Interim Guidelines for SARS-CoV-2 PCR laboratories in National Public Health Laboratory Network, Nepal

Approval from MoHP: 24 July 2020

This document is subject to updates, please use the hyperlink to confirm the latest information where appropriate. http://nphl.gov.np/

Acronyms:

COVID-19	Corona Virus Disease -19
DoHS	Department of Health Services
EDCD	Epidemiology and Disease Control Division
GoN	Government of Nepal
HO	Health Office
MoHP	Ministry of Health and Population
NPHL	National Public Health Laboratory
PPHL	Provincial Public Health Laboratory
q-PCR	real time Polymerase Chain Reaction
QA	Quality Assurance
WHO	World Health Organization

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Government of Nepal
Ministry of Health & Population
Department of Health Services
National Public Health Laboratory
Teku, kathmandu
2050

1. Background

In December 2019, a novel Coronavirus (COVID-19) outbreak started in Wuhan, China and soon, this virus started to spread to other parts of the world. The first case of COVID-19 was detected in Nepal on late January 2020. The first suspected case detected in Nepal was laboratory confirmed in Hong Kong because of lack of COVID-19 diagnostic facility within the country. National Public Health Laboratory (NPHL) started COVID-19 testing with real time PCR since 26th January 2020. As of date, q-PCR remains the gold standard confirmatory test for COVID-19. To meet increased need for COVID-19 testing, designated COVID-19 laboratories are now expanded to all the seven provinces of Nepal.

2. Aims and Objectives

The objective of this guideline is to provide guidance to all the designated COVID-19 testing laboratories in Nepal. It provides guidelines related to collection of samples, physical infrastructures needed, workflow development, quality assurance and the communication of the results to concerned authority.

3. Scope

The document aims to provide guidance to the laboratories involved in SARS-CoV-2 testing by real time PCR.

4. Requirements for setting up COVID-19 PCR laboratory.

The following requirements must be fulfilled before starting COVID-19 testing by real time PCR.

- The interested authorities must receive permission from the MoHP, GoN
- Laboratory must be established based on PCR laboratory establishment guideline produced by NPHL
- The established lab will commence operation after clearing the evaluation conducted by NPHL and/or nearest PPHL
- Laboratory report must be integrated with NPHL/PPHL

- The laboratory will be a member of COVID-19 testing molecular laboratory network under NPHL.
- Laboratory must follow the rules and regulations established by MoHP and NPHL.

4.1 Facility Design and Workflow:

Laboratory areas shall have adequate space and controlled environment. The laboratory should be designed and operated in a way that prevents contamination of reactions with amplified products from previous assays and cross-contamination between samples, both of which can lead to false-positive results. For minimal requirement, refer to **Annex-8**.

A laboratory space for performing PCR should be divided into at least three physically separate rooms:

a) Aliquoting and extraction *(if possible, with negative pressure)*
b) Master Mix preparation and template addition
c) Amplification and product detection

A unidirectional workflow will reduce the opportunity for contamination to occur.

- No materials, supplies, or equipment from the aliquoting and extraction room should be taken into the master mix preparation room.
- Nothing from the amplification and product detection room should be taken into the aliquoting and extraction room or the master mix preparation room.
- Equipment should not be moved between the rooms used for any PCR sample processing and analysis steps.

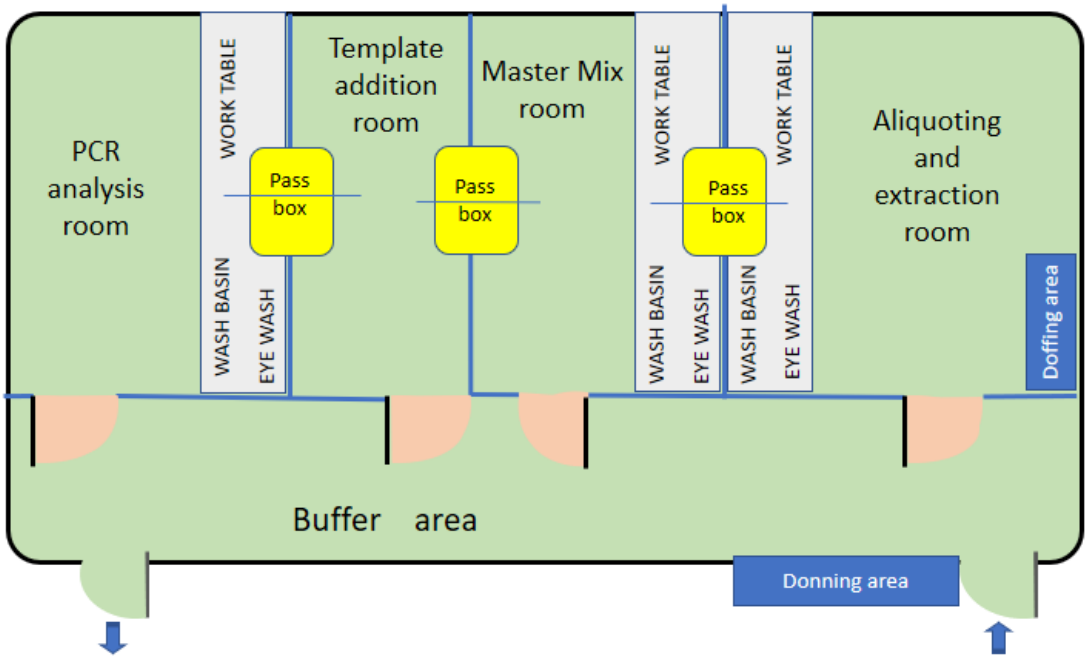


Fig-1: Example of Floor Plan of a PCR lab for COVID-19

4.2 Equipment, Reagents and Consumables:

4.2.1 Equipment

The equipment used to perform PCR should function properly to generate reliable data. Equipment should be dedicated to a specific laboratory room, and the instrument manuals from the manufacturer should be available.

Quality Assurance of the following equipment in the lab must be ensured:

a. Thermocyclers
b. Centrifuges
c. Biosafety cabinets/ Laminar flow cabinets
d. Pipettes
e. Temperature dependent instruments: Refrigerators/ Freezers

User log and temperature log should be maintained where applicable.* *(refer to sample document-II)***

4.2.2 Reagents:

- Care should be taken to ensure that the reagents are maintained contamination-free.

- All reagents should be clearly labeled with name, expiration date, and relevant safety information.
- Opening date, expiration date and the initials of the individual must be present on each reagent that are opened for use.
- Reagents from different lot numbers should not be interchanged without prior functional verification.
- All reagents from new lots should be tested to ensure that they work properly by running a PCR positive control using the new reagents. If the PCR positive control fails, new lots should be tested against the old lots.
- A copy of the manufacturers' specifications and procedures should be kept in the laboratory's SOP binder for every commercially available kit used in the laboratory.
- Logbooks should be maintained for all reagents and kits and should document all pertinent information needed to identify possible sources of contamination, including the following;
    - Product name, manufacturer, product number, lot number, all associated dates (receipt, preparation, open, expiration, when passed internal laboratory assessments, etc.), receiving person or test analyst name and initials, storage location and location of components.

4.2.3 Consumables

Disposable materials used in PCR analysis include pipette tips, eppendorf tubes, PCR tubes, and gloves. To reduce the contamination and degradation of the target nucleic acids, disposable materials should meet the standards as follows.

Pipette Tips

- Special tips for PCR analysis include barrier tips and aerosol-resistant tips, both of which minimize cross-contamination of samples during pipetting.

- These tips can be purchased pre-sterilized and pre-loaded in hinged racks to provide tip protection and easy access.
- Pipette tips for PCR analyses should be lot-certified, RNase-, DNase-, and pyrogen-free.

Sample and PCR Tubes

- Laboratories should use polypropylene tubes that are lot-certified DNase-, RNase-, and pyrogen-free.
- The size and style of PCR tubes or reaction plates recommended by the manufacturer for the thermocycler should be selected to ensure that the tubes are compatible with the block and lid height.

Gloves

- Disposable powder free gloves should be available in each section of laboratory used for PCR analysis.
- Gloves should be changed before leaving and entering each section of the laboratory and each time contaminating RNA is potentially encountered.

4.3 Personnel:

- Adequate number of trained personnel should be recruited for uninterrupted laboratory workflow
- Analysts involved in PCR analyses should have appropriate qualification and skills needed for PCR.
- Personnel responsible for report verification must have at least Master's degree and should be registered in their respective professional councils.
- Personnel should be oriented by a trained staff member before initiation of sample testing.
- Training regarding biosafety practices should be done periodically.

5. Hub and Satellite laboratories

The concept of hub and satellite laboratories network has been endorsed for regular coordination between the designated COVID-19 testing laboratories across the country. The hub laboratories will be responsible for close coordination with their respective satellite laboratories for technical assistance, logistic management support, bio-repository of positive sample and validation, etc.

Hub laboratories	National Public Health Laboratory (NPHL)	B.P Koirala Institute of Health Sciences (BPKIHS)	Bharatpur COVID-19 Diagnostic Laboratory, Chitwan	Bheri Hospital, Banke
Satellite Laboratories	NAMS/Bir hospital	Koshi Hospital, Biratnagar	Pokhara Academy of Health Sciences, Gandaki	Provincial Public Health Laboratory (Rupandehi)
	Patan Academy of Health Sciences (PAHS)	Gajendra Narayan Singh Hospital, Rajbiraj, Saptari	Vector Borne Disease Research and Training Center (VBDRTC), Hetauda	Seti Provincial Hospital, Dhangadhi
	Kathmandu University Teaching Hospital, Dhulikhel	Provincial Public Health Laboratory, Biratnagar	Narayani Hospital, Birgunj	Rapti Academy of Health Sciences, Dang
	Sukraraj Tropical infectious disease Hospital (STIDH), Teku		Provincial Public Health Laboratory, Janakpur	Provincial Hospital, Surkhet
	Tribhuvan University Teaching Hospital (TUTH)		Provincial Public Health Laboratory, Pokhara	Karnali Academy of Health Sciences (KAHS), Jumla
Total 22 sites (4 hub & 18 satellite labs)				

6. Sample collection

- Respiratory samples are recommended for real time PCR for diagnosis of COVID-19
- Samples should be collected with synthetic swabs with plastic shaft. Do NOT use cotton swabs with wooden sticks)

**Link to video on sample collection:* https://youtu.be/orRq1GbT0yI

Sample types and method of collection in **Annex 2**

- Swabs must be collected in leak proof container with VTM[1].
- Specimen must be correctly labelled and accompanied with correctly filled sample referral form **(*refer to Sample document III*)**
  - Specimen that can be delivered promptly to the laboratory can be stored and shipped at 2-8°C for up to 72 hours.

[1] When VTM is not available, 0.9% NaCl (2ml) in screw capped vials can be used.
(References: https://jcm.asm.org/content/jcm/58/6/e00590-20.full.pdf
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3295134/)

- Specimen must be kept at - 20°C or ideally -80°C till shipment to laboratory. It is important to avoid repeated freezing and thawing of specimen.
- The receiving laboratory where the samples are to be sent must be telephonically informed regarding the number of samples.

6.1 Sample Reception:

A record of sample received with regard to the number of samples, collection site, date and time of reception, person delivering and receiving the sample should be maintained.

6.2 Sample Collection Desk:

[Sample should be handled by personnel with proper Personal Protective Equipment (PPE) ***{refer Annex-3}*** *and within BSC-II]*

- Patient information on the sample tubes must match with that in the requisition form.
- Any discrepancies on patient identification should be documented.
- At least two patient identifiers like name, age, sex, date of birth, etc. are required.
- Make sure that the sample received is acceptable for processing
- For sample rejection criteria refer Annex-III. Records of all the samples rejected along with reason for rejection should be documented and should be timely communicated to the contact person.
- Patient's record should be maintained in computerized form (Softwares are available).
- Each sample should be given their unique laboratory identification number. *(**refer to Sample document IV**)*
- If the samples cannot be processed immediately, they must be stored at 2-8^{0}C for up to 72 hours, beyond which they must be stored at -20^{0}C for up to one month.

- All materials transported within and between laboratories should be placed in a secondary container to minimize the potential for breakage or a spill. Cold chain should be maintained during transportation.

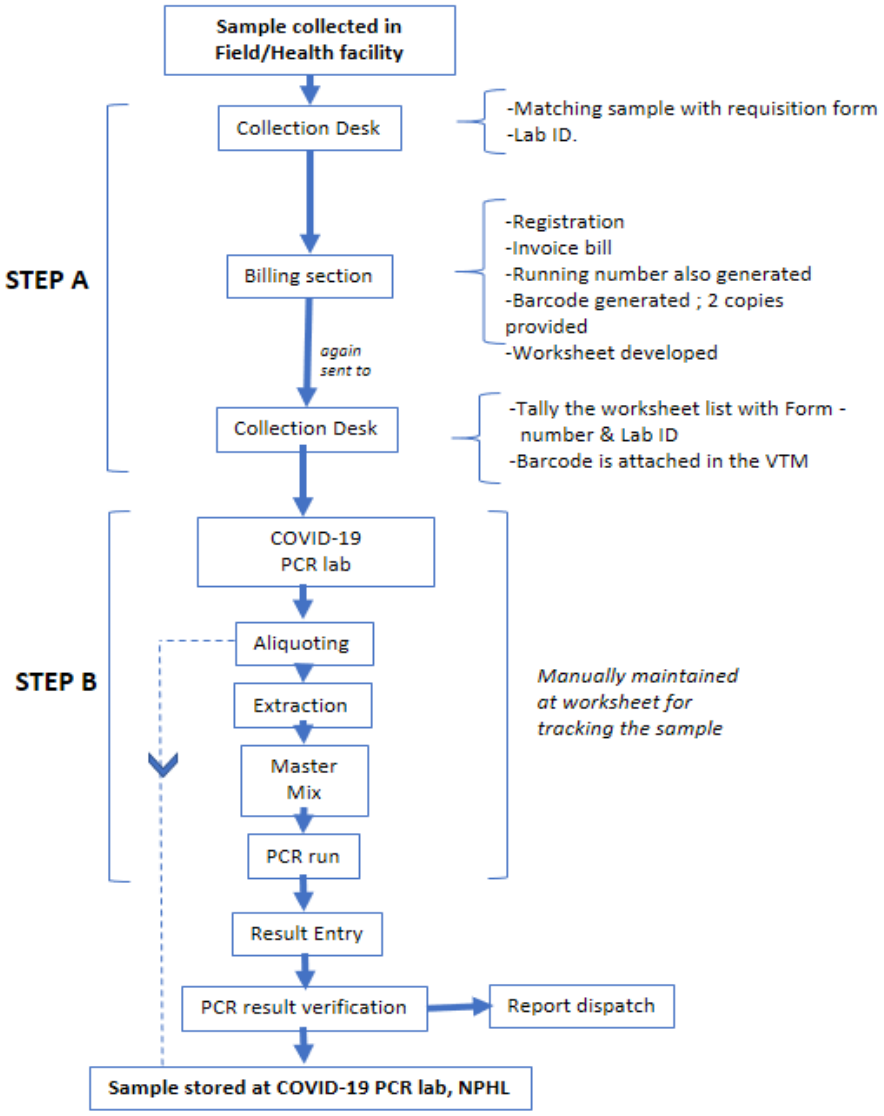


Fig-2: An example of the flow of samples from collection site till reporting at NPHL

7. Sample Processing:

All Standard Operating Procedures (SOPs) must be accessible to all the persons working in the laboratory. Bench guides can be pasted at the workstation. Appropriate PPEs must be worn, and safety guidelines must be followed all the time during handling COVID-19 specimens.

7.1 Aliquoting:

(Refer to sample document-V)

- Aliquot the sample into three vials, 200 microliters into two and 500 microliters into one).
- Use one vial (200 microliter) for immediate extraction and store the other two preferably at -80°C (-20°C for up to a month).
- Alternatively, specimen can be directly used from VTM and remaining VTM specimen can be directly stored. In this way, we can reduce the time and risk of contamination and infection.
- The used tips and tubes must be disposed safely in leak proof containers.

7.2 RNA Extraction

(Refer to sample document-VI)

Follow the protocol provided by the kit manufacturer

- The sample extraction should be performed under a Class II Biosafety cabinet (BSC).
- Use low or zero retention filtered tips for extraction.
- Use RNase free tubes and tips.
- For disinfecting the pipettes, use 70% ethanol. Do not use bleach for cleaning the pipettes.
- Vortexing should be done within the BSC.
- Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups.
- Extracted RNA should be stored at -20 ºC or lower temperature.
- The used tips and tubes must be disposed safely in leak proof containers.
- PCR reagents and amplified products must not be pipetted in this area.

7.3 Master-Mix

(Refer to sample document-VII)

- This is the cleanest step in PCR. It should be performed within a laminar flow/PCR cabinet equipped with UV light.

- Amplification reagents should be kept in a freezer (or refrigerator, as per manufacturer recommendations) in the same designated space, ideally next to the laminar flow cabinet.
- Reagents containing fluorescent probes from light to avoid degradation. The lights inside the cabinet should be put off while preparing the master mix.
- Cooler rack must be used for master mix preparation.
- The used tips and tubes must be disposed safely in leak proof containers.
- Gloves should be changed each time upon entering this area or inside laminar flow cabinet used for master mix preparation.

7.4 Template Addition

- Template should be added in the dedicated PCR cabinet.
- The used tips must be disposed safely in leak proof containers.

Bench space cleaning:

- Before and after use, wipe down all items in the cabinet, e.g. pipettes, tip boxes, vortex, centrifuge, tube racks, pens, etc. with 70% ethanol or a commercial nucleic acid destroying decontaminant and allow to dry.
- Bench spaces should be cleaned with 0.1% sodium hypochlorite followed by 70% ethanol or a validated commercially available decontaminant. Expose the hood to UV light for 30 minutes.

7.5 PCR Amplification

This area should be physically separated from the master-mix area.

- A separate set of gloves, lab coat, bins and other equipment should be available in this area.
- The PCR instrument must be validated before use.

7.6 PCR Analysis

Interpret the result as per protocol

- If any two genes (N, ORF1ab, E, RdRp) detected within the CT value defined in the package insert, the specimen should be considered positive for SARS-CoV-2.

8. Reporting:

- Record the results in the predesigned format. *(refer to sample document VII)*
- For positive results, keep and maintain a record of the CT value of the samples along with controls used.
- To minimize errors, it is recommended to enter and verify the positive and negative results at different times.

8.1 Review of Results:

Authorized laboratory personnel should review the results before release and evaluate them against internal quality control and, as appropriate, available clinical information and previous test results.

8.2 Report Content

Reporting of results must be done in accurate, clear and unambiguous manner. It should include information necessary for interpretation of test results. *(refer to Sample document VIII)*

Report content must include, but not be limited to:

- clear identification of test kit used and the testing technology
- identification of lab issuing the report
- patient identification
- date and time of primary sample collection
- type of primary sample
- interpretation of results, where applicable
- other comments as cautionary notes (e.g. quality or adequacy of primary samples that may have compromised results)

- identification of person(s) reviewing the results and authorizing the release of report
- date of report and time of release

Release of Results

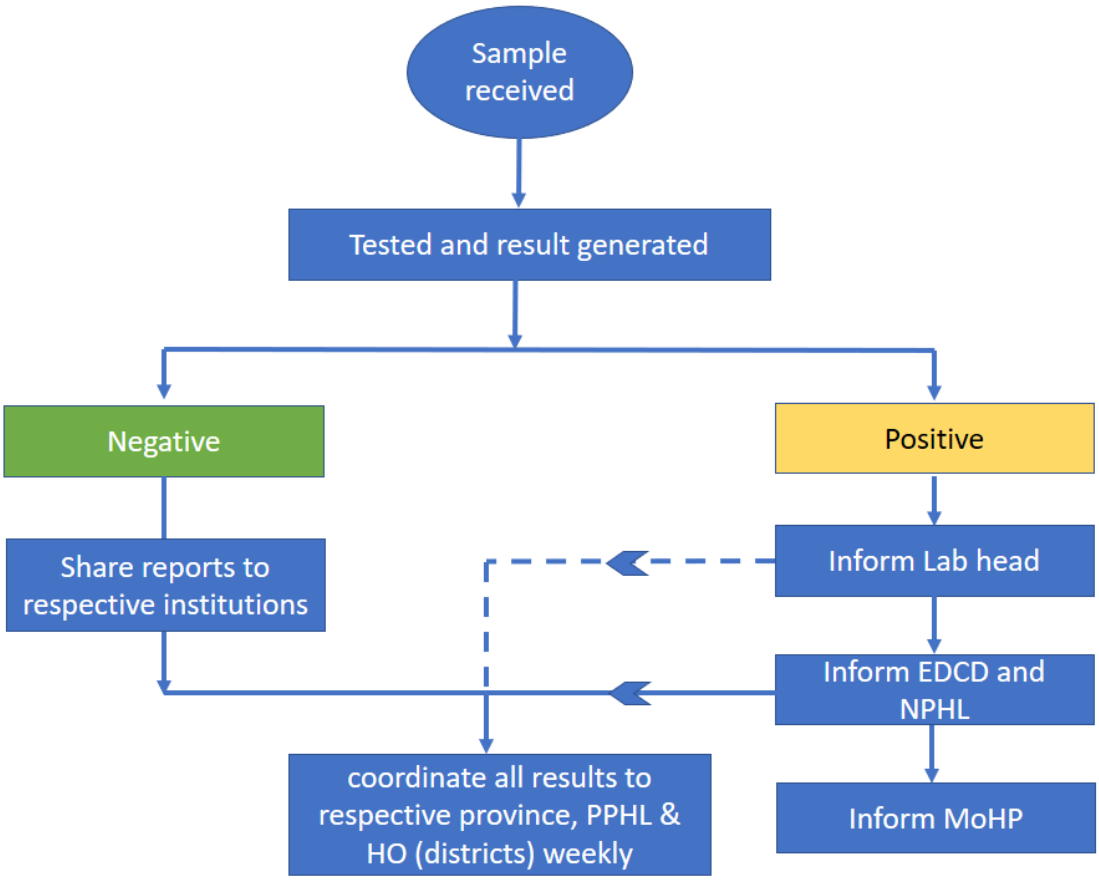


Fig-3: Flow chart for releasing the report

There should be a documented procedure for release of examination results, including details of who may release results and to whom.

Results can be released electronically, or they can be picked up from the testing laboratory.

The details of a positive result must also be reported to EDCD and NPHL via email.

8.3 Revised test records

When the original test is revised, the following instructions regarding revision should be followed

- revised test result should clearly be identified as a revision and should include reference to date and patient's identity in the original result
- the revised result will specify the date and time of revision and the name of person responsible for revision.
- original result entries should remain in record when revisions are made.

9. Storage, Retention and Disposal of Clinical Samples:

(Refer to sample document-IX)

- All positive samples must be stored at -20ºC *(up to one month)* for later transfer to NPHL
- All negative samples should be retained at -20^{0}C up to for 48 hours after report is released by laboratory. Then, all negative samples must be treated/autoclaved before they are disposed.

10. Ensuring Quality of Examination Results

10.1 Use of Internal Quality Control (IQC) Materials

- **PCR positive controls (PC)** are used to verify that the extraction, PCR master mix preparation and reagents preparation were done correctly to produce amplification of the target nucleic acid.
- **Negative control (NC)** samples are used to verify that no contaminating nucleic acid has been introduced into the master mix or into samples during sample processing. These negative controls are clinical samples earlier confirmed negative and do not contain target nucleic acid.
- **No template control (NTC)** is used to verify that no contaminating nucleic acid has been introduced into the master mix. These controls are prepared when no template is added to the master mix. They are prepared as separate samples to which aliquots of molecular-grade water or buffer are added to the master mix in place of clinical sample. A negative result with this control indicates that the master mix and final processing reagents are not contaminated.
- QC results should be documented with every run.

- For prevention of false positive and negative results refer ***Annex-5***

10.2 CORRECTIVE ACTION

A. If any positive control fails,

- All samples associated with the control should be considered invalid, and negative samples should be listed as potentially false-negative samples.
- If amplification of a positive control fails to produce the specific amplification product, the integrity of the control and the PCR design should be examined to determine the reason for the failure.
- When determined, the reason for the failure should be documented and the controls and samples must be repeated.

B. If PCR negative controls or method blanks produce specific amplification products,

- All samples associated with the failed controls should be considered invalid, and all positive samples should be listed as potentially false-positive samples.
- The source of contamination should be identified and eliminated.
- Once determined, the source of the contamination should be documented, and the samples in the batch should be reanalyzed.

10.3 External Quality Assurance

- Quality Assurance is a planned system of review of procedures, preferably performed by an independent third-party laboratory, which assesses procedures and verifies that quality objectives have been and are being met.
- A newly established laboratory should have at least 10 positive and 10 negative samples tested on different days verified from NPHL or another laboratory designated by NPHL. Verification will be done by re-testing same sample (split sample analysis method[2]).

[2] *In split-sample testing, a single biologic sample (e.g., tube of blood) is divided into aliquots. If testing is performed on different biologic samples obtained at the same time (e.g., two tubes of blood obtained at the same venipuncture) then the term split specimen testing is used. The aliquots are sent to another laboratory(ies) for testing. Split-sample*

- Each month 5 negative samples and all positive samples should be sent to NPHL for quality assurance. (Out of all the positive samples from a facility, 5 will be verified at NPHL for QA).
- While selecting the positive samples, include samples with different CT values (ranging from low to high).
- Every laboratory should develop a mechanism of inter-laboratory result comparison with the nearest laboratory, once a month (*minimum 2 random samples*); discrepancies if any will be reviewed by NPHL.
- The laboratory should mandatorily participate in the NEQAS/EQAS program conducted by NPHL. (Annex 10)

11. Laboratory Information Management System:

Lab must have documented procedure to ensure confidentiality of patient information. It should also be ensured that data is

- protected from unauthorized access
- safeguarded against tampering and loss
- readily retrievable at the time of need to authorized users.

Authorities and responsibilities for management of information system are defined, especially for those who can:

- access patient data and information
- enter patient data and examination results
- change patient data and examination results
- authorize release of examination results and reports

12. Data Recording and Record Keeping

Laboratories should retain record of test results for five years either electronically or hardcopies.

If data are maintained electronically, data should be backed up on a regular basis and stored in a separate location from the original data, if possible.

procedures evaluate interlaboratory agreement and testing errors, but do not evaluate trueness (i.e., bias) per se unless the outside laboratory uses a method that is calibrated to a reference method or reference material.

All data recording should be checked by the laboratory supervisor for correctness and completeness, and each entry should be checked for accuracy of transcription.

All these data storage must be password protected with limited access to protect the patient information.

13. Biosafety Practices

All the testing for the presence of SARS-CoV-2, or of clinical specimens from patients meeting the suspected case definition for COVID-19 should be performed in appropriately equipped laboratories, by staff trained in the relevant technical and safety procedures.

- Each laboratory should conduct an institutional risk assessment to ensure it is competent to safely perform the intended testing with appropriate risk control measures in place. ***Annex 6***
- When handling and processing specimens, safe laboratory practices and procedures should be followed.
- PCR should be conducted at a facility using procedures equivalent to Biosafety Level 2 (BSL-2) adopting the practices and procedures of "core requirements", as detailed in ***Annex 7***
- All procedures must be performed based on risk assessment and only by personnel with demonstrated capability, following relevant protocols at all times. Orientation training and periodic refresher trainings must be conducted.
- Initial processing of specimens should take place in a validated biological safety cabinet (BSC) or primary containment device.
- Patient specimens should be transported in a leak proof triple layer pack maintaining cold chain.
- A dedicated hand-wash sink should be available in the laboratory
- Appropriate personal protective equipment (PPE), as determined by a detailed risk assessment **(*refer Annex 8*)**, should be worn by all laboratory personnel handling these specimens. Dedicated areas for donning and doffing must be identified.
- Appropriate disinfectants with proven activity against enveloped viruses should be used (for example, hypochlorite [bleach], alcohol,

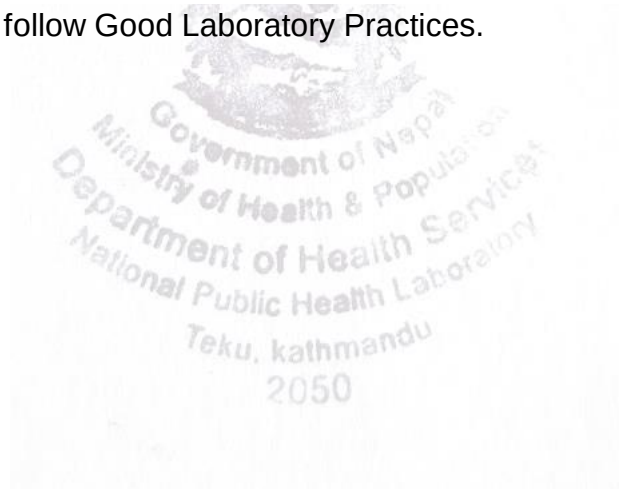
hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds). They should be used for the recommended contact time, at the correct dilution and within the expiry date after the working solution is prepared.

- All technical procedures should be performed in a way that minimizes the generation of aerosols and droplets
- Centrifugation of specimens should be performed using sealed centrifuge rotors or sample cups. These rotors or cups should be loaded and unloaded in a BSC.
- All materials transported within and between laboratories should be placed in a secondary packaging, to minimize the potential for breakage or a spill. Specimens leaving the BSC should be surface decontaminated.
- Wastes must be segregated at the point of generation. Appropriate methods for decontamination of waste must be available close to the laboratory.

14. Dos & Donts in PCR laboratory

- Always use separate consumables and equipment for each step (RNA extraction, Master mix preparation and amplification)
- Use separate compartment spaces for each step used in PCR.
- Avoid any sources of RNase contamination.
- Use RNase free tubes and tips.
- In case of withdrawing microcentrifuge tubes from a beaker/container, disinfect the gloves first or tap the tilted container to drop the required number of tubes on sterile surface.
- It is advised to label the instruments such as pipettes, with the name of the laboratory it is being used in. This will avoid the confusion of exchanging pipettes between laboratories, thus reducing chances of contamination.
- Remember to always keep reagents and components capped whenever possible.

- Correct pipetting technique can minimize contamination between samples that can lead to false positive results.
- Change gloves especially if it is suspect to have become soiled with solutions containing template RNA.
- Always disinfect the instruments, materials and environment with proper disinfection method.
- Use low or zero retention filtered tips to avoid cross contamination.
- Tubes containing stored samples and reagents should be centrifuged briefly before opening to ensure that all liquids are at the bottom of the tubes.
- Always wear a full-length, long-sleeved laboratory coat or appropriate PPE.
- Remove PPE before leaving lab
- Remove respiratory protection after leaving the dirty zone.
- Always follow Good Laboratory Practices.



Annexes

Annex 1: Interim Guideline for the establishment and operationalization of molecular laboratory for COVID-19 testing in Nepal.
https://drive.google.com/file/d/157Q7JK3rFTgQRFk3DCuVpw_-owRCBI65/view

Annex 2: Types of sample and collection methods

http://nphl.gov.np/uploads/Types%20of%20Samples%20and%20Collection%20Methods-min.pdf

Annex 3: Personal Protective Equipment (Donning and Doffing)
http://nphl.gov.np/uploads/Donning%20%26%20doffing.pdf

Annex 4

Sample Rejection Criteria

1) Sample tube without labels/ Patient identifiers
2) Mismatch of Patient indentifiers on the tube and the form
3) Inadequate sample volume
4) Sample in broken containers
5) Sample collected with inappropriate swab stick
6) Sample sent without maintaining cold chain
7) All samples from patient who do not meet the testing criteria laid down by EDCD.

Annex 5: Prevention of False positives and false negative results
http://nphl.gov.np/uploads/Prevention%20of%20False%20Positives%20and%20False%20Negatives.pdf

Annex 6: Biosafety Risk Assessment
http://nphl.gov.np/uploads/Biosafety%20Risk%20Assessment%20Template.pdf

Annex 7: Core requirement: Good microbiological practices and procedures

http://nphl.gov.np/uploads/Core%20Requirements%20Good%20Microbiological-min.pdf

	Sample	Sample	Aliquoting	Extraction	Master mix	Amplification	Lab Floor cleaning
Gloves	√	√	√	√	√	√	√ heavy duty gloves
Gown	√		√	√	√		
Apron							√
Surgical mask		√			√	√	√
N95	√		√	√			
Face shield/goggles	√		√	√			
Head cover	√		√	√			
Shoe cover	√		√	√			

Annex: 8. Checklist for minimal PPE required for different activities.

ANNEX-9

Sample Document-I
Laboratory Evaluation:
Covid-19 Diagnostic Testing Capability

Institute/Laboratory being evaluated:		**Location:**	
Evaluation conducted by:		**Personnel consulted:**	
1.0 – Laboratory Overview *i.e. Type/level of laboratory. Routine diagnostics offered, etc.*			
2.0 – Dedicated Facilities (Y/N)			
Sample reception:		**Template room:**	

Inactivation room:		**PCR/Equipment room:**	
Extraction room:		***Other:***	
Master Mix room:			

3.0 Equipment & Consumables

Note: document if there is in place an equipment calibration/maintenance programme

Item:	**Make/Model:**	**Number available:**	**Item:**	**Make/Model:**	**Number available:**
Biological Safety Cabinet* (Class II or III)			**+4C storage (fridge)**		
Centrifuge (1.5ml tubes)			**-20C (freezer)**		
PCR Hood			**-80C (freezer)**		
RT-PCR Machines			**VTM tubes**		
Pipettes			**PPE** (gloves, face shields, masks, goggles, tyveks/gowns)	**	
Pipette tips	**		**Biohazardous waste sacs**	**	
Autoclave			**Disinfectants**	**	
UPS			**Collection tubes** (for extraction)	**	
Extraction Kits			**1.5ml tubes**	**	

**Date of certification and its expiry :*

***General notes about availability acceptable*

Notes - Equipment and consumables

4.0 Trained Personnel			
Task:	**No. of staff currently performing task:**	**No. that could be brought into perform task if required (surge):**	**Names/ Qualification/ council number**
Sample collection/ Reception			
Sample inactivation			
Extraction (manual)			
Extraction (automated)			
PCR set-up (inc use of RT-PCR machines)			
RT-PCR results interpretation			
Result reporting			
Sample Archiving			
Notes - Trained Personnel			

5.0 Waste Management		
Item:	Y/N:	Notes:
Waste system in place?		
Waste segregated?		
Inactivated at source?		
Autoclave present?		
Incinerator available?		
All waste dealt with on site?		
Waste disposal sub-contracted?		
Staff trained on spills/loss of containment?		

Notes – Waste management

7.0 Quality Systems		
Item:	Y/N:	Notes:
Code of Practice		
Standard Operating Procedures		
Risk Assessments		
Quality Management Policy		
LIMS system		
Sample tracking sheets		
Training & Competency documented		
Other:		

Notes - Quality systems

8. RECOMMENDATION BY EVALUATION TEAM

Sample document -II a

Department:…….. **Year/**

National Public Health Laboratory	LIST OF FORMATS	FORMAT. NO.: Com3
	NPHL	Date of issue: 28-08-2019
		Rev.No.: 00
	ISO 15189:2012	Effective Date: 01-09-2019

month:……...

TECHNICAL FORMS

ROOM TEMPREATURE CHART

DAY	ROOM TEMPREATURE 18-24 ˚ C	HUMIDITY 40-60%	OBSERVED BY:	REMARKS
1				
2				
3				
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6				
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31				
32				

Verified by:…….. Date:……..

REFRIGERATOR TEMPERATURE CHART

Department:
Refrigerator no: **Month/ year**
- ……..

DAY	TEMP.AT 9:00 AM	OBSERVED BY	TEMP.AT 3:00 PM	OBSERVED BY	Remarks
1					
2					
3					
4					
5					
6					
7					
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17					
18					
19					
20					
21					
22					
23					
24					
25					
26					
27					
28					
29					
30					

Verified by: ……..date:…….....

STERILIZATION LOG SHEET

Month/Year: _____ Location/Unit: _____

Date *(dd/mm/yy)*	Time			Temp. °F or °C	Pressure	Temperature-sensitive indicator: *Colour Change Observed (Y/N)*	Operator's Initials	Comments
	Start	*End*	*Cycle Length*					

☐ **Biweekly spore strip tests submitted:** *(dd/mm/yy)* ☐ Result received *(dd/mm/yy)* ☐ Result received *(dd/mm/yy)* ☐ Result received

PLEASE NOTE:

1. Pressure cookers, glass-bead sterilizers, microwaves, U.V. sterilizers, boiling water and dry-heat domestic ovens are **NOT** approved means of sterilizing.
2. The manufacturer's instructions for the sterilizing equipment must be onsite and easily accessible.
3. A record book for monitoring each load placed in the sterilizer (temperature, pressure and cycle length) must be maintained for a minimum of five years, with the last 12 months onsite.
4. Biweekly spore strip test results must be kept for a minimum of five years, with the last 12 months onsite.
5. Failed spore strip tests – refer to *Biological Monitoring of Sterilizer (Spore Test)* poster.

Sample Document-III (Sample Referral Form)

Government of Nepal
Ministry of Health & Population
Department of Health Service
National Public Health Laboratory
Teku, Kathmandu

Phone 4252421
Fax: 4252375
E-mail: nphl@nphl.gov.np

Laboratory Sample Collection Form for Suspected COVID-19 Case

Date: __/__/_____ S.No.....

Patient's Name		
Patient's Age	Sex:- ☐ Male ☐ Female	DOB:
Patient' s Temporary address	Province:_____ District: _____ Municipality:_____ Ward: _____	
Patient's Permanent address	Province:_____ District: _____ Municipality:_____ Ward: _____	
Patient's Contact Details	Landline: _____ Mobile: _____ Email: _____	
Name of hospital where patient is admitted		
Patient's Hospital ID		
Type of Collected Sample	Nasopharyngeal ☐ Oropharyngeal (Throat) ☐ Sputum ☐ Endotracheal Aspirate ☐ Bronchioalveolar ☐ Serum ☐ Others *If others, Please Specify*_____	

Symptoms:

ILI ☐	Fever ☐	Cough ☐
SARI ☐	Duration :-	Duration :-
Co morbidity	Temp. recorded (°F)	Sputum:- Yes ☐ No ☐

Additional symptoms? If any, specify _____

Travel History in last 14 days?
☐No ☐Yes

Country visited by you (If yes) -

H/O close contact with positive COVID-19 patient?
☐No ☐Yes

Is the patient admitted in isolation ward/unit in hospital?
☐No ☐Yes

Lab result to be communicated:-
Name:-
Phone No.:-

Chest X-ray and CT Scan finding if any:-

**This form is to be filled mandatory by clinicians to send sample for COVID-19 test.*
**Sample from patient not meeting WHO case definition and not in isolation facility won't be accepted for COVID-19 testing.*
**Sample should be collected and transported in VTM with triple layer packaging and cold chain maintenance.*

Attending Physician:
Signature:
NMC number:
Contact Number:

To be filled by NPHL:
Sample Received: Without Cold Chain Management ☐ Without proper patient information ☐ Others: .....

For further information please visit https://www.nphl.gov.np/
Contact Person: Mr. Rajesh Kumar Gupta (9851239988)

Sample Document-IV (Sample Tracking Form)

National Influenza Center

Date: 2020-06-11 / 2077-02-29

DF110001 Patient Id: 471649 Name here Corona Virus Test 25 Y Male 9851180778 Q45	SAMPLE Entry	ALIQUOTING	EXTRACTION	MM PREPARATION TEMPLATE ADDITION	PCR RUN	Pt info entry by:	Result entry by:	RESULT VALIDATION:
	Entry date/ time:	Done by:	Done by:	MM prepared by-	Platform-	User:	User:	By-
	Labelling done by: 1. 2	Completed on: Date/time- Aliquot for extract- Fridge no	Kit/ Platform used	Template added by:	Date/Time-	Date/ time-	Date/ time-	Date/ time-
	Remarks:	Archive aliquot- fridge no:	Date/ time-	Date/ time-	User:	Remarks	Remarks	Remarks
DF110002 Patient Id: 471650 Name here Corona Virus Test 40 Y Male 9851180778 Q46	SAMPLE Entry	ALIQUOTING	EXTRACTION	MM PREPARATION TEMPLATE ADDITION	PCR RUN	Pt info entry by:	Result entry by:	RESULT VALIDATION:
	Entry date/ time:	Done by:	Done by:	MM prepared by-	Platform-	User:	User:	By-
	Labelling done by: 1. 2	Completed on: Date/time- Aliquot for extract- Fridge no	Kit/ Platform used	Template added by:	Date/Time-	Date/ time-	Date/ time-	Date/ time-
	Remarks:	Archive aliquot- fridge no:	Date/ time-	Date/ time-	User:	Remarks	Remarks	Remarks
DF110003 Patient Id: 471652 Name here Corona Virus Test 34 Y Male 9851180778 Q47	SAMPLE Entry	ALIQUOTING	EXTRACTION	MM PREPARATION TEMPLATE ADDITION	PCR RUN	Pt info entry by:	Result entry by:	RESULT VALIDATION:
	Entry date/ time:	Done by:	Done by:	MM prepared by-	Platform-	User:	User:	By-
	Labelling done by: 1. 2	Completed on: Date/time- Aliquot for extract- Fridge no	Kit/ Platform used	Template added by:	Date/Time-	Date/ time-	Date/ time-	Date/ time-
	Remarks:	Archive aliquot- fridge no:	Date/ time-	Date/ time-	User:	Remarks	Remarks	Remarks
DF110004 Patient Id: 471653 Name here Corona Virus Test 35 Y Male 9851180778 Q48	SAMPLE Entry	ALIQUOTING	EXTRACTION	MM PREPARATION TEMPLATE ADDITION	PCR RUN	Pt info entry by:	Result entry by:	RESULT VALIDATION:
	Entry date/ time:	Done by:	Done by:	MM prepared by-	Platform-	User:	User:	By-
	Labelling done by: 1. 2	Completed on: Date/time- Aliquot for extract- Fridge no	Kit/ Platform used	Template added by:	Date/Time-	Date/ time-	Date/ time-	Date/ time-
	Remarks:	Archive aliquot- fridge no:	Date/ time-	Date/ time-	User:	Remarks	Remarks	Remarks

Sample Document-V

Aliquoting Worksheet

Analyst/ Team: **Date & Time:**

Name of aliquoting site: **Sample volume:**

SN	Covid Lab ID	SN	Covid Lab ID	SN	Covid Lab ID
1		16		31	
2		17		32	
3		18		33	
4		19		34	
5		20		35	
6		21		36	
7		22		37	
8		23		38	
9		24		39	
10		25		40	
11		26		41	
12		27		42	
13		28		43	
14		29		44	
15		30		45	

TOTAL NUMBER OF SAMPLE ALIQUOTED: **SAMPLE HANDOVER TO:**

Sample Document- VI

VIRAL RNA EXTRACTION WORKSHEET

Name of Laboratory: BSL-3, NPHL	Extraction kit:
Kit Lot No:	Date of expiry:
Number of sample:	Sample volume:
Date of extraction:	Analyst:

S.N.	SPECIMEN ID NO.	S.N.	SPECIMEN ID NO.
1		21	
2		22	
3		23	
4		24	
5		25	
6		26	
7		27	
8		28	
9		29	
10		30	
11		31	
12		32	
13		33	
14		34	
15		35	
16		36	
17		37	
18		38	
19		39	
20		40	

NOTE(if Any) :

Sample Document -VII
(Master mix /PCR worksheet)

Initial _____
Date_____
Experiment No._____
Signature_____

Master Mix Protocol for 2019-nCoV (PLEASE WRITE YOUR COMPANY KIT NAME)

Components	Per rxn µl	Total amount	Cycling Condition:	Positive: FAM, VIC, Cy5: ≤32
PCR reaction Solution A	----		Hold 1 50ºC for 15 mins Hold 1 95ºC for 15 mins Cycle - 45 94ºC for 15 sec 55ºC for 45 sec Reporter Dyes: FAM: N gene VIC : ORF1ab gene Cy5/ ROX: Internal Control (IC) OTHER DYE NAME: …… *RUN PC value(For all: <32* *RUN NC Value(For all: >40* Invalid if >40 or N/A	Negative: FAM, VIC, Cy5/ROX: > 40 OR … Re-test: Cy5/ROX (IC/RP) > 40 or N/A Inconclusive result: *If FAM & VIC in between 32-40 repeat the test.*
PCR reaction Solution B	---			
Total mater mix	----			
Template	---			
Total volume:	----			

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

REMARKS:

(FRONT PAGE)

PLEASE WRITE CT VALUE OF POSITIVE & CONTROL OR STANDARD

LAB ID	Cy 5/ROX	FAM	VIC	OTHER DYE	Remark	LAB ID	Cy5	FAM	VIC	OTHER DYE	Remark
PC						PC					
NC						NC					

(BACK SIDE PAGE)

Sample Document- VIII (Report Format)

ABC Laboratory, Kathmandu-10, Nepal
Ph. Number: 01-123345

REPORT FOR SARS Cov-2 VIRUS DETECTION

PATIENT INFORMATION

Patient Name: XXX
Address: XXX
Age: XX
Gender: X

Address: XX
Phone: XXXXXX
email: _____@gmail.com
Referring Physician: Dr. XX

SPECIMEN INFORMATION

Institute where sample is collected: *YY site*
Transportation condition: *cold chain maintained in ice box*
Specimen: *NP in VTM*
Sample ID: *101/A2020*

Sample collection date: __/__/_____
Sample received date: __/__/_____

RT-PCR RESULT*			
Sample type	Oropharyngeal swab/Nasopharyngeal swab	**Assay Kit**	Sansure Biotech Inc., Hunan, China
Reporting Date	__/__/_____	**Result**	**SARS CoV-2:** DETECTED
Diagnosis and Interpretation		**Positive**	

Assay Description: This kit is used for qualitative 2 detection of the ORF1ab and N genes of SARS-CoV-2 RNA. Internal control targeting the RNase P gene monitor the sample collection, sample handling and rRT-PCR process to avoid false-negative results. The LoD of the kit is 200 copies/mL.

_____ _____

Performed by Verified by

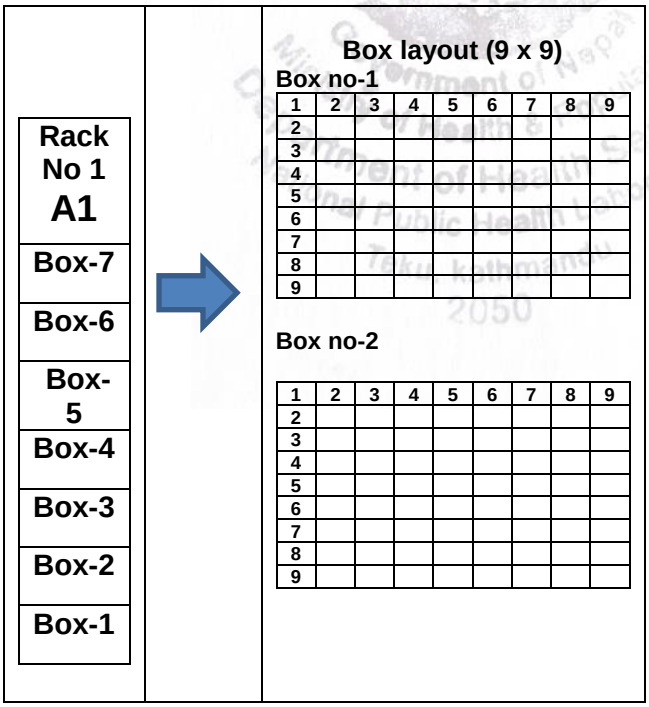
Sample Document-IX (Sample archiving Sheet)

NATIONAL PUBLIC HEALTH LABORATORY

FREEZER LOG (-80^{0}C)

FREEZER NAME..... MODEL NO..... .REQUIRED TEMP^r.....
INSTALLATION DATE..... DEPARTMNT.....AUTHORITY.....

COMPARTMENTS: ROW 1-3, COLUMN-11(A1 – K1)										
1	2	3	4	5	6	7	8	9	10	11
A1	B1	C1	D1	E1	F1	G1	H1	I1	J1	K1
A2	B2	C2	D2	E2	F2	G2	H2	I2	J2	K2
A3	B3	C3	D3	E3	F3	G3	H3	I3	J3	K3



Sample Document-X

(Incident Reporting Form)

National Public Health Laboratory	LIST OF FORMATS	FORMAT. NO.: Com10
	NPHL	Date of Issue.: 28-08-2019
		Rev.No.: 00
	ISO 15189:2012	Effective Date.: 01-09-2019

Date of Incident	Staff Name	Age/sex	Contact no	Time of incident	Accident/ incident type causes	Notifiable occurrence	Action taken	Reported to supervisor name	Remark

Annex 10 EQA PROGRAM FOR COVID-l9 PCR TESTING LABS
http://nphl.gov.np/uploads/EQA%20PROGRAM%20FOR%20COVID-19%20PCR%20TESTING%20LABS-min.pdf

List of Contributors

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5) Ms. Lilee Shrestha, Head- QC and training unit NPHL
6) Dr. Saugat Shrestha, WHO Nepal
7) Dr. Shankar Kafle, Consultant Pathologist, NPHL