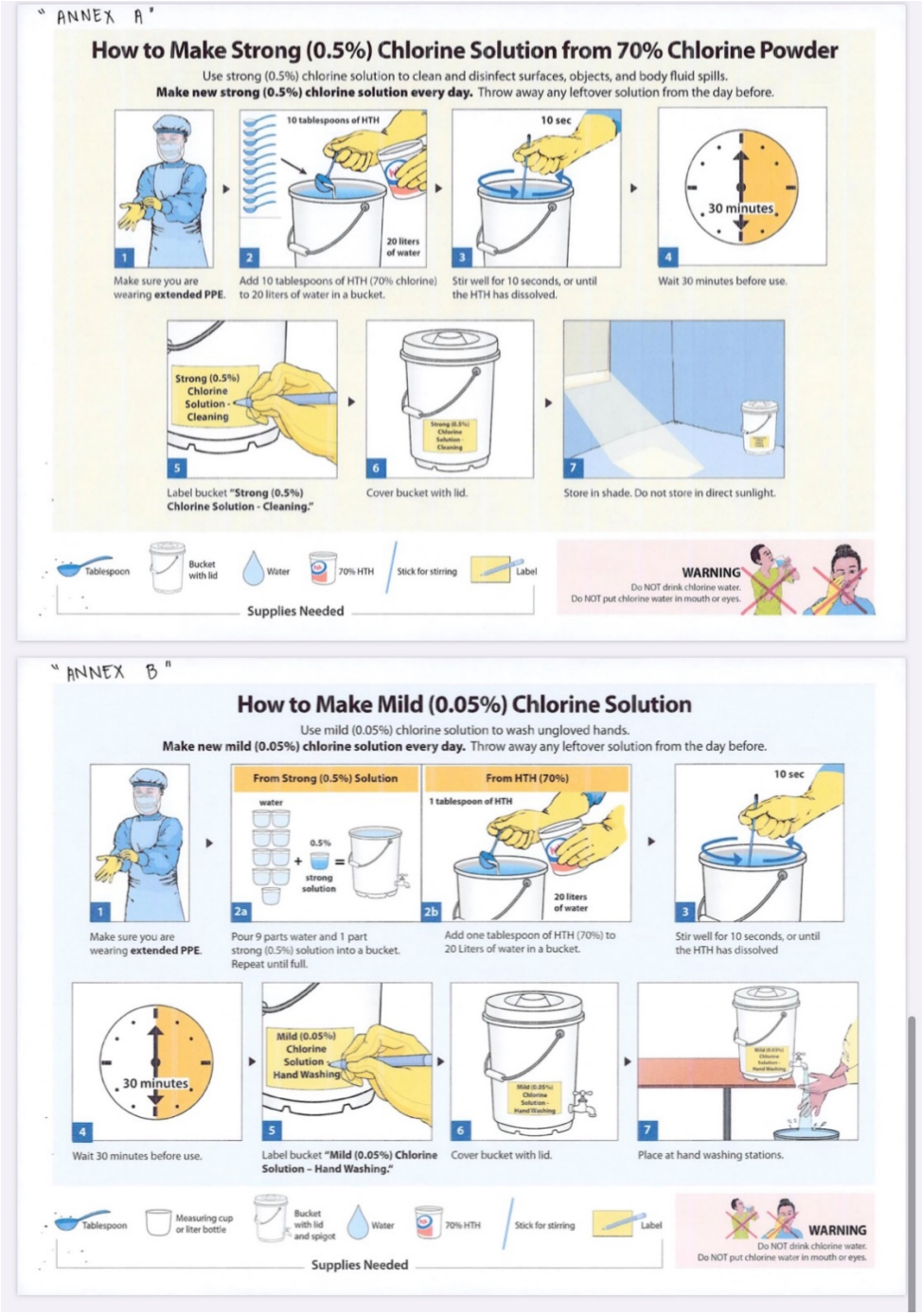
Laboratory must prepare for accidental spills and related laboratory emergencies while working with COVID-19 specimens.
Spilled blood and body fluids/substances and infective agents may be encountered in many situations in the laboratory. Management of these spills may require specialize procedures. All biohazard spills must be attended to immediately.

L. 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 to 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 for respiratory protection (N95, P100 and 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.

Annex



Annex 1.

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PART 5: POST-POSTANALYTICAL STAGE

Section 1: Retention, Retrieval, and Disposal of Laboratory Records and Postanalytical Specimens

Introduction and background

Retained laboratory specimens and laboratory records are integral by-products of laboratory operations hence a good management is needed to prevent these from unnecessary build-up thereby taking up the limited space of the laboratory storage area. Moreover, there are biosafety and biosecurity risks involved in stored and retained specimens. The laboratory, being the custodian of these by-products, shall establish a retention policy for records and specimens.

Objectives

A retention policy shall be implemented in order to secure the integrity and accessibility of laboratory records and retained specimens for purposes of review, recall, or inquiry that will arise later. The policy shall likewise constitute biosafety and biosecurity measures to prevent the spread of laboratory-acquired infections and the malicious use of retained biological specimens, respectively. These records and specimens that are retained at a predefined period by the laboratory shall be discarded or disposed off following standard decontamination procedures mentioned in this manual and correct recording to maintain traceability.

Definition of terms

Decontamination: reduction of microbiological contamination to a safe level.

Disposal: discharge or release of specimens or deletion of laboratory records.

Primary or original specimen: the specimen submitted in its original form.

Records: are documents (paper or electronic) that provide evidence of activities performed in the laboratory. Examples are quality control results, filled-out logbook, or completed test report.

Specimen product: sample aliquots, nucleic acid extracts, serum, or plasma separated from original specimen submitted (whole blood) or the isolates obtained from culturing clinical specimens.

Specimens with unusual results (SWUR): specimens that are particularly retained due to specific instruction from relevant agencies or specimens showing peculiar patterns or results that are relevant in management and control of disease.

Scope

These guidelines shall apply to all laboratory personnel involved in the storage and retention of specimens and records after analytical and postanalytical steps and until disposal of such records or retained specimens.

Responsibility

Document control custodian (DCC) or record control custodian (RCC) is a laboratory personnel specifically designated with lead authority in controlling the disposition and management of laboratory document or records.

Laboratory administrator (LA) or chief medical technologist (CMT) shall be responsible in ensuring that all laboratory personnel are oriented on the retention, retrieval, and disposal policy of laboratory records and retained samples.

Laboratory aide shall be responsible in the proper disposal of records or samples after its retention period. Recording of disposed samples and records are required as they form traceability for future reference.

Laboratory biosafety/biorisk officer is a laboratory personnel specifically designated with lead authority in the implementation of biosafety and biosecurity culture and practices.

Laboratory director or pathologist shall be responsible that all laboratory personnel follow the established retention, retrieval, and disposal policy of laboratory records and retained samples.

Laboratory section head/supervisor shall be the main custodian of section records and retained specimens, hence responsible in ensuring that section records and specimens are intact and accessible only to allowed laboratory personnel. Shall ensure that limited-access records shall be destroyed (i.e., shredded) and retained specimen for disposal shall be decontaminated prior to disposal after the predefined retention period.

Laboratory technical staff shall be responsible in securing limited-access or classified records, accessibility of open-access records, and secure integrity of stored samples while being retained by the laboratory.

Procedures and guidelines

1. Retention, retrieval, and disposal of postanalytic specimens
    1.1. The LA or CMT shall conduct or facilitate orientation/reorientation of all laboratory staff on the retention, retrieval, and disposal policy of laboratory records and retained samples prior to deployment of staff in the laboratory. The LA/CMT shall maintain record of staff attendance to orientation/reorientation conducted.

1.2. Laboratory personnel in-charged in handling postanalytic samples shall properly identify samples for retention following Table 1 and endorses the samples to the laboratory section head/supervisor.

Specimen	Retention period and storage condition of original specimen	Retention period and storage condition of specimen product/s	Retention period and storage condition of specimens with unusual results
Blood for viral serology	1 week refrigerated (2–8°C)	All positive serum/plasma are stored for 3 years at −80°C or at −20°C to −30°C for 3 months.	Stored 5 years at −80°C.
Respiratory specimens for molecular/PCR-based testing	1 week refrigerated (2–8°C).	Extracted nucleic acid (positive and negative) 2 years at −80°C or at −20°C to −30°C for 3 months.	Extracted nucleic acid retained 2 years at negative −80°C.
Other specimens	Do not retain. Discard after testing process following decontamination procedure.	Do not retain. Discard after testing process following decontamination procedure.	2 years at −80°C or at −20°C to −30°C for 3 months.

Table 1. Retention period and storage condition of retained SARS-CoV-2 testing postanalytic specimens.

1.3. Laboratory section head/supervisor shall store retained postanalytic specimens following storage condition indicated in the above table while implementing appropriate biosafety practices during the storage process.

1.4. The laboratory section head/supervisor who stored the retained postanalytic specimen shall be the custodian of such retained specimen and shall personally record the details of the retained specimen including the end of its retention period in the specimen retention logbook.

1.5. Upon request for retrieval of retained specimen, the laboratory section head/supervisor in consultation with laboratory biosafety/biorisk officer shall screen and grant specimen access or clearance to individuals requesting such based on the biosecurity protocol of the laboratory. Granting of access to retained specimens posting biosecurity risk shall require approval and clearance from BOTH the section head/supervisor and biosafety/biorisk officer.

1.6. The laboratory biosafety/biorisk officer shall record all requests for access to retained specimens and document such event in the biosecurity logbook whether the request is granted or denied.

1.7. When retained specimens are beyond retention period, the laboratory section head/supervisor shall retrieve stored specimens, decontaminate the retrieved specimen/s following decontamination protocol defined by the laboratory, and endorse them to the laboratory aide for disposal by indicating in the disposal of retained specimen logbook.
1.8. The laboratory aide shall dispose off retrieved and decontaminated postanalytic specimens that are passed retention period following specimen disposal guidelines indicated in the waste management portion of this manual.
1.9. The laboratory aide shall record details of disposed postretention specimen using the disposal of retained specimen logbook.

1.10. The laboratory section head/supervisor shall provide monthly report of retention, retrieval, and disposal of postanalytic specimens to the laboratory biosafety/biorisk officer copy furnished CMT and/or laboratory director/pathologist.

1.11. The biosafety/biorisk officer shall review the monthly reports from section head/supervisor and recommend findings to the CMT and laboratory director or pathologist.

1.12. The laboratory director or pathologist shall assess monthly reports submitted by the biosafety/biorisk officer and shall conduct quarterly review of policy implementation together with the LA/CMT and laboratory biosafety/biorisk officer. The laboratory director/pathologist, after proper review of the reports submitted, shall likewise recommend improvement plans and action items for implementation.

2. Biosecurity procedures for retained specimens/samples

2.1. Section head/supervisor in consultation with biosafety/biorisk officer shall designate limited-access area, refrigerator or freezer in the laboratory to store controlled or limited-access retained specimens and shall ensure that these storage areas are properly labeled or identified so activities or personnel not allowed to access are prohibited from entering.

2.2. Granting of individuals or requests to access controlled retained specimen shall require clearance or approval of BOTH section head/supervisor and biosafety/biorisk officer.

2.3. The section head/supervisor shall provide a separate access logbook to monitor who entered the limited-access specimen storage area/s.

2.4. All laboratory staff shall fill out the access logbook when entering limited-access storage area.

2.5. The accessing staff shall indicate in the logbook her/his name, date and time of the accession, the purpose of the access and actual activities done inside the limited-access area.

2.6. The section supervisor shall conduct monthly review, or weekly when appropriate, of the access logbook, specimen retention logbook with that of the actual specimens stored in the limited-access storage area and shall provide report and recommendation to biosafety/biorisk officer.

2.7. When warranted, the biorisk/biosafety officer shall take action (i.e., investigation or mitigation) based on the report or recommendation of the section supervisor and shall notify the CMT and pathologist/laboratory director of any outcome of the action or investigation.

2.8. All biosecurity actions, investigations, and outcomes shall be recorded in a separate biosafety/biosecurity logbook under the custody of biosafety/biorisk officer of the laboratory and shall be included in the monthly review and report of biosafety/biorisk officer to the LA/CMT and laboratory director/pathologist.

3. Retention, retrieval, and disposal of laboratory records

3.1. Paper-based records that are generated in an area with exposure to SARS-CoV-2, such as worksheets and testing checklist, shall be decontaminated by the laboratory staff using disinfectant machines such as portable virus disinfectant machine or UV light prior to segregation.

3.2. Electronic hardware and input devices such as keyboard or mouse that are touched by personnel when accessing digitized records that may possibly harbor SARS-CoV-2 shall likewise undergo decontamination using alcohol-based wipes or spray containing 70% alcohol if no manufacturer guidance is indicated or available.

3.3. Laboratory section head/supervisor shall be the custodian of section records and shall therefore secure integrity of records while being stored.

3.4. Laboratory section head/supervisor shall designate records keeping area and shall label the same whether free-access for laboratory staff or limited access. Storage area for limited-access records shall be secured with lock or physical barrier.

3.5. Laboratory section head/supervisor shall compile and index all laboratory records related to COVID-19 testing operations.

3.6. Laboratory section head/supervisor shall segregate records if open-access or limited-access and shall store it in designated storage area that secures integrity and easy retrieval of the records when necessary and when access is permitted.

3.7. Access to electronic records shall be role based and require access password. Electronic records shall be backed-up for safety and stored preferably in separate secured location or encrypted in cloud for easy retrieval when necessary and when access is permitted.

3.8. The laboratory section head/supervisor shall ensure retention of records following table:

Laboratory record	Access category	Retention period
Proficiency testing results and other records related to proficiency testing	Free access	5 years
Quality control records, calibration, remedial action, records of preventive and corrective maintenance	Free access	2 years
Test requisition, CIF, test records	Limited access	2 years
Records of near-miss, incidents and non-conformances, adverse events	Limited access	5 years
Method performance validation, equipment evaluation	Free access	5 years
Test reports	Limited access	15 years

Table 2. Retention period and access category of laboratory records.

3.9. Upon request for retrieval of free-access records, the laboratory section head/supervisor shall be responsible in granting record access clearance to individuals requesting such records. The laboratory section head/supervisor shall have the right to decline access to records following data privacy act and confidentiality issues.

3.10. Upon request for retrieval of limited-access records, the laboratory section head/supervisor in consultation with document/record control custodian or the CMT (in the absence of DCC/RCC) shall be responsible in granting record access clearance to individuals requesting such records using the access category indicated in Table 2. Requests for limited-access records shall require approval from BOTH laboratory section head/supervisor AND document/record control custodian or the CMT in the absence of DCC/RCC.

3.11. All approved or denied access requests to records that are limited access shall be recorded in a logbook provided by the record custodian/section supervisor for reporting and review purposes.

3.12. Upon termination of retention period of particular group or range of record, the laboratory section head/supervisor shall retrieve and prepare records for disposal.

3.13. The laboratory section head/supervisor shall destroy confidential or limited-access records by using shredder prior to endorsement to laboratory aide for disposal.

3.14. The laboratory aide shall dispose endorsed paper-based records using existing disposal system for records as used in disposal of non-hazardous healthcare wastes or material recovery and recycling procedure of the facility.

3.15. The laboratory aide shall record disposal mode, date, and type of the disposed records in physical records disposal logbook.

3.16. Electronic records that are passed the retention period shall likewise be deleted by the laboratory section head/supervisor. Details of the deleted electronic records, such as type of record and date range, shall be recorded by the laboratory section head/supervisor in the digital record disposal logbook.

3.17. The laboratory section head/supervisor shall provide monthly report of retention, retrieval, and disposal of laboratory records to the DCC/RCC, CMT, and laboratory director/pathologist.

3.18. The laboratory director or pathologist shall assess monthly reports submitted and shall conduct quarterly review of policy implementation together with DCC/RCC and LA/CMT. The laboratory director or pathologist, after proper review of the reports submitted, shall likewise recommend improvement plans and action items for implementation.

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Section 2: Contingency and Biosecurity Emergency Preparedness

Introduction

The purpose of this guidelines is to establish proper procedure on how to respond when there is equipment breakdown, unavailability of power supply, and when there is an emergency/accident that will be encountered during the tour of duty.

Objectives

1. To be well prepared and avoid panic in cases of equipment breakdown, emergencies, and accidents.
2. To establish proper procedure for reporting, investigation, corrective actions, and tracking associated with equipment breakdown, emergencies, or accidents inside the COVID-19 laboratory.

Definition of terms

Automated external defibrillation: sophisticated computerized devices that can analyze heart rhythm and general high voltage electric shocks.

Basic life support (BLS): an emergency procedure that consists of recognizing an arrest and initiating proper cardiopulmonary resuscitation techniques to maintain life without the use of drugs until the victim either recovers or is transported to a medical facility where advance life support measures are available.

Compression–airway–breathing (CAB): compression: create blood flow by increasing intrathoracic pressure and directly compress the heart; generate blood flow and oxygen delivery to the myocardium and brain; airway: this must be done to ensure an open passage for spontaneous breathing or mouth to mouth during CPR; breathing: the process of respiration, during which air is inhaled into lungs through the mouth or nose due to muscle contraction and then exhaled due to muscle relaxation.

Cardiac arrest (CA): the condition in which circulation ceases and vital organs are deprived of oxygen.

Cardiopulmonary resuscitation (CPR): a series of assessments and interventions using techniques and maneuvers made to bring victims of cardiac and respiratory arrest back to life.

Contingency plan: a plan that is made for dealing with an emergency or with something that might possibly happen and cause problem in the future.

Emergency preparedness: the steps taken to be ready to respond to and survive during emergency.

Scope

This protocol is applicable to all laboratory personnel that will engage on the examination of COVID 19 specimens. It entails step-by-step procedure on how to deal with different emergencies or accidents as well as equipment breakdown inside the laboratory.

Responsibility

Head of the laboratory: ensures staff attend training on emergency preparedness and basic life support.

Biosafety officer: receives and conducts investigation of accident reports. Presents the results of the accident investigation to the head of the laboratory. Tracks corrective actions to completion and report progress.

Medical technologist/laboratory personnel: reports all accidents and dangerous conditions to supervisors or biosafety officer.

Procedures and guidelines

1. Contingency plan

In the event of equipment breakdown, the following instructions must be followed:

a. The PCR COVID-19 testing laboratory shall have a notarized Memorandum of Agreement with another Department of Health (DOH) licensed PCR COVID-19 testing laboratory.
b. A written report shall be given to the DOH-HFSRB or CHD-RLED informing the temporary suspension of the PCR COVID-19 testing.
c. The laboratory must be ready for any system breakdown or shortage of reagent supplies since the situation covers the entire globe. The collected specimen shall be referred immediately to the nearest PCR COVID-19 testing laboratory with strict adherence guidelines on specimen handling and transport. Received specimen from referring centers both government and private must be notified.
d. The PCR COVID-19 testing laboratory shall not accept specimens from any patient or referral health facility.
e. The PCR COVID-19 testing laboratory shall inform their patients and refer to another DOH-licensed rapid PCR COVID-19 testing laboratory.
f. Once equipment breakdown was resolved, the operation of the COVID-19 laboratory shall be restored maximally 3 months.

2. Emergency/incident response plan

In order to reduce the likelihood exposure to/release of a biological agent, a contingency plan must be provided in each COVID-19 laboratory for specific standard

operating procedures to be followed in possible emergency cases that maybe apply to work. All laboratory personnel must be trained on these procedures and have a routine drill scenario (spill drill) to maintain competency of the personnel.

The first aid kits with complete medical supplies must be available and accessible to the personnel in the COVID-19 laboratory. The expiration dates must be regularly checked to ensure its use and availability in the central supply department.

All incidents must be reported to the biosafety officer. Accidents and incidents must be documented and investigated in a timely manner and taken into consideration when updating laboratory procedures and emergency response plans.

Laboratory staff should have immediate access to spill kits, including those containing disinfectant. It will depend on the size, location, concentration, or volume of the spill; different protocols maybe applied. Written procedures for cleaning and decontaminating spills was developed and followed by adequate training of personnel.

Emergency procedures for COVID-19 laboratory

1. Biological safety cabinet (BSC) failure

   Biosafety cabinet requires a constant power supply to function correctly as a primary containment device (uninterrupted power supply (UPS) with power conditioner if possible). A sudden loss of power to the biosafety cabinet requires laboratory personnel to take quick action to minimize the likelihood scape of biological materials from the BSC. Remember not to panic. If cabinet power is lost, stop the work immediately. The following steps must be followed:

   1. Secure first any infectious material by capping VTM/UTM/or any viral transport tubes, covering cryo tubes and closing any opening bottles.
   2. Disinfect and remove gloves inside the BSC. Remove hands from the cabinet.
   3. Close the window sash and leave the area until the power goes back on.
   4. Notify others in the area of potential exposure to biological materials and place signage on cabinet indicating it is OUT OF ORDER.
   5. Once power returns, reopen the window sash and allow 5 minutes for the air to purge and stabilize before recommencing work.
   6. If power is not re-establish, wait for 30 minutes for possible aerosols to settle. Don additional PPE. Open the window sash and decontaminate the surface of the BSC and all work items prior to their removal allowing for sufficient contact time.

7. Do not attempt to use the BSC until the issue has been resolved.
8. A highly useful tool to reduce the risk of power loss of BSC is a UPS. A UPS provides equipment with limited amount of continuous energy when there is a periodic instability in the building's energy supply. A UPS can provide staff working at the BSC with enough time to properly secured infectious materials. Generally, a UPS is capable of providing a BSC with 10 minutes of uninterrupted manner that provides the worker enough time to shut down the BSC before power is lost. Consult the manufacturer's specification for the cabinet electrical requirements to select the most appropriate UPS.
9. Disinfect cabinet surfaces and other items and appropriately segregate waste materials in a quick but safe manner.

2. Accidents and medical emergency

There are other unexpected instances or events such as medical emergency, building alarms, fire, or immediate threat where laboratory personnel may need to suddenly stop the work. If safe to do so, secure infectious material within the BSC, remove PPE as standard protocol, and exit the laboratory. However, the most important thing to remember in any emergency situation is that personal safety comes first. After any incident, it is important to report the situation to the biosafety officer as soon as possible. The evaluation report and investigation of the biosafety officer is submitted to the head of the laboratory for necessary recommendations to reduce the likelihood in the future.

2.1. Puncture wounds, cuts, abrasions, and needle prick

The first aid kit treatment of the type of injury must be sustained in the COVID-19 laboratory. Such injuries are caused not only on biological and chemical substances; they often consist of cuts from broken glass tube or apparatus, burns from the alcohol lamp, and abrasions caused by contact with packaging cases. The treatment suggested in the table must be considered as first aid:

Type of injury	First aid measures/interventions
1. Needle prick	Irrigate thoroughly with water and normal saline. Antiseptic like betadine can be apply to the injured area. Obtain medical attention.

	• Then the doctor will assess for the proper dressing and 0.5 tetanus toxoid should be given. This management can also be done for abrasions and shallow cuts that is not bleeding. No need for oral antibiotics. Topical ointment (muciprucin ointment can be applied) is important and cover it with sterile dressing to avoid infections. • Report this incident to infection control committee.
2. Deep clean cuts	Immediately apply pressure to arrest bleeding and send to emergency room. Irrigation with normal saline, sometimes with incorporation of betadine. Then local anesthesia and suturing (staples are normally used due to less SSI). 0.5 Tetanus toxoid will be given plus 3000 units of antitetanus depending on the severity of the wound. Oral antibiotics should be given.
3. Dirty wound	The doctor will irrigate, and debridement of devitalized tissues will be performed. Then they could do secondary healing intentions wherein the wound will not close to prevent infection. They let it granulate. Report this incident to infection control committee.
4. Burns	Different management for different types and degree of burns such as chemical burns, flame burns, scald burns, and so on. Medical intervention as follows: • Irrigate with normal saline. The doctor will give toxoids and apply 1% silver sulfadiazine. Then wet to dry dressing. Removing blisters by pricking will be performed by the doctor to prevent further infections. Report this incident to infection control committee.
5. Eye injuries	• Irrigate of at least 1 L of normal saline then refer for ophthalmologist for further evaluation and management. Report this incident to infection control committee.

The laboratory first aid kit box should contain the following: 1 eye irrigation bottle (0.5 L capacity), 1 tablespoon, 1 bottle of common salt, 1 bottle of magnesium sulphate (Epsom salts), 1 bottle of milk magnesia (dose: two tablespoons), and 1 bottle of vinegar of 1% acetic acid.

2.2. Potentially infectious aerosol release (outside a BSC)

All laboratory personnel must immediately vacate the affected area and any exposed persons should be

referred for medical treatment. The head of the laboratory and biosafety officer must be informed at once. Restriction to enter the room for 1 hour to allow aerosols to be carried away and heaviest particle to settle. Signs are posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed and supervised by biosafety officer. Appropriate personal protective equipment (PPE) and respiratory protection must be worn.

2.3. Broken containers and spilled infectious substances

When responding with broken containers contaminated with infectious substances and spilled infectious materials, put "Do not Panic" sign. Alert people in the immediate area of the spill. Remove contaminated clothing and identify any agents for specific issue.

Spill clean up procedure is as follows:

1. Allow the spill to settle the aerosol for at least an hour.
2. Wear PPE.
3. Use tongs/forceps to pick up sharp objects (broken glass and so on) that may puncture gloves.
4. Starting at the edges and working toward the center of the spill, cover the spill with a paper towel or other absorbent material.
5. Carefully pour disinfectant over the absorbed material and spill starting around the edges and working toward the center. Saturate the area with disinfectant.
6. Allow sufficient contact time. Non-viscous spills: 15–20 minutes; viscous spills: 30 minutes.
7. Wipe up spill with paper towels, working from edge to center.
8. Clean the spill area with fresh paper towels soaked in disinfectant. Wipe down all reachable cabinet surfaces with disinfectant.
9. If dustpans are used to clear away the broken material, it should be autoclaved or placed an effective disinfectant. Paper towels or other absorbent materials used for cleaning up must be place in biohazard bags or heat-resistant containers with lids before autoclaving and further clean up.

10. If laboratory forms or other printed or written matter are contaminated, the information must be copied onto another form and the original is discarded into contaminated waste container.

2.4. Fainted staff/cardiac arrest (CA)

Once you recognized that an emergency has occurred and decided to act, you must make sure that an emergency is safe for you as well as the victim. If working in a BSC, close the sash window, remove gloves, and use a new pair of gloves to attend to the victim. Check for responsiveness by tapping the victim's shoulder and ask loudly. "Are you OK?" If unresponsive, shout for nearby help or call a BLS healthcare provider. Recognition of CA such as unresponsive, no breathing or gasping, and no pulse. Check for breathing and pulse simultaneously for no more than 10 seconds. Check for the responsiveness (look, listen, and feel), perform compression (CAB), open the airway, and perform rescue breathing. Do CPR if the staff is trained BLS. If an emergency respondent or BLS healthcare provider arrives, he or she will then assess and continue CPR or automated external defibrillation. Then proper referral to advanced medical authority for further evaluation and management.

2.5. Fire and natural disaster

The laboratory personnel must be properly oriented in the event of sudden fire due to electric malfunction and other circumstances that may be involved in the development of emergency preparedness. They should be aware of which rooms contain potentially infectious materials and should be secured at once. Emergency exit signages must be clear and free from blockage.

After a natural disaster, local emergency services should be warned of the potential hazards within and/or near laboratory buildings. An outside responder should always be accompanied by a trained laboratory worker or a biosafety officer. Infectious materials should be collected in leakproof

boxes or biohazard bags. The final disposal should be determined by biosafety officer.

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Notes

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