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Strengthening Laboratory Capacity to Improve the Availability and Accuracy of COVID-19 Diagnosis in Peru: Recommendations and Training Plan to Improve Specimen Collection, Handling, and Transport

Local Health System Sustainability Project

February 2023

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The Local Health System Sustainability Project (LHSS) under the USAID Integrated Health Systems IDIQ helps low- and middle-income countries transition to sustainable, self-financed health systems as a means to support access to universal health coverage. The project works with partner countries and local stakeholders to reduce financial barriers to care and treatment, ensure equitable access to essential health services for all people, and improve the quality of health services. Led by Abt Associates, the five-year project will strengthen local capacity to sustain strong health system performance, supporting countries on their journey to self-reliance and prosperity.

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Executive Summary

Based on the short-term recommendations, a specific training proposal is presented for specimen collection, specimen transport, and specimen analysis. For each of these, the proposal includes: a general description of the training process; the purpose of the training; the objectives of the training; the main contents of the training; and a proposal for training evaluation (pre- and post-training measurements).

For specimen collection, short-term recommendations include the formation of a committee to develop standard guidelines for which INS will provide continuous consultation and review as these guidelines are implemented. As a mid- to long-term strategy, Seoul Clinical Laboratories (SCL)also suggests using a unified document format, establishing a quick-response (QR) code system, and improving infection control during specimen collection.

With respect to specimen transport, Peru needs a transportation system that provides primary cold chain service and enables real-time temperature and location tracking of specimens in. A short- or mid-term recommendation to address this is to develop and implement a global positioning system-based real-time temperature and location tracking cold chain transport system. Bar code systems and electronic document management programs can improve efficiency. In addition, INS needs to develop a standard guideline for implementing the recommended specimen transport system through consultation with national and international experts.

As a short-term recommendation for specimen analysis, SCL suggests improving the reagent storage and management system of laboratories, as well as the temperature management of refrigerators for storing reagents and specimens. They also recommended that a system for checking and confirming test results be established. Long-term recommendations include increasing testing capacity by expanding real-time thermal cyclers in the network laboratories and expanding Biosafety Level 2 laboratory facilities in each region to increase capacity for specimen analysis.

Finally, SCL suggests directions for strengthening diagnostic test capacity and establishing a diagnostic test system based on infectious disease prevention strategies, as part of a proposal for establishing an improved diagnostic test strategy overall. In addition, in order to establish a strong national diagnostic test system in Peru, INS should establish an integrated diagnostic test management strategy in terms of governance and workforce, as well as a test report system for integrated quality control and operation monitoring.

Introduction

The COVID-19 pandemic has had a severe impact on Peru despite the early implementation of a national lockdown and other restrictions. Peru is one of the countries with the highest excess mortality per million people because of the epidemic. Strengthening the capability of the health system to detect cases in a timely manner and to reduce disease transmission is essential for preparing and responding to health emergencies. The purpose of this paper is to propose recommendations for the Instituto Nacional de Salud (INS, National Institute of Health) to increase laboratory capacity in preparation for future epidemics and pandemics. Proper specimen collection and transport play an important role in the accurate and timely identification of a newly emerging infectious disease. This report contains recommendations for improving the SARS-CoV-2 specimen collection and transport system in Peru, based on the findings of a field assessment conducted by a team from Seoul Clinical Laboratories in some of the Peruvian laboratories between October 15 and 21, 2022. The document highlights short-term, mid-term, and long-term recommendations for specimen collection, storage, transportation, and laboratory analysis, and the establishment of a diagnostic test strategy for the SARS-CoV-2 virus. Short-term recommendations can be adopted in 6 to 12 months, while medium-term and long-term recommendations can be achieved in two and five years, respectively.

Recommendations for Pre-Analytic Phase

Operation of a transportation system that provides primary cold chain service and enables real-time tracking of transported specimens

In Peru, the establishment of a reliable specimen transport system for SARS-CoV-2 is urgently needed. Sampling sites are far apart from the testing facilities. The transport system should be equipped with a warning alarm that alerts both the transporter and the person monitoring the transport remotely if the temperature in the vehicle deviates from the allowable range. Specimens should be tracked from the time of departure from the specimen collection site to the time of their arrival at the INS or local reference laboratory. Transport vehicles should be equipped with a thermometer, a tracking device that reports on temperature change as measured by the thermometer, and a global positioning system-based tracking system that reports on transport time and exact location. An INS manager should be able to monitor these, along with the estimated time of arrival, through their own personal computer.

If it is not viable to execute this system throughout Peru, INS can launch a small-scale pilot project in the Lima metro area and expand it to other regions in the future.

Expansion of storage facilities and establishment of a monitoring system for specimen stability

Peru has a system for transporting specimens from collection sites to testing facilities once a day. However, when the number of test participants suddenly increases due to the emergence of a new infectious disease, the collection site has to store the specimens for longer. To do this they need to expand their refrigeration facilities and specimen storage space to keep the specimens stable. To meet the criteria for storing specimens the overall system has to be able to rapidly report on whether specimens are stored at an appropriate temperature, and to know why if they are not. A manager needs to routinely monitor the specimen storage status and a computer needs to record it. If there is a problem with the storage system, the INS should be notified immediately, and the institution storing the specimen should provide training for its employees so that they can make sure it does not happen again.

Table 1: The recommended temperatures for transportation and storage of various types of specimens for SARS-CoV-2 testing [World Health Organization 2020]

Specimen type	Collection material	Recommended temperature during transportation	Recommended temperature during storage in the laboratory
Nasopharyngeal and oropharyngeal swabs	Dacron or polyester flocked swabs*	4°C	≤5 days: 4°C >5 days: -70°C

Specimen type	Collection material	Recommended temperature during transportation	Recommended temperature during storage in the laboratory
Bronchoalveolar lavage	Sterile container*	4°C	≤48 hours: 4°C >48 hours: -70°C
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
Tissue from biopsy or autopsy including from lung	Sterile container*	4°C	≤24 hours: 4°C >24 hours: -70°C
Serum (two types of specimen: acute and convalescent)	Serum separator tubes (adults: collect 3–5 mL whole blood)	4°C	≤5 days: 4°C >5 days: –70°C

* *For transport of specimens for viral detection, use viral transport medium containing antifungal and antibiotic supplements. Avoid repeated freezing and thawing of specimens.*

Development and implementation of INS-recommended standard packaging system for specimen transfer

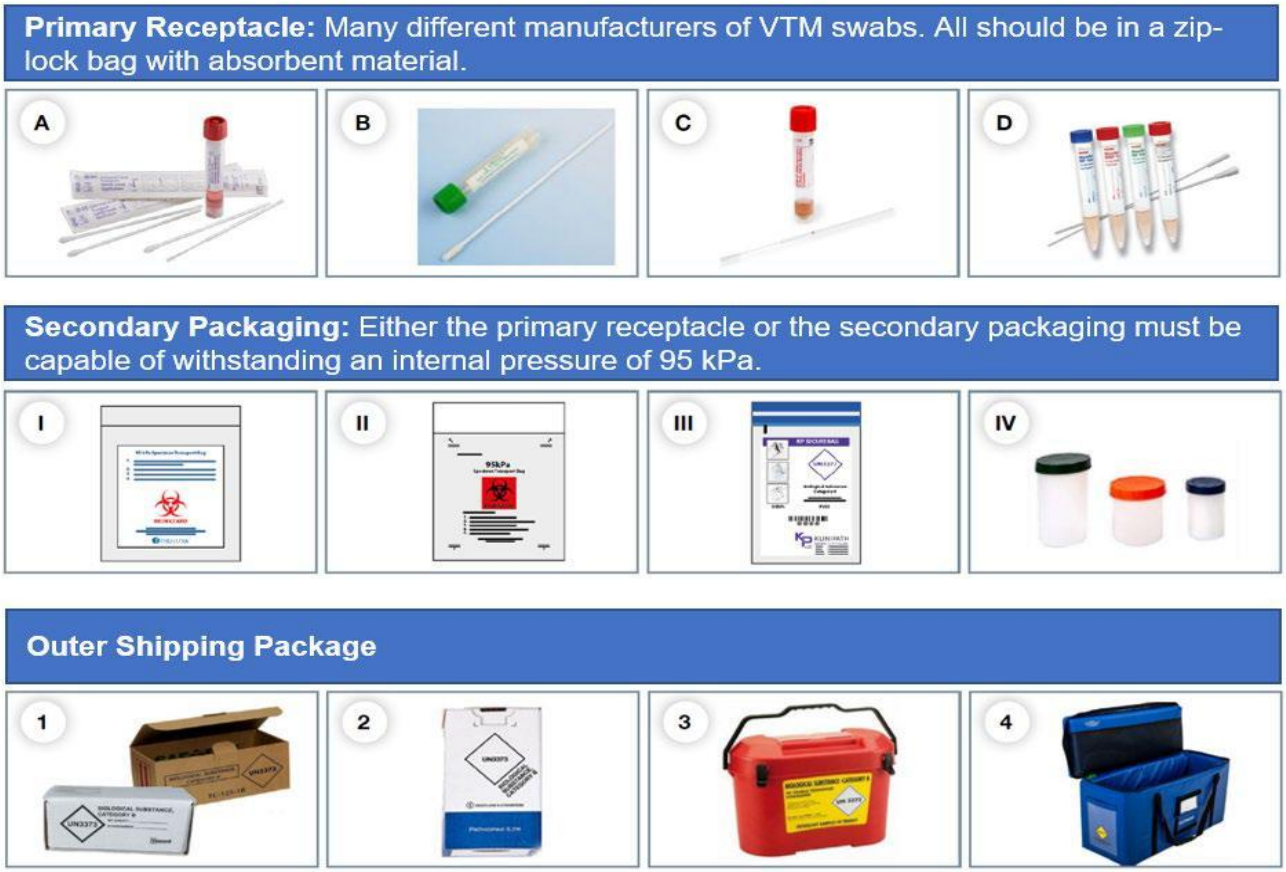
The packaging system for specimen transfer must be standardized under a separate guideline produced by the INS through the formation of the relevant committee, and the guideline must meet the requirements recommended by the United Nations (UN) for a triple-layered packaging system for infectious substances. Diagnostic specimens for COVID-19 are classified as category B (UN3373) following packaging instruction P650.

The institution's staff receiving specimens inspect the triple-layered packaged specimens using barcodes attached to each specimen in accordance with the UN recommendations. Through the primary separation, it is important to implement a computerized operating system in the specimen-receiving institution to assess the existence or lack of abnormality in the specimen through the barcode attached.

Table 2: Requirements for triple- layered packaging system for the transportation of SARS-CoV-2 specimens [World Health Organization 2021]

Layers	Requirements
Primary receptacle	A labeled, watertight, leak-proof primary receptacle containing the specimen, wrapped in sufficient absorbent material to absorb all fluid in case of breakage.
Secondary packaging	A second, durable, watertight, leak-proof receptacle to enclose and protect the primary receptacle(s).
Outer shipping package	The secondary receptacle must be placed in an outer shipping package, which protects it and its contents from outside influences such as physical damage and water while in transit.

Figure 1: Examples of receptacles, canisters, and containers that can be used for triple-layered packaging systems [World Health Organization 2021]



Note: Rigid tertiary packaging applies to specimens being transported by road (examples 3 and 4). The specimens may be transferred out of a rigid box at the reception area.

Implementation of an electronic document management system and barcode system

In the current system, specimens are inspected using three separate handwritten documents, and the person responsible for the test at the receiving institution must manually enter the paper-based information into an electronic system to generate a barcode for the specimens. This manual approach will take too long if the number of specimens increases rapidly, hence the need for a computerized alternative.

Use of a unified document format and establishment of a QR code system

It is important to use a uniform document format to collect the information of a patient (or a suspected patient) at the time of SARS-CoV-2 specimen collection. Furthermore, a barcode- or QR code-based electronic health questionnaire system can be established so that the patients can submit their information electronically. Introducing an e-questionnaire system using QR codes can reduce the pressure on frontline health care workers caused by handwritten responses to health questionnaires and minimize problems such as patients writing erroneous information. Such a system could also reduce the risk of infection caused by the high concentration of people waiting to be tested in the COVID- 19 screening site. However, the regular hard copy questionnaire should be available at each screening site for those who do not own or use smartphones.

Example case: Use of digitalized system in South Korea

In South Korea, after a patient or potential patient completes the e -questionnaire the system generates the QR code for that person, and the information on that individual is transferred and delivered electronically throughout the entire specimen collection and transport process [Ministry of Health and Welfare of the Republic of Korea 2020] This eliminates the need for a separate printed document for use in tracking the specimen.

In USA, diagnostic results from COVID-19 testing are crucial for decision-making at all levels, from school boards and doctors to employers and local government officials. However, diagnostic devices and COVID-19 test results have not yet been paired with digital software tools for an easy transmission of information. To focus on this national challenge, the U.S. Department of Health and Human Services (HHS), in partnership with the U.S. Census Bureau, facilitated a COVID-19 TOPx tech sprint [1] to help develop innovative digital solutions for data capture, harmonization and reporting of COVID-19 tests [United States of America Centers for Disease Control and Prevention 2019].

In Australia, although clinical care is driven by the EMR, initial triage and screening requires patient-staff interaction. They decided to digitalize this process to deliver online screening and a registration tool using an established REDCap™ electronic data capture system [5]. This was done for three key reasons – to allow a complete and standardized minimum dataset to be collected from all patients, to reduce the face-to-face contact time between symptomatic patients and clerical staff unfamiliar with PPE precautions, and to maximize overall clinic throughput and clinician workflow efficiency [Sung et al 2020].

It is important to review how to establish and operate an integrated server system to implement this.

[1] https://www.hhs.gov/blog/2021/04/01/covid-19-topx-tech-sprint-final-demos-showcasing-digital-diagnostic-tools.html

Using a standard online format for documentation would help streamline the process for producing various documents and entering data on each specimen into the system, while also making it easier for staff. In addition, an automated system would enable the laboratory to promptly notify medical institutions involved in specimen collection if the test result has problems such as an indeterminate result. This in turn would help the medical institution uncover any problems that might be occurring during specimen collection [Hong et al 2019].

Improvement of infection control in the specimen collection process

The core of infection control in the SARS-CoV-2 specimen collection process is preventing SARS-CoV-2 infection among personnel handling the specimens and among other patients. Current Infection control problems in the specimen collection process in Peru can be divided into two major categories.

First, some health care facilities have established a specimen collection site very close to the area where other, non-COVID-19 patients receive medical treatment. Space limitations can also create overlap between the movement routes of COVID-19 patients and the general patient population.

Second, specimen collection facilities at some health care institutions were not well ventilated for infection control and had insufficient sanitation. Ventilation and sanitation are critical in preventing infection among high-risk patients. The environment of specimen collection sites in Peru shows a risk of cross-infection among people to be tested, the person in charge of specimen collection, and other hospital patients. In order to prevent the spread of such infections, local labs must implement ventilation system and surface disinfection measures.

Example case: Ventilation and disinfection system in South Korea

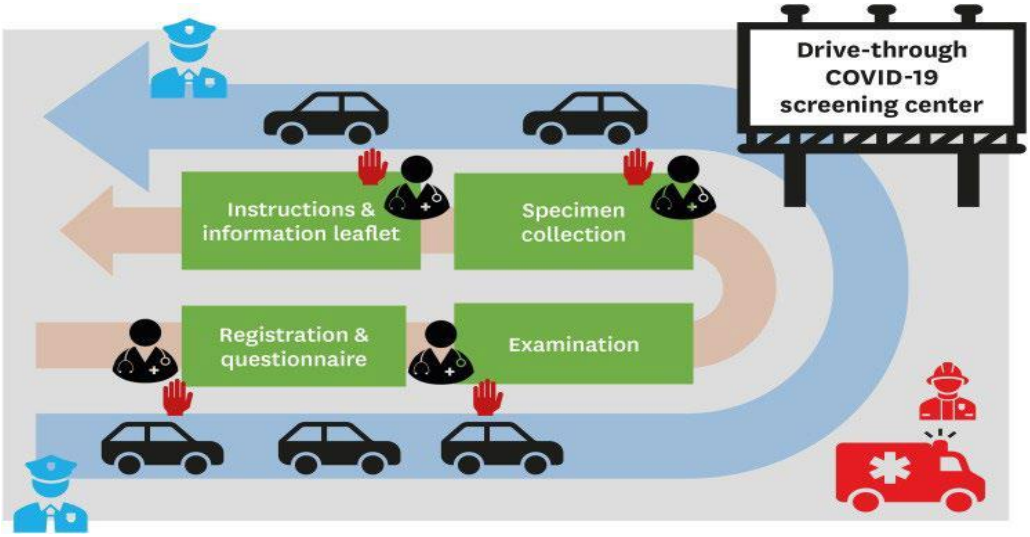
South Korea has implemented an automated ventilation system, and separate ventilation is performed around six times per hour. High-efficiency particulate air filters are also installed in the screening booth to prevent cross-infection. UV-C lamps and alcohol are used to disinfect booth surfaces contaminated with SARS- CoV-2. [Ministry of Health and Welfare of the Republic of Korea 2020]; [World health Organization 2021]

Based on South Korea's experience, establishing walk-through and drive-through screening centers in resource-constrained situations can be a safe and efficient approach to reducing community transmission of the SARS-CoV-2 virus during specimen collection [Ministry of Health and Welfare of the Republic of Korea 2020].

A drive-through screening center may be effective in big cities such as Lima. It should be operated away from highly populated areas. A large parking lot is a good choice. The entrance and exit should be strictly guarded, and movement should be controlled at every stage. People will not need to leave their cars. All communication can be by mobile phone except during the specimen collection. Either an open tent or a temporary building can hold the work booths. Health care workers can collect specimens from six to eight people per hour [Ministry of Health and Welfare of the Republic of Korea 2020]; [Sung et al 2020].

Compared to drive-through screening centers, walk-through screening centers can be more effective at primary health care facilities or their branches in local communities. Walk-through screening centers can also effectively reduce the consumption of limited personal protective equipment (PPE) resources as well as the discomfort and risk of infection associated with frequent PPE replacement. People waiting for the test outside and inside the screening locations are advised to stay at least two meters away from others to minimize the possibility of droplet transmission. All patients should wear masks and move under the supervision of health care staff from the entrance to the exit. See Figure 2.

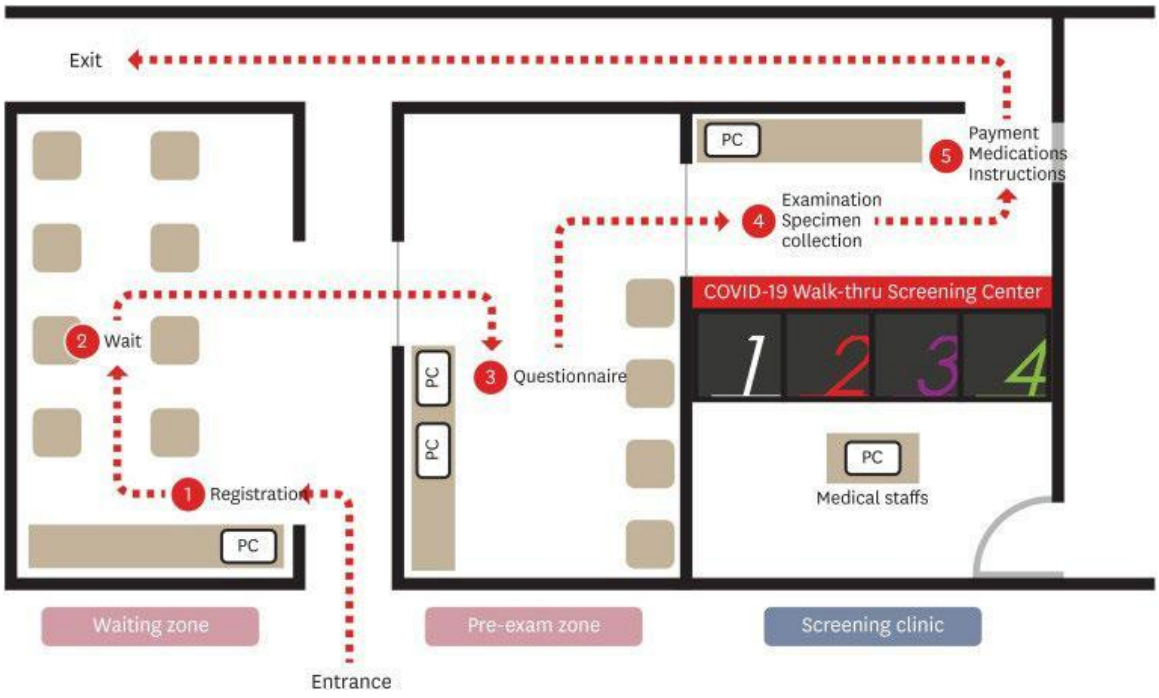
Figure 2: Flow of drive-through screen center implemented in South Korea during COVID-19 pandemic



Local environmental considerations must be considered when planning drive-through and walk- through test-collection sites. Tent structures without wind barriers, adequate heating systems, and natural ventilation systems must be considered while installing both types of collection sites.

In addition, medical personnel who perform specimen collection for SARS-CoV-2 must change clothes in a dedicated space not used by other medical staff. The local labs must implement detailed evaluation of the specimen collection site, as it follows the principle that the specimen collection site must not be the cause of the spread of infection [Korea Centers for Disease Control and Prevention 2020].

Figure 3: Flowchart of a walk-through screening center implemented in South Korea during COVID-19 pandemic [Sung et al 2020]



Lastly, a system for regular consultation with infection control experts, should be established, including strategies for effective and efficient specimen collection methods and prevention of infection within the health care institution and screening centers, considering local circumstances.

Recommendations for Analytic and Post-analytic Phase

Storage and management of reagents for specimen analysis

The people in charge of specimen analysis need to become more efficient and attentive in managing reagents. Identical reagents must be stored in the same refrigerator. All refrigerators must have a notice attached stating that food or flammable items should not be stored.

Temperature management of refrigerators for storing reagents and specimens

In using a refrigerator for storage of specimens or reagents, it is necessary to accurately control the temperature. A temperature management checklist and records of tests including time should be posted on the refrigerator. The manufacturer's instruction manual for the refrigerator should be kept nearby and consulted as needed. The personnel of the labs should report any aberration in temperature to the chief of the laboratory.

Also posted on the refrigerator should be a record of reagent use.

Establishment of a system for checking and confirming analysis results

In order to prevent human errors that may occur during the analysis process, a computer system is required to automatically transmit the analysis results confirmed through the CFX manager[2] using an interface. If this is not possible, exporting/importing data in the form of an electronic document such as an Excel file can be considered. The results must be transferred as a unified system to the Laboratory Information System (LIS) established by the INS or regional reference laboratory, and a system to compare the transferred data with the raw data at the stage of result analysis must be established. Furthermore, the criteria for retesting must be documented and printed so that the person in charge can quickly check and decide.

[2] Bio-Rad CFX Manager is a program designed for an intuitive experiment setup and data analysis with the following Bio-Rad real-time PCR detection system

Recommendations for Establishing a Diagnostic Test Strategy

Directions for strengthening diagnostic test capacity and establishing a diagnostic test system based on infectious disease prevention strategies.

The establishment of a diagnostic test system and strengthening of testing capacity is crucial to build quarantine strategies for national-level response for infectious disease. Diagnostic testing capacity is an important factor in formulating national quarantine policies. It will require expansion of the workforce and development of a system for management and evaluation for mid- to long-term development.

Recommendations for Establishing National Diagnostic Test System

Formation of a committee to develop standard guidelines and consultation.

It is necessary that INS form a committee to regulate matters related to specimen collection and management. The committee should include leaders and laboratory personnel from relevant departments within the INS, as well as representatives from associated societies and organizations and experts from various relevant fields. "Associated societies and organizations" may include academic societies or organizations related to laboratory medicine, pathology, clinical microbiology, and epidemiology. The committee should include national and international experts from such areas. It should develop a standard guideline and serve as a national consultative body for specimen collection and management.

The guideline should incorporate scientific evidence, new government policies, and other relevant information based on expert opinion and ideas. Developing a guideline should be a continuous process that includes regular review by relevant committees to reflect new policies and scientific evidence by adding required provisions and removing irrelevant ones. The guideline should be implemented uniformly in all laboratories of Peru, and laboratory personnel should be trained on the guideline on a regular basis. It is recommended that INS and the Escuela Nacional de Salud Pública (ENSAP, National School of Public Health) establish a system, such as a web-based education platform or videos, that can provide continuing education about the guideline for laboratory personnel.

Lastly, post-training monitoring should be carried out to provide continuous feedback on whether each laboratory is correctly following the guidelines. Based on the monitoring, INS should establish a system for management and supervision that can address and rectify errors during the specimen collection and transport process.

Governance and Workforce: Direction of integrated diagnostic test management system based on INS

Peru is implementing a plan, centered on the INS, to develop an integrated diagnostic testing management system, but is encountering problems related to budget and human resources. INS has already established its own management system, NetLab, but needs to strengthen the monitoring and diagnostic testing system for new infectious diseases.

Recommendations regarding the diagnostic test management system are as follows:

- Expand human resources and budget for use by INS diagnosing emerging infectious diseases.
- The INS needs an initial diagnostic methodology for infectious diseases of unknown cause, and n eeds to train lab technicians on this through NetLab. Emergency Use Authorization of reagents s hould also be introduced and implemented.
- Through these steps, INS can continue to expand the scope of network construction to conduct monitoring and operation of training and quality control through a well-trained laboratory in the early implementation stage. The INS will be responsible for formal approval for reagents manage ment and for establishing a system for training and enhancing overall test capacity in Peru.

System: Establishment of a test report system for integrated quality control and monitoring of operations

For the establishment and operation of an integrated network, the INS should establish a reporting system for all laboratories on the network (to be expanded to private laboratories in the future) and establish a system that can evaluate daily test results. In particular, it is necessary to establish a system that reports laboratory capabilities on the network at least once a month to evaluate the current and demanded test capability in the region. This data will provide a basis for continuously securing human resources or equipment. The INS needs to carry out quality control for all laboratories on the network.

Strengthening testing capacity through expansion of real-time thermal cyclers

South Korea has gradually strengthened its RT-PCR testing capacity to respond to SARS-CoV- 2 in proportion to its population (approximately 51 million, as of 2021). Korea's RT-PCT test capability was 25,000 cases per day within the first month of SARS-CoV- 2 onset, 50,000 cases per day within three months thereafter, 100,000 cases per day after nine months, and 200,000 cases per day after 12 months.

Despite differences in each country's COVID-19 outbreak situation and response strategy, the Korean experience suggests that within 12 months of an infectious disease outbreak, approximately 0.4 percent of the total population should be tested per day. If this standard is applied to Peru, it is estimated that at least 10,000 cases per day should be tested within one month of the outbreak, 30,000 cases per day within three months, and at least 100,000 cases per day within 12 months. If the early response to an infectious disease is delayed, it is obvious that more testing capacity will be required over time.

During the Q&A session in Peru, it was mentioned that the national capacity for RT- PCR testing is up to 50,000 cases per day., So, at least 150 to 200 real-time thermal cyclers are required. Furthermore, given the regional distribution, having more than 250 units is ideal.

Therefore, INS needs to calculate the number of real-time thermal cyclers required according to regional distribution based on its own accurate data and strengthen its capacity for initial diagnosis in the event of an emerging infectious disease such as COVID-19. However, it is understood that the real-time thermal cyclers being used in Peru are rented from an overseas manufacturing company on the condition of purchasing reagents and renting a real-time thermal cycler under certain conditions is not a sustainable strategy.

The previously mentioned capacity for 50,000 RT-PCR tests per day is difficult to view as Peru's own testing capabilities. When renting out real-time thermal cyclers as a condition of purchasing reagents, the reagent manufacturer can seek the return of the rented device if the purchase of the reagent is stopped for some reason (e.g., a new infectious disease appears for which the reagent cannot be used). Therefore,

it is necessary to secure an adequate budget to purchase thermal cyclers that can be used continuously and without constraints. In addition, the real-time thermal cyclers acquired through this mode of procurement can have the advantage of having the standard specification desired by the INS.

Expanding Biosafety Level 2 laboratory facilities

Laboratories should each have a negative pressure test room in order to maintain testing capacity by preventing infection among laboratory personnel. Molecular detection of SARS-CoV-2 must be performed in a Biosafety Level 2 laboratory with Biosafety Level 3 equivalent infection prevention and control practices. Reference laboratories established in each region of Peru need to upgrade to the minimum standard of Biosafety Level that is required for molecular detection of SARS-CoV-2. In order to create such facilities, a budget for construction and maintenance must be allocated and testing performed. Furthermore, the INS should develop and implement a national guideline for Biosafety Level 2 laboratories' structure and operation, to strengthen laboratory capacity. To do this the INS will need training on the concept of biosafety and its importance, and to invest in establishing Biosafety Level 2 laboratories.

Training proposal for Pre-analytic Phase: Specimen collection

Training overview

The most important part in responding to the constantly emerging situation of COVID-19 is quick and accurate diagnosis and testing. Accordingly, training on specimen collection aims to educate personnel, specially at the primary care, about processes and scientific evidence for use in the diagnosis of COVID-19. The training program is based on effective and safe specimen collection methods specified in the guidelines presented by the World Health Organization, the United States Centers for Disease Control and Prevention (CDC), the Korea Centers for Disease Control and Prevention, and the Korean Society for Laboratory Medicine

Training objectives

The main objective of the training is to prevent exposure to the source of infection by providing education on specimen collection and handling following the outbreak of new infectious diseases such as COVID-19. The prevention of infectious disease should be implemented while maximally securing the safety of medical workers as well as patients during specimen collection.

General aspects of training

The training should include safety-securing methods in the specimen collection process and cover establishment of a standard procedure for this and for using different methods for specimen collection (nasopharyngeal swab, oropharyngeal swab, sputum specimen collection).

Pre- and post-training assessments must be conducted to assess changes in knowledge. Additional training can be conducted if needed in areas where knowledge and behavior have not improved.

Training Contents

Pre- and Post-Training Assessments

The pre-/post-training assessment should include a simple test on use of PPE and on specimen collection. Participants should be encouraged to recognize, and ask for help with, any problems they have in these two areas.

Example case: Questions for Assessment

Q1. COVID-19 diagnostic tests mainly focus on collecting respiratory specimens. When is the best time to collect specimens?

A1. Earliest time after symptom onset.

Q2. Which type of hygiene must be addressed before and after patient contact and when removing PPE?

A2. Hand sanitizing.

Q3. What are the recommended locations for collecting COVID-19 specimens?

A3. A negative pressure room for one person is recommended. If there is no negative pressure room, an independent space with good natural ventilation Is recommended.

Q4. How long should a negative pressure room be left constantly empty after disinfection, based on air circulation?

A4. Approximately 30 minutes based on air circulation of 12 times per hour.

Q5. Please describe the type of PPE the specimen collector wears to prevent infectious disease.

A5. N95 grade or higher-grade mask, gloves, lab coat (full body, long sleeves, opening at the back), goggles or face shield.

General Guidelines for Collection of COVID-19 Specimens

The training should emphasize the importance of early collection of respiratory specimens after symptom onset and importance of having medical personnel conduct the testing. The latter includes emphasizing the need to protect oneself and others from exposure to aerosolized virus during airway aspiration, bronchoscopy, and certain other procedures. Aerosolized transmission appears to have been implicated in the spread of infection from patients to medical personnel in the early stage of COVID-19 in Peru.

Example case: Prevention and Management Guidelines for Aerosol-Generating Procedures from the Korea Centers for Disease Control and Prevention

1. Aerosol-Generating Procedures

   Sputum collection, airway aspiration, bronchoscopypositive pressure ventilation, tracheal intubation, etc.

2. Infection Prevention Principles

   1) Patient treatment in a way that minimizes physical contact between patients and staff performed only for essential personnel
   2) Wearing of PPE: N95, KF94 or equivalent grade or higher-grade respirator, all-inclusive protective clothing, disposable gloves, eye protection (goggles or face shield), disposable lab coats can be worn after apply procedures to virus inactivation
   3) Compliance with hand hygiene before and after contact with patients and after removing PPE

3. Treatment Location

   1) For patient treatment, it is recommended to use a negative pressure room for one person that can supply and exhaust more than 12 total room air changes every hour. If it is difficult to use a negative pressure room, use an independent space with natural ventilation of 160 L/s per patient ((hourly average ventilation rate).

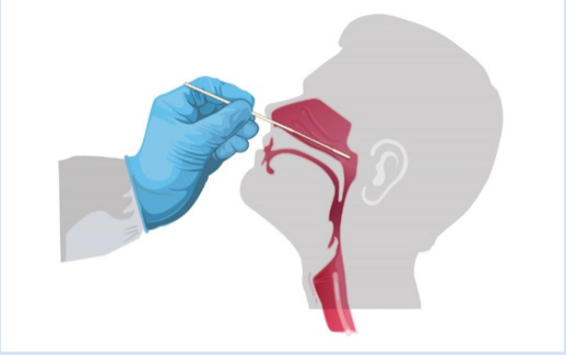
   2) After the isolation room has been used it must be disinfected and left empty about 30 minutes based on 12 air circulation per hour.

   3) Minimize access to the isolation room during treatment.

Training on Specimen Collection Method

Specimen collection methods for COVID-19 testing generally consist of nasopharyngeal swabs, oropharyngeal swabs, and sputum specimen collection. Trainees need to learn how to use these.

Example case: Specimen collection of nasopharyngeal swabs

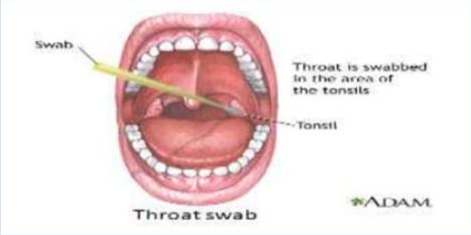


1. Use a thin synthetic material swab (about 20 cm) with a wire shaft for specimen collection.

2. Tilt the patient's head back 70 degrees, and then slowly and gently insert the cotton swab through the nostril parallel to the palate. This must be done at the same distance as from the patient's nostril to the ear, and the swab needs to be pushed deep into the nasopharynx. The insertion depth is usually about half the distance from the nasal cavity to the ear canal orifice.

3. Once the nasopharynx is touched, rotate it gently two or three times or leave it there for 10–15 seconds so that the specimen is sufficiently absorbed into the swab. Gently rub and roll the swab, then leave the swab for a few seconds to absorb secretions. After this, slowly remove the swab with rotation.

Example case: Specimen collection of oropharyngeal swab



1. Insert a cotton swab into the posterior pharynx and tonsil area.
2. Rub the tonsil pillar and the back of the oropharynx with the cotton swab and collect secretions by pressing the tongue and scraping secretions from the posterior wall of the pharynx.
3. In this step, be careful not to touch teeth and gums.

Example case: Sputum specimen collection

1. After washing the inside of the mouth with clean water, take a deep breath and cough deeply. Any secretions need to be spit out directly into the sputum container.
2. Difference between sputum (phlegm after deep coughing) and oral secretions (saliva) needs to be explained to the patient so that saliva is not included.
3. Sputum should not be induced during collection, as there is a risk of transmission of infection due to the production of aerosols.

The training should also cover the different types of containers for specimens—for example, use of a sterilized container with a screw cap for sputum collection.

Training on Requesting Specimen

As a final step in delivering information about the specimen to the testing institution, following the collection of the specimen, information on the specimen will be recorded. Trainees should learn how to transmit information in the system in a standardized format and how to attach barcodes on the specimen collection container, or, if the system does not use barcodes, to label the specimen with the patient's name and patient registration number, and, if the system so specifies, other demographic characteristics such as sex. If in this process the specimen information is erased or the barcode is damaged by ethanol disinfection on the surface of the primary container where the specimen is stored, it must be recorded again to ensure accurate identification. The training should emphasize the importance of recording the necessary information.

Training proposal for Pre-analytic Phase: Transportation

Overview of the training process

This training is based on the transportation and storage methods specified in the guidelines presented by the WHO, the CDC, the Korea Centers for Disease Control and Prevention, and the COVID-19 Response Task Force of the Society of Laboratory Medicine of the Republic of Korea.

Training Objectives

The goal of this training is to teach trainees how to store specimens when a new infectious disease emerges (e.g., COVID-19) and what precautions to take when transferring them to ensure safe transportation to the testing institution and minimize the possibility of problems with the specimen.

General aspects of training

The training includes processes such as removal of contamination and disinfection due to leakage of the specimen that may occur during specimen transfer. The instructor should also solicit information from the trainees about any problems that have experienced with storage and transportation. In addition, it is possible to improve the content of training in a positive way by recognizing problems with the storage method of the specimen currently being used by the subject of training, packaging for transportation, and transportation stage.

In order to increase the effectiveness of the training, the same as the Specimen Collection training, pre- and post- evaluation must be conducted on the use of packaging containers for transportation and storage methods for safe storage of specimens within the training process. It is important that these parts include training in the content. Changes in knowledge according to training can be measured through the results of pre- and post-evaluation. Through this, the performance of training can be identified through the result of measuring changes in knowledge, and additional training is possible for the part where knowledge and behavior have not changed among the training contents.

Training Contents

Assessment of the Training (Pre- and Post-training)

A pre- and post -training assessment should be conducted. The assessment should include knowledge on: maintenance of the temperature required during the storage process for the specimen, the challenges involved in maintaining the right storage temperature during transportation, and the use of packaging containers in transportation.

Example case Questions for Assessment

Q1. What are the storage conditions for specimens when transporting COVID-19 specimens to reverse transcription polymerase chain reaction (RT-PCR)?

A1. For delivery within 48 hours, specimens need to be stored at 2~8℃. If delivery is expected to take more than 72 hours, specimens need to be stored at -20℃ or lower.

Q2. What are the principles for packaging in transporting COVID-19 specimens?

A2. Triple safety packaging in compliance with P650 UN packaging standard.

Q3. Choose all that are correct for the packaging and transport of COVID-19 specimens.

1) Triple safety packaging in accordance with Category B, Infectious Substance Packaging Standards (P650 UN Packaging Standard).
2) Disinfect the outer surface of the primary container with 70 percent ethanol. This container needs to be wrapped with a sufficient amount of absorbent material to prepare for an emergency of infectious material leakage.
3) On the outer surface of the tertiary transport container, attach the UN3373 mark indicating that it is a category B infectious substance. This also shows the sender, recipient, and emergency contact information.
4) If there is no visible leakage in the secondary container when the specimen arrives, the container can be reused after an appropriate disinfection process with 70 percent ethanol or with sodium hypochlorite (chlorine 1.0 percent).

Standards of Specimen Storage

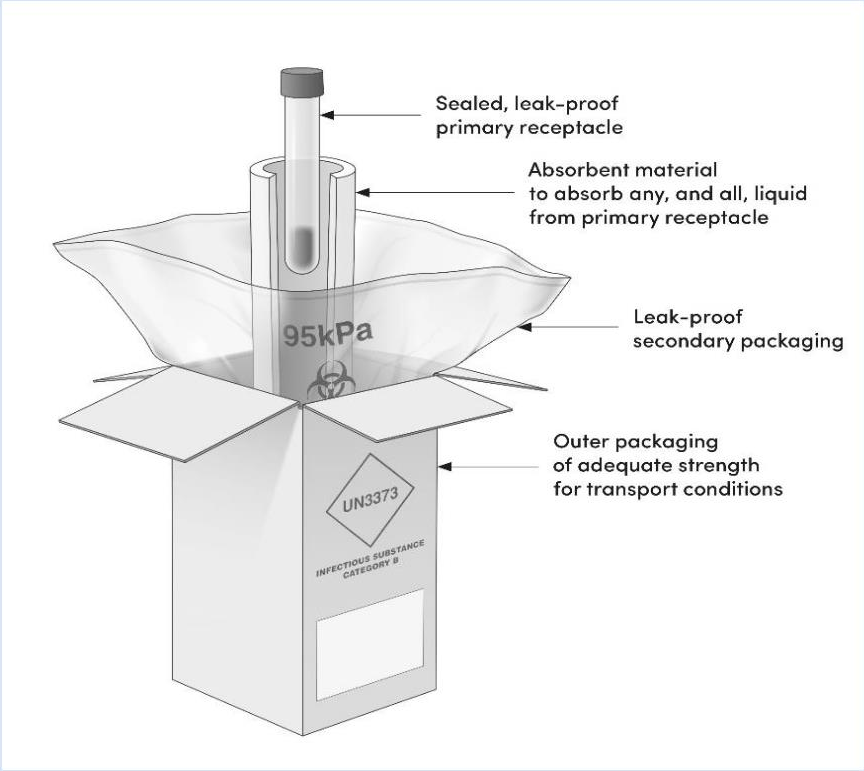
Site visits to institutions in Peru uncovered problems in verifying temperature changes during storage and transportation.

The WHO, European Centers for Disease Control and Prevention, USA CDC, and Korea Centers for Disease Control °Cand Prevention, in tests for infectious diseases such as COVID-19, specify to store specimens at 2-8 and transport them immediately. If the specimen is expected to be delayed for more than 72 hours, it should be stored frozen at -20°C or ideally -70°C, and transported on dry ice. For the safe storage of these specimens, a standard of procedure is in place, such as avoiding repeated freezing and thawing of specimens. The training should address all this.

Specimen Packaging and Transportation

COVID-19 specimens should be transferred within a facility using a separate dedicated elevator or staircase if possible. Staff should be trained on spill decontamination in the event a specimen leaks.

Example case: Instructions for primary packaging

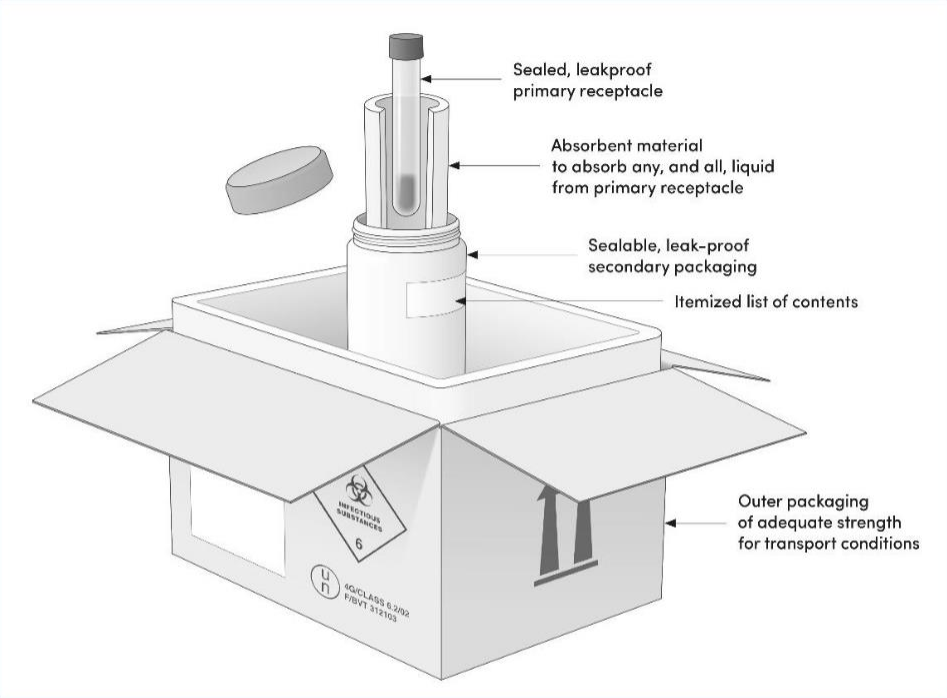


1. Whatever the intended temperature of the consignment, the primary receptacle OR the secondary packaging must be capable of withstanding a pressure differential of not less than 95kPA (0.95 bar), as well as temperatures in the range of -40°C to +55°C.
2. When the shipment is being carried at ambient temperature (or above), the primary receptacle must be glass, metal, or plastic. Effective means of ensuring a leak-proof seal should be provided— e.g., a heat seal, skirted stopper, or metal crimp seal. If screw caps are used, they must be secured, for example by paraffin sealing, tape, or a manufactured locking closure.
3. Lyophilized substances may also be transported in primary receptacles that are flame-sealed glass ampoules or rubber-stopped glass vials fitted with metal seals.
4. Packing method in primary containers:

   1) The primary container should be made of plastic material with low risk of breakage and sealed with a screw cap stopper with an O-ring. Disinfect the outer surface of the primary container with 70 percent ethanol, cover it with a sufficient amount of absorbent material, and place it into the secondary container with the container stopper facing up. At this time, the absorbent material should be enough to absorb even the entire amount of the infectious substance in the container.

   2) Then, put the primary container in a Ziplock bag, pack it in a secondary container, and transport it. Secondary containers must be marked to identify infectious substances and must be able to withstand impact without breaking.

Example case: Instructions for Triple Packaging



1. If there is no visible leakage in the secondary container upon receipt of the specimen, it can be reused after an appropriate disinfection process. Disinfection for secondary container reuse: spray or immerse uniformly in 70 percent ethanol or in sodium hypochlorite (chlorine 0.1 percent or 1,000 ppm) and let it react for 1 minute.
2. In the tertiary container to contain the second container, put a shock absorber such as air vinyl to reduce external impact during transportation, and fix the second container in place. Place the specimen test request form between the secondary container and the tertiary packaging container.
3. On the outside of the tertiary transport container, attach the UN3373 label indicating the sender, recipient, emergency contact information, and presence of category B infectious substances.

All personnel in charge of transporting specimens must wear N95 grade gas masks and gloves. The training should also ask trainees to consider the type of specimen, collection time, and transportation time before transporting the specimen.

Training proposal for Analytic and Post-analytic Phase

Overview of the Training Process

Rapid and accurate COVID-19 testing must be conducted on a large scale. Each laboratory in Peru needs to use standard COVID-19 testing procedures based on scientific evidence, as provided by the INS. Training related to practice-based analysis of the overall contents of the standardized COVID-19 test should be conducted by the INS to the staff of their labs.

Training Objectives

Trainees should learn the overall procedures and methods for analyzing specimens collected following the outbreak of a new infectious disease such as COVID-19. In this case trainees will learn, and practice, the entire process from specimen preparation to nucleic acid extraction, real-time RT-PCR test methods, and accurate reporting of results.

General aspects of Training

It is necessary to learn the preparation process according to the type of specimen being used in the COVID-19 diagnostic test. Standardized nucleic acid extraction and real-time RT-PCR test methods for COVID-19 diagnostic tests should be included. The training should include ways to increase the accuracy of the results, by reviewing and discussing the factors that can affect the reading of test results and the test. It should include an evaluation of trainees' performance of the real-time RT-PCR test.

Training Contents

Pre- and Post-training Assessment

Example case: Example questions for assessment

Q1. Explain the principle of real-time reverse transcription polymerase chain reaction (RT-PCR) among COVID-19 diagnostic tests.

A1. In RT-PCR, PCR testing is performed after making cDNA from ribnonucleic acid (RNA). During the DNA amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers.

In the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase decomposes the probe, and the reporter dye is separated from the quencher dye to generate a fluorescence signal. At each cycle, an additional reporter dye molecule is cleaved from the corresponding probe to increase the fluorescence intensity. The fluorescence intensity is monitored at each PCR cycle by a PCR thermocycler and reported quantitatively graphically in the reaction program.

Q2. Choose all of the following statements that are correct regarding preparation for real-time RT-PCR testing.

1) Specimen distribution for nucleic acid extraction is done within the biosafety cabinet.
2) All pipette tips and reaction tubes must be sterilized before use.
3) The pipette uses a tip with a barrier to prevent aerosolization during pipetting.
4) If pipetting is difficult due to the viscosity of the specimen, add PBS buffer and mix well.

Specimen Preparation Stage

Example case: Caution in specimen handling

1. Continuing care should be taken not to change the specimen in all stages of the test, from specimen collection to reporting results.
2. For all specimens received, check records (e.g., barcode) of patient name, registration number, date of birth, gender, date of collection, specimen container, and test items.
3. When transferring material from one container with a barcode to another container, write the test date and work number on the container to avoid confusion.
4. Determination of unsuitability of the specimen:
   1) Inappropriate container
   2) Lack of specimen volume
   3) If the specimen is leaked
   4) In case the patient name, registration number, etc. cannot be recognized

In training staff to prepare specimens, focus on how to determine whether the specimen is suitable for a particular kind of test. Cover the precautions to be employed in specimen collection, storage, and transportation. In view of past experience in Peru, it is especially important to determine whether all records for the received specimens match; include in the training the process for checking whether or not there is a problem with barcode registration.

In addition, it is necessary to clearly designate the criteria for determining when a specimen cannot be used because of problems in the storage and transportation stages and provide training on this.

Implementation Stage of Tests

This will cover verification of a laboratory's testing capacity, quality control procedures, and the range of permitted reagents.

Training for the test execution stage should include the entire RT-PCR process, including reagent preparation, specimen pretreatment, nucleic acid extraction, and nucleic acid amplification, according to the test order, including safety management and measures to improve the reliability of the test, with attention to possible contamination of the test result, false negatives, and false positives, and what to do about any of these.

Example case: managing test implementation

1. The examiner wears appropriate PPE (N95, KF94 or equivalent or higher respiratory protection (mask), full body protective clothing, disposable gloves, etc.) and is in a Biosafety Level 2 (BL2) examination room at a Class II or higher biosafety workstation. (A biosafety cabinet is recommended.)
2. PCR is a very sensitive method, so there needs to be caution about contamination, such as carry-over contamination.
3. It is recommended to use two or more different sites for real-time RT-PCR testing. The test should use a positive control substance, a negative control substance, and an internal control substance. The internal control substance should be contained in the same container (well) as the specimen.

Example case: examples for managing false negative cases

1. The incubation period of coronavirus is known to be four to six days, but there is large variation among people, and it is difficult to know the exact timing of exposure. Therefore, if the first test is negative but the person is clinically liable to have COVID-19, the upper respiratory tract specimen is repeatedly collected after a certain period of time and tested.
2. Causes of false negative:

   1) Early or late specimen collection

   2) Inappropriate specimen quality and quantity

   3) Mutant virus

   4) Presence of inhibitory substances in the specimen

   5) Administration of antiviral drugs or other drugs that may affect the result of the test
3. In case of a negative result

   1) If only the upper respiratory tract specimen is negative, take another specimen or additionally collect a lower respiratory tract specimen.

   2) Retest using other genetic test reagents.

   3) Residual specimens are sent to the reference laboratory for examination.

   4) An antibody test is considered if repeated negative results are found in clinically and epidemiologically suspected patients.

Example case: examples for managing false positive cases

1. There are errors in the pre-analysis stage and errors in the analysis stage.

   1) Pre-analysis step: specimen change, cross-contamination from other positive specimens in the pre-processing step.

   2) Analysis step: non-specific amplification of genes, fluorescence errors, cross-reactivity.
2. Solution to the above:

   1) After retesting, if the results are the same, the case can be judged as positive, and if not, it can be judged as a false positive. Specimens should be re-extracted or re-collected if there is a possibility of cross-contamination.

   2) The patient history and clinical findings can be confirmed.

In addition, training should cover criteria for re-examination and for reporting of results. In addition, trainees should be taught that serious office errors, analysis errors, and abnormal results must be immediately reported to the person in charge and corrected according to the procedures of the laboratory.

Conclusion

This proposal has been built from the assessment of the process of sampling and transport of samples in the field and considering the current conditions of the country´s health system.

Thus, the recommendations and the training plan presented here are adapted to what needs to be done, in the country, to ensure adequate sampling, sample transport and biosafety conditions for patients and health personnel.

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Annexes

Annex A: Training Plan for Specimen Collection and Transport

Category	Content	Method
Learning objectives	- To present, and train on, guidelines based on scientific evidence for COVID-19 specimen collection, storage, and transportation for proper performance of diagnostic tests - To prevent test errors occurring in specimen collection, and dangerous accidents caused by poor specimen handling, through accurate training on specimen collection, request, storage, transportation, and disposal	
Participant	- Laboratory personnel in charge of institutions that collect, store, and transport COVID-19 specimens - Laboratory personnel of institutions performing RT-PCR for COVID-19	
Topics	- The participants will be able to establish and use standard operating procedures for specimen collection and handling, storage and transportation, and disposal for COVID-19 diagnostic tests. - Participants will learn how to properly collect nasopharyngeal swabs, oropharyngeal swabs, and sputum specimens for COVID-19 diagnostic tests, and how to safely handle and store each specimen. - Participants will learn how to implement packaging and transportation guidelines and proper specimen disposal methods that follow biosafety regulations.	
Contents and methods	Pre-assessment	Online pre-test
	Safety issues related to specimen collection	Lecture (PowerPoint)
	Location of specimen collection 1. Upper respiratory tract specimen collection 2. Lower respiratory tract specimen collection 3. Other specimen collection	Lecture (PowerPoint) Practical training on specimen collection method

Category	Content	Method
	Storage standards for specimen collection	Lecture (PowerPoint) Discussion of specimen storage methods
	Packaging method for specimen transport	Lecture (PowerPoint) ractical training on specimen packaging method
	Post-assessment	Conduct overall practical assessment of specimen collection, storage, and transportation
Period	Theoretical training: One hour for each topic	Lecture (PowerPoint)
	Practical training: 30 minutes per required section	Hands-on practice using tools
Continuing education	Repeated training sessions in every six months	Include real-life feedback

Annex B: Training Plan for Specimen Analysis

Category	Content	Method
Learning objectives	- To present and train on the entire process of COVID-19 RT-PCR testing, including specimen preparation, nucleic acid extraction, and RT-PCR test method. The processes of quality control, result reporting, and problem solving are also discussed with respect to proper performance of diagnostic tests. - To enable participants to perform the entire process of the COVID-19 RT-PCR test after the training.	
Participant	- Laboratory personnel of institutions performing RT-PCR related to COVID-19	
Topics	- Participants will learn the entire process for the COVID-19 RT-PCR test, including specimen secretion, reagents and equipment, pre-test processing, nucleic acid extraction, nucleic acid amplification, judging results, and problem solving. - Participants will learn processing and preparation of specimens through theoretical lecture and practical training. - Participants will learn nucleic acid extraction and real-time RT-PCR test methods for COVID-19 diagnostic tests through theoretical lecture and practical training.	
Contents and methods	Pre-assessment	Online based pre-test
	Specimen types, collection containers, precautions for specimen handling, and rejection criteria	Lecture (PowerPoint)
	Use of equipment and reagents 1. Reagent information and preparation 2. Composition and preparation of reagents	Lecture (PowerPointt) Practical training on reagent composition and preparation Practical training on test equipment operation

Category	Content	Method
	Test procedures 1. Specimen processing 2. Nucleic acid extraction 3. Nucleic acid amplification	Lecture (PowerPoint) Practical training on RT-PCR equipment and nucleic acid extraction equipment
	Analysis of test results 1. Determination of status of single sample 2. Determination of status of mixed samples 3. Classification of re-examination criteria 4. Analysis of false negative cases	Lecture (PowerPoint) Practical training on reading test results Reading (evaluation) example test results
	Post-assessment	Overall assessment is conducted based on both practical aspects of test procedures and the theorical aspect of RT-PCR.
Period	Theoretical training: one hour for each topic	Lecture (PowerPoint)
	Practical training: 30 minutes per required section	Hands-on practice using tools
Continuing education	Repeated learning every six months	Include real-life feedback