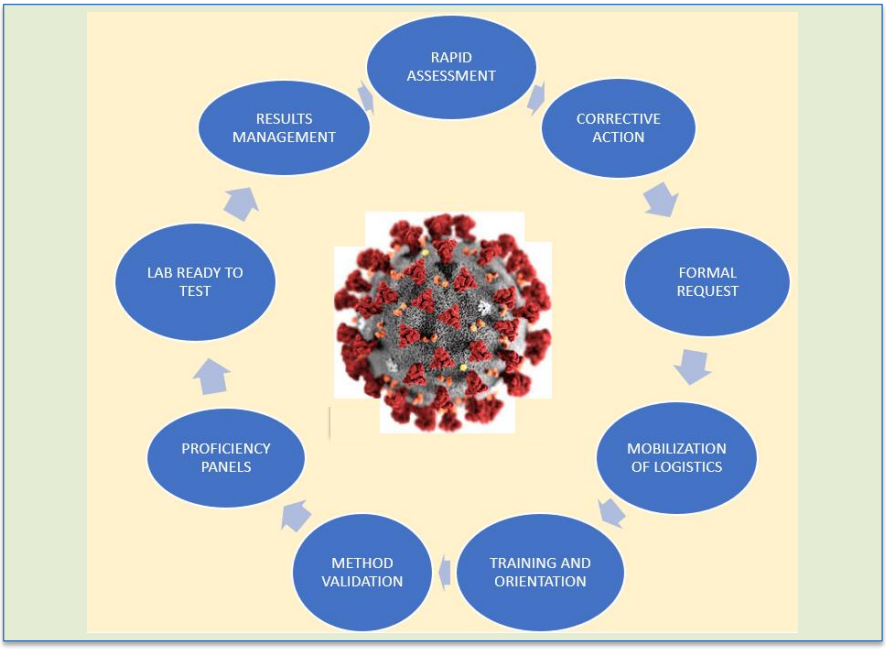
THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

MINISTRY OF HEALTH

STANDARD OPERATING PROCEDURE (SOP) MANUAL FOR ACTIVATING AND IMPLEMENTING DECENTRALIZED COVID-19 LABORATORY TESTING



MAY 2020

PREFACE

The Ministry of Health through the COVID-19 National Task Force recommended to make testing services accessible to all its citizens by decentralizing COVID-19 testing to lower facilities in addition to the larger central national referral testing laboratories in Kampala. This will improve access to testing as well as reducing the waiting periods for results.

These Standard Operating Procedures (SOPs) are intended to systematically guide the conduct of activities related to COVID-19 Laboratory Testing and the associated Quality Assurance activities. Any laboratory that will be allowed to test for COVID 19 will ensure that the laboratory staff read, understand and comply with the individual procedures and adopt them into their laboratory Quality Management System.

The SOPs have been developed with information and guidelines that are currently available. However, when more and newer information becomes available, the SOPs will require to be reviewed, updated, and validated on a regular basis

These SOPs should be adapted for use as reference SOPs by the respective laboratories customized to their local settings without diminishing the operational guidance of the coordination ~~between the~~ by central MOH ~~and the districts~~.

These SOPs are applicable for use by both public health facilities, research institutions, academic institutions such as university laboratories, private-not-for-profit (PNFP) and private laboratories

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INSTITUTION NAME	**SOP NAME:** **LABORATORY CERTIFICTION FOR COVID TESTING**
SOP Number:	**Effective Date:**

	Name	Designation	Signature	Date
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1.0 Background

SARS-Cov-2, the virus that causes Coronavirus disease-19 (COVID-19) disease is new and laboratory-based diagnosis is an area undergoing rapid evolution based on research and refinement over time to increase the accuracy, acceptability, reliability and efficiency of test results.

Laboratories need to be assessed and certified to ensure that certain requirements are met for them to conduct COVID-19 testing in order to ensure quality results.

2.0 Purpose

The purpose of this document is to describe the steps to be taken to certify a laboratory for COVID-19 testing in Uganda.

3.0 Abbreviations

WHO- World Health Organization

NIC- National Influenza Centre

PCR- Polymerase Chain Reaction

UVRI- Uganda Virus Research Institute

4.0 Definition of terms

SARS-CoV-2: The strain of coronavirus that causes coronavirus disease 2019 (COVID-19), a respiratory illness.

COVID-19: A disease caused by a new strain of coronavirus. 'CO' stands for corona, 'VI' for virus, 'D' for disease, 19 for 2019.

Validation: The process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Laboratory certification: The process of confirming that the laboratory meets specific regulations and standards.

5.0 Responsibilities

NIC: Conduct training to laboratories certified for COVID-19 testing

Perform validation of protocols to be used by certified labs in COVID-19 testing.

MOH: Conduct rapid assessment and issue certification of labs for COVID-19 testing

Certified laboratory: Conduct COVID-19 testing

6.0 Procedure for Lab Certification

6.1 Initiation of certification process

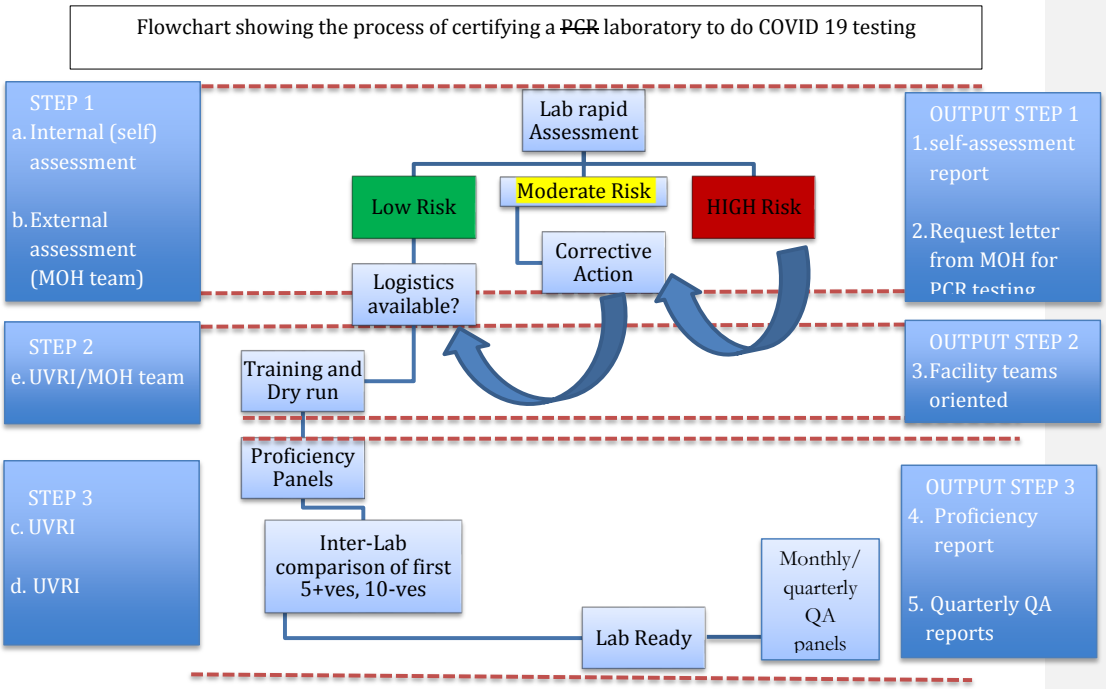
All laboratories intending to enroll for COVID-19 patient testing both public and private sector, shall make their intentions known to the Ministry of Health through an official letter from the responsible offices of such laboratories. Laboratories in the private for profit sector shall procure their own test kits and use only such test kits as have been validated and authorized by the MoH for use in Uganda.

6.2 Risk assessment

A team of officials from the MOH will conduct a rapid assessment to determine the level of risk of exposure for the laboratory.

The standardized assessment tool focuses on five major areas i.e, human resource capacity, infrastructure, logistics, biosafety standards and quality assurance.

As shown in figure below, only laboratories ranked as **Low risk** using a WHO recommended country customized assessment tool will be recommended for the next steps of certification for COVID-19 testing.



Step 1: Following successful risk assessment, the Ministry of Health will select qualifying laboratories and provide letters of recommendation to start the activation process for COVID-19 testing. This letter will equally define test logistic modalities and accessibility through the national lab logistics support system on a case by case basis. The laboratory shall provide a written acceptance/non-acceptance response within an appropriate time period.

Step 2: The proposed testing lab shall be visited by UVRI/MOH teams to orient the staff on the additional procedures of testing and results return in compliance with outbreak response reporting. Alternatively, key lab team members shall visit UVRI for this process orientation.

Step 3: Following orientation, the proposed lab shall be supplied with Proficiency Panels to do comparative testing on their devices prior to initiation of full-scale testing. Upon receipt of results, UVRI shall provide certification confirming the lab's preparedness to test. Despite this certification, the lab shall ensure to return to UVRI their first five (05) positives and ten (10) negative COVID 19 samples for Inter-Lab comparison.

7.0 References

WHO- Laboratory biosafety guidance related to coronavirus disease (COVID-19)

8.0 Appendices None

Institution Name	**SOP NAME:** **SOP FOR REAL TIME PCR COVID 19 DIAGNOSIS**
SOP Number:	**Effective Date:**

	Name	Designation	Signature	Date
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1.0 Introduction

In the wake of exponential spread of COVID-19, efforts have been vested in development of specific primers and standardized laboratory protocols for COVID-19 to facilitate the increasing need for laboratory diagnosis. The National Influenzas Center (NIC) based at Uganda Virus Research Institute, is the WHO accredited regional referral laboratory for COVID-19 testing in Uganda using the Berlin protocol, a real time PCR protocol. With decentralization of testing, other laboratories with different protocols may participate after thorough assay verification to qualify for COVID-19 diagnosis. These assays include other real time PCR protocols all designed to target different virus genes. Among the first real-time RT-PCR assays is the Berlin protocol (Charité, Germany) assays targeting the RNA-dependent RNA polymerase (RdRp) in the Open reading frame ORF1ab region, Envelope (E), and Nucleocapsid (N) genes of SARS-CoV-2. Literature provides that RdRp assay among these gene assays had the highest analytical sensitivity with RdRp probe 1 also called "pan Sarbeco-Probe" capable of detecting SARS-CoV-2, SARS-CoV, and bat SARS-related coronaviruses, and probe 2, termed as "RdRp-P2" reported to be specific for SARS-CoV-2 and not any other. Table 1 below describes such protocols.

Table 1: Examples of established protocols with their respective target genes

Institute	Gene targets
China CDC, China	ORF1ab and N
Institut Pasteur, Paris, France	Two targets in RdRP
US CDC, USA	Three targets in N gene
National Institute of Infectious Diseases, Japan	Pancorona and multiple targets, Spike protein
Charité, Germany (Berlin Protocol)	RdRP, E, N
HKU, Hong Kong SAR	ORF1b-nsp14, N
National Institute of Health, Thailand	N

Sourced from: WHO inhouse assays document

2.0 Objectives and Scope

2.1 Objectives

To provide procedure for PCR methods used in testing appropriate samples for COVID-19..

2.2 Scope

This document describes the steps to be taken to perform PCR testing in laboratories that meet the testing criteria for diagnosing COVID-19 in Uganda.

3. Principle of Reverse Transcriptase PCR

The process involves the reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the cDNA.. The test tube reaction amplifies a specific (desired) DNA segment a millions times to detectable levels. The process goes through the major steps of denaturation (strand separation), annealing (primer hybridization) and extension (amplification by thermostable polymerase), the process is repeated 30 to 40 times (PCR cycles). All the processes are performed at regulated optimal temperatures.

In quantitative PCR (qPCR), DNA amplification is monitored at each cycle of PCR. When the DNA is in the log linear phase of amplification, the amount of fluorescence increases above the background. The point at which the fluorescence becomes measurable is called the threshold cycle (CT) or crossing point. By using multiple dilutions of a known amount of standard DNA, a standard curve can be generated of log concentration against CT. The amount of DNA or cDNA in an unknown sample can then be calculated from its CT value.

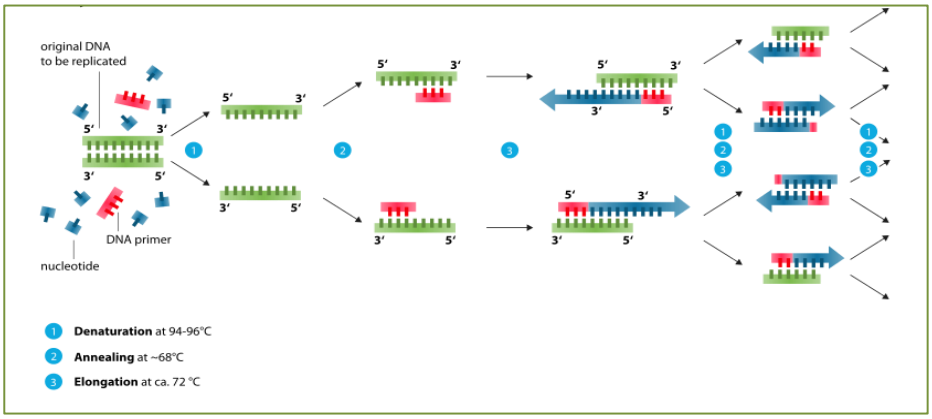


Figure 1: Illustration of PCR Reaction

3. Abbreviations, Terms and Definitions

Ct: Cycle Threshold

RT PCR: Reverse Transcriptase PCR

qPCR: Quantitative PCR or Real time PCR

CV: Coefficient of Variation

IQC: Internal Quality Control

ISO: International Organization for Standardization

LoD: Limit of detection

NIC: National Influenza Centre

PCR: Polymerase Chain Reaction

PPE: Personal Protective Equipment

QA: Quality assurance

QC: Quality Control

dNTPs: Deoxynucleotide 5' triphosphates

SOP: Standard operating procedure

UVRI: Uganda Virus Research Institute

WHO: World Health Organization

Analytical Sensitivity (LoD): the lowest concentration of analyte that could be reliably detected at least 95% of the time

Analytical Specificity: ability of an assay to measure on particular organism or substance, rather than others, in a sample.

Coefficient of Variation (CV): ratio of the standard deviation to the mean usually expressed in percentage.

Cycle threshold: the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level)**.** Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

Reverse Transcriptase PCR: involves the reverse transcription of SARS-CoV-2 RNA into complementary DNA (cDNA) strands, followed by amplification of specific regions of the cDNA.

Real Time PCR or Quantitative PCR: is achieved by monitoring the amplification reaction using fluorescence. Combined RT-PCR and qPCR are used for analysis of gene expression and quantification of viral RNA.

4. Responsibilities

Task	**Responsible Party**
Select and procure new methods/equipment	Ministry of Health/Laboratory Director
Performing the test	Trained and competent Technologist at bench
Review of results reports	Trained competent Technologist
Approves results release	Laboratory Director

5. Requirements

5.1 Specimen

Respiratory material such as nasopharyngeal and oropharyngeal swabs are suitable for the detection of respiratory pathogens. Upper respiratory samples are broadly recommended, although lower respiratory samples are recommended for patients exhibiting productive cough. Upper respiratory tract samples include nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal washes, and nasal aspirates.

5.2 Components of the PCR

- Deoxynucleotide 5' triphosphates (dNTPs) for example Deoxyadenosine 5' triphosphates (dATP), Deoxycytide 5' triphosphates (dCTP), Deoxythymide 5' triphosphates (dTTP) and Deoxyguanosine 5' triphosphates (dGTP).
- DNA polymerase (thermostable, from *Thermus aquaticas*)
- Primers, forward and reverse
- Ions (Mg2+)
- DNA template (contains your target sequence)
- Buffer
- Water

6. Safety and Environment

Handle all types of specimens (including venous and capillary whole blood, serum/plasma, oral fluid, etc.) as potentially infectious. Apply appropriate universal precautions to minimize exposure to infectious hazards. Put on appropriate PPE at all times when dealing with equipment, reagents, control materials and patient samples during this procedure.

Follow specific guidelines for COVID-19 sample and biowaste handling as established by the laboratory.

7. Quality Control

Quality control procedures are intended to monitor reagent and assay performance.

Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.

Always include a negative control and the appropriate positive control in each amplification and detection run. All clinical samples should be tested for human RNAse P gene to control for specimen quality and extraction. Viral transport media or negative specimen can be used as a negative control.

Good clinical laboratory practice (GCLP) recommends including a positive extraction control in each nucleic acid isolation batch.

8. Procedure for Real time PCR

8.1 Determine RT-PCR approach

In performing RT-PCR, one-step and two-step methods are the two common approaches, each with its own advantages and disadvantages.

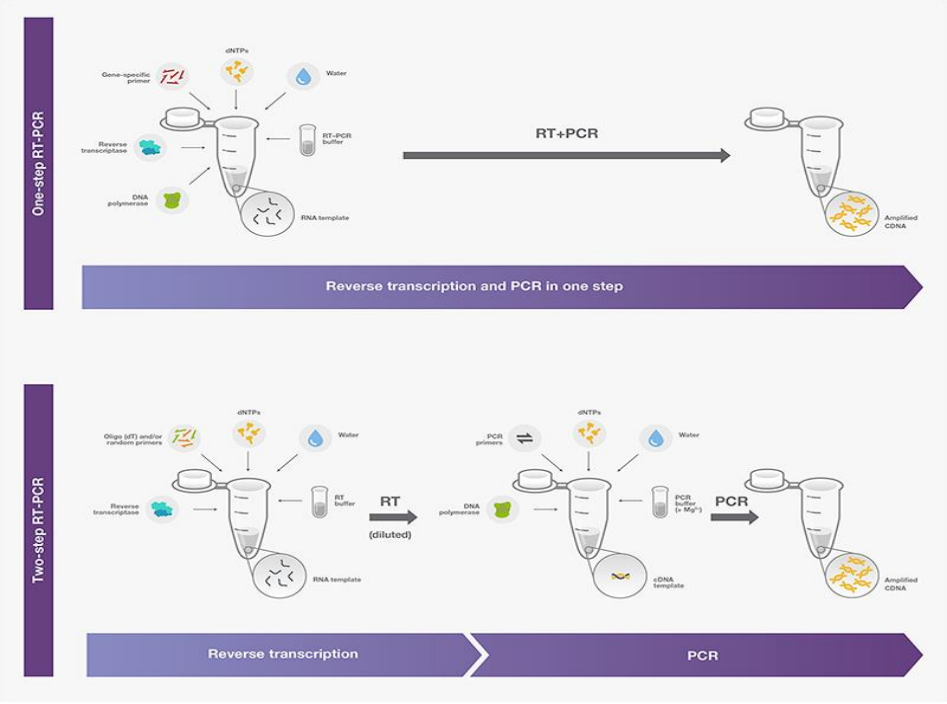


Figure 2: Illustration of One step and two step RT PCR Reactions

One-step RT-PCR combines first-strand cDNA synthesis (RT) and subsequent PCR in a single reaction tube. However, two-step RT-PCR entails two separate reactions, beginning with first strand cDNA synthesis (RT), followed by amplification of a portion of the resulting cDNA by PCR in a separate tube.

8.2 General Sample analysis

Treat the sample with the provided extraction buffer as to remove substances, such as proteins and fats, and extracts only the RNA present in the sample. This extracted RNA is a mix of a person's own genetic material and, if present, the coronavirus' RNA. RNA is reverse transcribed to DNA using a reverse transcriptase enzyme.

Then add primers which are oligonucleotides complementary to specific parts of the transcribed viral DNA. These primers target sections of the viral DNA if the virus is present in a sample. Some of the added oligonucleotides are for building DNA strands during amplification, while the others are for building the DNA and adding marker labels to the strands, which are then used to detect the virus.

The mixture is then placed in a RT-PCR machine. The thermocycler cycles through temperatures that heat and cool the mixture to trigger amplification of the target sections of viral DNA facilitated by polymerase enzyme. The cycle repeats and each cycle doubles the previous amount: two copies become four, four copies become eight, and so on. A standard real time RT-PCR setup usually goes through 35 cycles, which means that by the end of the process, around 35 billion new copies of the sections of viral DNA are created from each strand of the virus present in the sample.

Amplicons of the viral DNA sections are built, the marker labels attach to the DNA strands and then release a fluorescent dye, which is measured by the machine's computer as and presented in real time on the screen. The computer tracks the amount of fluorescence in the sample after each cycle.

8.3 Specific Sample processing and analysis BERLIN Protocol

Set up a 25-µl reaction containing 5 µl of RNA, 12.5 µl of 2 X reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen; containing 0.4 mM of each deoxyribonucleotide triphosphates (dNTP) and 3.2 mM magnesium sulfate), 1 µl of reverse transcriptase/Taq mixture from the kit, 0.4 µl of a 50 mM magnesium sulfate solution (Invitrogen – not provided with the kit), and 1 µg of nonacetylated bovine serum albumin (Roche).

Perform thermal cycling at 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15 s, 58°C for 30s.

8.4 Data analysis and Results interpretation

In a real time PCR assay a positive reaction is detected by accumulation of a fluorescent signal measured in form of Ct values. Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level). Ct levels are inversely proportional to the amount of target nucleic acid in the sample (ie the lower the Ct level the greater the amount of target nucleic acid in the sample).

Ensure threshold setting is above the maximum level of Blank Control

Quality control: Prior to evaluating the specimen results, the Positive Control and Blank Control should be interpreted. Negative control should be undetected. Positive control should be detected with Ct > 38

If the Positive Control and Blank Control do not meet the criteria, the entire run is invalid and results should not be reported. Repeat the entire process (specimen and control preparation, amplification and detection).

Positive Results

Cts < 29 are strong positive reactions indicative of abundant target nucleic acid in the sample

Cts of 30-37 are positive reactions indicative of moderate amounts of target nucleic acid

Cts of 38-40 are weak reactions indicative of minimal amounts of target nucleic acid which could represent an infection state or environmental contamination.

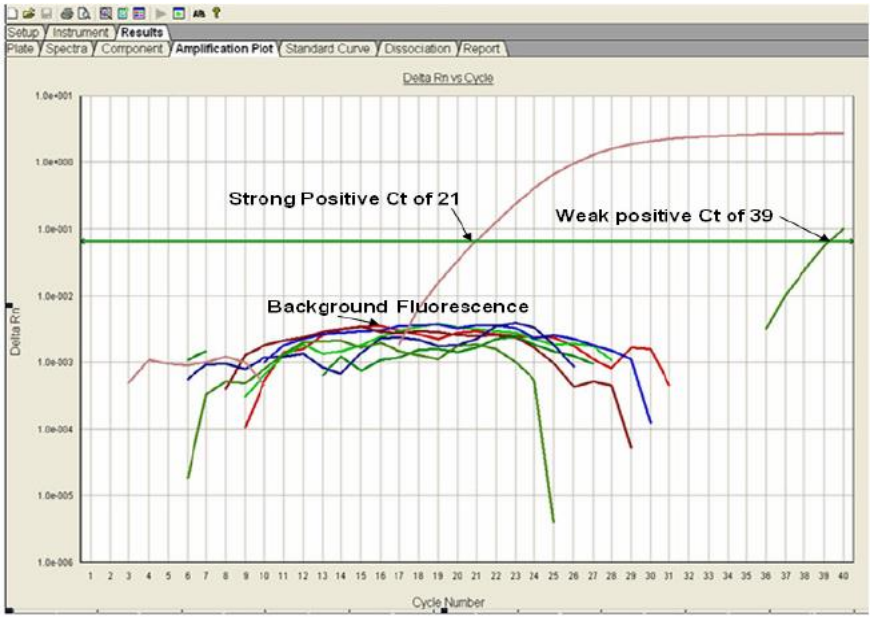


Figure 3: Illustration of amplification plot

NOTE

1. **Every laboratory shall send their first ten (10) negative and five (05) reactive tests for independent verification testing at UVRI.**

7.0 Limitations

• All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.

• Negative results do not preclude 2019-nCoV infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.

• A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.

• Do not use any reagent past the expiration date.

• If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.

• Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nCoV is the causative agent for clinical symptoms.

7.0 Related documents

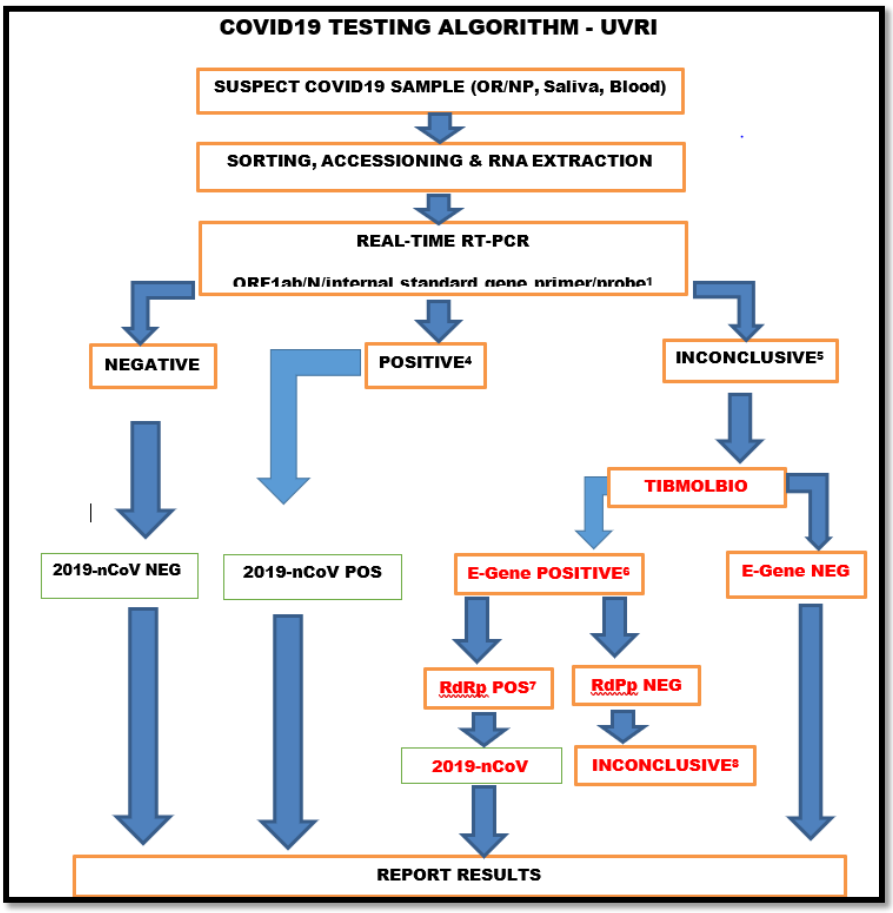
None

8.0 References

- ISO 15189: 2012: Medical laboratories — Requirements for quality and competence
- https://www.who.int/docs/defaultsource/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2 accessed on 17/5/2020
- COVID 19 PCR Charité, Germany (Berlin) protocol
- Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated *In Vitro* and with Clinical Specimens. Jasper Fuk-Woo Chan, Cyril Chik-Yan Yip, Kelvin Kai-Wang To, Tommy Hing-Cheung Tang, Sally Cheuk-Ying Wong, Kit-Hang Leung, Agnes Yim-

Fong Fung, Anthony Chin-Ki Ng, Zijiao Zou, Hoi-Wah Tsoi, Garnet Kwan-Yue Choi, Anthony Raymond Tam, Vincent Chi-Chung Cheng, Kwok-Hung Chan, Owen Tak-Yin Tsang, Kwok-Yung Yuen
Journal of Clinical Microbiology Apr 2020, 58 (5) e00310-20; **DOI:** 10.1128/JCM.00310-20

9.0 Appendices



[1]Specific primers and fluorescent probes are designed (N gene probe is labeled with FAM and ORF1ab probe VIC) for the detection of 2019-nCoV in the specimens.

[2] Negative control (ORF1ab/N): no obvious amplification curves for FAM and VIC detection channels, and obvious amplification curve for Cy5 channel.

[2] Positive control (ORF1ab/N): obvious amplification curves for FAM and VIC detection channels and Ct ≤ 32, and amplification curve for Cy5 channel

Institution Name	**SOP NAME:** **METHOD VERIFICATION FOR COVID 19 LABORATORY DIAGNIOSIS**
SOP Number:	**Effective Date:**

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1.0 Introduction

SARS-Cov-2, the virus that causes Coronavirus disease-19 (COVID-19) has continued to spread globally leading to health and economic ramifications. SARS-Cov-2, a novel Corona virus requires early detection and therefore good quality diagnostic tools that offer high sensitivity and specificity. In Uganda, the National Influenza Center (NIC) based at Uganda Virus Research Institute, is a WHO accredited regional referral laboratory for COVID-19 testing in Uganda using the Berlin protocol, a real time PCR protocol as the gold standard. The dynamics surrounding the spread of COVID-19 infections in Uganda has prompted health and other experts to quickly benchmark and increase laboratory diagnosis, a critical phase to curb the exponential spread of disease throughout the county. Other laboratories in country have thus been identified and recommended by the Ministry of Health to decentralize COVID-19 testing some of which implement similar quantitative PCR protocols to the Berlin protocol implemented at NIC while others are implementing test methods including GeneXpert (rapid qualitative PCR test), and Rapid Diagnostic Tests (RDTs) which are immunoassays.

These different methods have been validated by manufacturers for COVID-19 testing and their verification is aimed at providing evidence as to whether the level of performance, claimed by the manufacturer is achievable within these laboratories selected for testing decentralization.

2.0 Objectives and Scope

2.1 Objectives

This procedure is applicable for verification of equipment and methods used in COVID-19 samples testing.

2.2 Scope

This document describes the steps to be taken to perform method verification for methods and equipment that will used for COVID-19 testing in laboratories identified for testing decentralization in Uganda.

3. Abbreviations, Terms and Definitions

CLIA : Clinical Laboratory Improvement Amendments

CLSI: Clinical and Laboratory Standards Institute

Ct: Cycle Threshold

CV: Coefficient of Variation

IQC: Internal Quality Control

ISO: International Organization for Standardization

LoD: Limit of detection

NIC: National Influenza Centre

PCR: Polymerase Chain Reaction

PPE: Personal Protective Equipment

QA: Quality assurance

QC: Quality Control

SD: Standard Deviation

SOP: Standard operating procedure

UVRI: Uganda Virus Research Institute

WHO: World Health Organization

Analytical Sensitivity (LoD): the lowest concentration of analyte that could be reliably detected at least 95% of the time

Analytical Specificity: ability of an assay to measure one particular organism or substance, rather than others, in a sample.

Coefficient of Variation (CV): ratio of the standard deviation to the mean usually expressed in percentage.

Cycle threshold: the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level)

Precision: Reapitability or reproducibility of a measurement. The arithmetic average of a group of values. This is measured by the coefficient of variation.

Sensitivity: Ability of a test to correctly identify those with the disease as diseased (identify positive as positive).

Specificity: Ability of a test to correctly identify those without the disease as not diseased (identify negative as negative).

Standard Deviation: A statistic which describes the dispersion about the mean.

Validation: confirmation, through the provision of objective evidence that the requirements for a specific intended use or application have been fulfilled (ISO 15189:2012). This process is used to confirm that the analytical procedure employed for a specific test is suitable for its intended use.

Verification: confirmation, through provision of objective evidence, that specified requirements have been fulfilled. (ISO 15189:2012). It is confirmation of the manufacturer's claims about method's performance.

4. Responsibilities

Task	Responsible Party
Select and procure new methods/equipment	Ministry of Health/Laboratory Director
Verify new methods/equipment	Technologist at bench
Review method/equipment verification reports	Trained competent Technologist
Approves methods and equipment for use	Laboratory Director

5. Safety and Environment

Handle all types of specimens (including venous and capillary whole blood, serum/plasma, oral fluid, etc.) as potentially infectious. Apply appropriate universal precautions to minimize exposure to infectious hazards. Put on appropriate PPE at all times when dealing with equipment, reagents, control materials and patient samples during this procedure.

Follow specific guidelines for COVID-19 sample and biowaste handling as established by the laboratory.

6. Procedure

6.1 Selection of Testing Procedures

The methods/ equipment selected are standard methods which have been validated for COVID-19 testing.

6.4 Performance characteristics to verify

Performance characteristics verified for a particular method or equipment will depend on those validated by the manufacturer. Some of the performance characteristics to be verified include;

Qualitative tests; Sensitivity, Specificity, positive and negative predictive values.

Quantitative tests; Accuracy, Precision (Precision within run & Precision between run), Analytical sensitivity (Limit of detection – LoD), Analytical Specificity, Clinical Evaluation. Linearity, Analytical Measurement Range (AMR) and Reportable ranges (RR)

6.5 Source of data used for verification of the methods and equipment.

Data used for verification is obtained from running of quality control materials or other known COVID-19 samples or Proficiency testing samples supplied by UVRI. While testing these samples, ensure they are a mix of both positive and negative samples.

6.6 Performing Method Verification for SARS-CoV-2 RDTs and GeneXpert

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens collected from individuals suspected of COVID-19 by their healthcare provider.

6.6.1 Sensitivity and Specificity

Sensitivity: ability of a test to correctly identify those with the disease as diseased (ability to identify true positives). It is expressed as a percentage.

Specificity: ability of a test to correctly identify those without the disease as not diseased (ability to identify true negatives). It is expressed as a percentage.

6.6.1.1 Methodology

Obtain a minimum of 20 samples from UVRI. Use at least 10 positive and 10 negative samples. Analyze the data comparing the expected test result to the laboratory obtained results for the same sample.
Count the number of Laboratory results that conform to the expected result as and those that do not conform.

6.6.1.2: Results

Enter results into the following 2x2 sensitivity/specificity table

Expected Results (Reference method)

Laboratory result (Method/Equipment being verified)		**Those with disease Positive (P)**	**Those without disease Negative (N)**	**Total**
	Positive (P)	True Positive (TP)	False Positive (FP)	**TP+FP**
	Negative (N)	False Negative (FN)	True Negative (TN)	**FN+TN**
	Total	**TP+FN**	**FP+TN**	**TP+FN+FP+TN**

Sensitivity = $\frac{\text{True Positive}}{\text{True positive + False Negative}}$ x 100 %

= $\frac{TP}{TP + FN}$ X 100 %

Specificity = $\frac{\text{True Negative}}{\text{True negative + False Positive}}$ x 100 %

= $\frac{TN}{TN + FP}$ X 100 %

6.6.1.3 Reporting

Percentages obtained are the respective sensitivity and specificity of the method being verified.

Compare the sensitivity and specificity obtained with manufacturer's claims using the table template below.

	Sensitivity	Specificity

	Manufacturer's Claims /Laboratory set criteria	Laboratory Result	Acceptance Criteria	Manufacturer's Claims/ laboratory set criteria	Laboratory Result	Acceptance Criteria
Method / Equipment being verified						

The Laboratory accepts a method or equipment when its specificity and sensitivity are greater than or equal to (> or =) manufacturer's claims for the method or equipment.

When the analysis is completed, write a verification report inclusive of an acceptance statement at the end of the report. The report is then reviewed by a trained and competent person and then approved by the Laboratory director. Approval by the Laboratory Director gives confirmation that the method or equipment can then be used by the laboratory for testing patients' samples.

When a method's sensitivity and specificity don't meet the established acceptance criteria, the cause of the failure is investigated, and corrective action is implemented. Verification is repeated after implementation of corrective action.

Note:

1. If the results meet the established acceptance criteria, the method is accepted for use within the laboratory.
2. If the results do not meet the established acceptance criteria, the method is rejected and cannot be used for testing in the laboratory

6.7 Performing Method and Equipment Verification for Quantitative PCR Protocols.

6.7.1 Analytical Sensitivity (LoD)

Obtain a sample with a known concentration.

Make percentage concentrations by diluting the samples with an appropriate sample diluent. Make up to at least 5 tubes.

Run samples from each of the tubes using the laboratory's documented procedure. For each tube, make two (2) runs and enter results into the table as test result 1 and test result 2.

Obtain the mean of test result 1 and test result 2 for both the copies/µl and CT values.

Obtain the tentative LoD as the lowest concentration at which all replicates were positive.

Confirm this tentative LoD by testing at least 10 replicates with concentrations at the tentative limit of detection. The final LoD is confirmed to be the lowest concentration resulting in positive detection with a minimum 95% positivity.

Compare the LoD to manufacturer claims.

Note: *Use the manufacturer's claimed LoD if Laboratory obtained LoD is smaller than what the manufacturer provided.*

6.7.2 Analytical Specificity

Test the protocol with other pathogens that are likely to be present in the clinical specimen to identify the homology between the primers/probe of the assay and the pathogens.

Check for cross-reactivity with non-SARS-CoV-2 species if available.

6.7.3 Clinical evaluation

Use at least 10 positive and 10 negative samples.

Positive samples used should span from (1-2x LoD and higher) if possible.

Test the samples using the established procedure

Populate a 2x2 table below

Expected Results (Reference method)

Laboratory result (Method/Equipment being verified)		**Positive (P)**	**Negative (N)**	**Total**
	Positive (P)	True Positive (TP)	False Positive (FP)	**TP+FP**
	Negative (N)	False Negative (FN)	True Negative (TN)	**FN+TN**
	Total	**TP+FN**	**FP+TN**	**TP+FN+FP+TN**

Calculate the sensitivity and specificity using the formulae in 6.6.1.2 and compare results to manufacturer's claims for acceptance.

6.7.4 Precision

6.7.4.1 Precision within run

Obtain at least 3 known positive samples with differing viral loads

Make at least of 10 replicates from each sample on the same plate and analyze using the established laboratory SOP on the same day.

Obtain the Ct values for each test and enter the data into an excel sheet

The mean, standard deviation (SD) and coefficient of variation (CV) and % CV are calculated.

Enter the information into the template below;

Sample ID	Concentration (Copies/µl)	Mean Ct	%Replicate detection	Ct Coefficient of Variation (CV)	Ct percentage CV
1					
2					
3					

Depending on what the manufacturer has provided as the measure of precision, compare the laboratory results to the manufacturers.

Result interpretation

If Laboratory's SD or CV or % CV ≤ Manufacturer's SD or CV or %CV, within run precision is acceptable.

6.7.4.2 Precision between run

Obtain at least 3 known positive samples with differing viral loads

Make at least 5 replicates of each sample on the same plate and analyze using the established laboratory SOP.

Repeat testing on the same samples across at least four days.

Obtain the Ct values for each test and enter the data into an excel sheet

The mean, standard deviation (SD) and coefficient of variation (CV) and % CV are calculated.

Enter the information into the template below;

Sample ID	Concentration (Copies/µl)	Mean Ct	%Replicate detection	Ct Coefficient of Variation (CV)	Ct percentage CV
1					
2					
3					

Depending on what the manufacturer has provided as the measure of precision, compare the laboratory results to the manufacturers.

Result interpretation

If Laboratory's SD or CV or % CV ≤ Manufacturer's SD or CV or %CV, between run precision is acceptable.

When the analysis is completed, write a verification report inclusive of an acceptance statement at the end of the report. The report is then reviewed by a trained and competent person and then approved by the Laboratory director. Approval by the Laboratory Director gives confirmation that the method or equipment can then be used by the laboratory for testing patients' samples.

When a method's performance does not meet the established acceptance criteria, the cause of the failure is investigated, and corrective action is implemented. Verification is repeated after implementation of corrective action.

Note:

2. If the results meet the established acceptance criteria, the method is accepted for use within the laboratory.
3. If the results do not meet the established acceptance criteria, the method is rejected and cannot be used for testing in the laboratory

7.0 Related documents

1. Equipment operator manuals
2. Equipment SOPs
3. Methods inserts

8.0 References

- ISO 15189: 2012: Medical laboratories — Requirements for quality and competence
- Equipment and methods validation reports

9.0 Appendices

- None

Institution Name	**SOP NAME:** **EXTERNAL QUALITY ASSURANCE FOR COVID-19**
SOP Number:	**Effective Date:**

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Date	**Version**	**Created by**	**Description of change**
May 15, 2020	1.0		Original Document

1.0 Introduction

To ensure that laboratories provide accurate and reliable results and reduce the risk of errors, implementing a quality management system (QMS) is important. However, in resource-constrained settings, the majority of laboratories are not accredited to international standards and may only be partially implementing elements of a QMS. Introducing a new test, particularly under outbreak conditions may therefore come with a high risk of errors.

External quality assurance is a critical element that laboratories should put in place to rapidly identify and minimize the risk of laboratory errors. In the absence of quality assurance (QA), use of inaccurate test results can lead to the wrong treatment and management decisions and lapses in surveillance of disease outbreaks.

2.0 Purpose

The purpose of this SOP is to define external assurance procedures for all COVID-19 testing laboratories.

3.0 Scope

The SOP covers frequency of EQA, management of COVID-19 EQA samples, reporting of results, management of results, evaluation of performance of the participating laboratory, provision of feedback by the EQA provider, confidentiality, management of unsatisfactory results and safety considerations.

4.0 Abbreviation

EQA- External Quality Assurance

QMS- Quality Management System

UVRI- Uganda Virus Research Institute

5.0 Definition

QMS- Is a set of interrelated or interacting elements that organizations use to formulate quality policies and quality objectives and to establish the processes that are needed to ensure that policies are followed and objectives are achieved.

External quality assurance: A system for objectively checking the laboratory's performance using an external agency or facility.

6.0 Responsibilities

Participating laboratory: Test the provided EQA samples/material and send back results to the EQA provider.

UVRI: Provides EQA material to the participating laboratory, evaluates performance and gives feedback.

7.0 Safety considerations

All EQA samples provided should be treated as highly infectious samples and appropriate safety measures should be practiced while handling the samples.

Disposal of leftover samples should be done according to the established laboratory procedure.

8.0 Procedure

8.1 Frequency of EQA

EQA panels shall be provided to all certified testing laboratories on a quarterly basis.

8.2 Management of EQA samples

EQA samples should be treated in the same manner as patient samples i.e they should go through the pre-examination, examination and post-examination processes.

8.2.1 Pre-examination process.

The samples should be accessioned and evaluated according to the acceptance and rejection criteria of the laboratory.

Samples that meet the acceptance criteria should then be taken through the examination process.

8.2.2 Examination process

COVID-19 EQA samples should not be tested by selected individuals within the laboratory. All laboratory staff should be given an opportunity to test EQA samples.

However only trained and competent staff should be allowed to test the EQA samples.

Equipment used in testing EQA samples should be in good working condition.

Reagents and consumables used in testing should be viable and potent. Expired and deteriorated materials should not be used.

EQA samples should only be tested after quality control has been done and met the acceptance criteria.

A report that is clear and legible should be developed using the provided results template.

8.2.3 Post examination process

Results should be reviewed by responsible personnel before they are submitted back to UVRI.

Results should be recorded in the appropriate information system of the testing laboratory.

Results should be submitted to UVRI within 5 days from the day of receiving the samples.

8.3 Evaluation of results.

Results will be evaluated using statistical methods and the laboratory overall performance will be determined.

8.4 Evaluation criteria

Performance of the laboratory will be defined as "**Satisfactory**" or "**unsatisfactory**".

A score of 100% will be regarded as satisfactory while any score less than 100% will be regarded as unsatisfactory for serological tests.

For PCR tests the results shall be compared to the results of the recommended method mean and shall preferably be within 95% Confidence interval.

A detailed feedback report will be sent back to the participating laboratory within the same month.

8.5 Confidentiality

The identity of participants in the EQA program shall be confidential and known only to persons involved in the operation of the EQA scheme.

All information supplied by a participant to the proficiency testing provider shall be treated as confidential.

8.6 Management of unsatisfactory results.

In case of unsatisfactory performance, the laboratory shall perform a root cause analysis and institute a suitable corrective action.

All remedial action/discussions shall be handled between the individual lab and UVRI

The feedback reports should be maintained at the participating laboratory and managed appropriately.

9.0 References

ISO 15189: 2012

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance

10.0 Appendices

- None

Institution Name	**SOP NAME:** **COVID-19 TESTING USING THE GENE XPERT**
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1.0 Introduction

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in either nasopharyngeal swab and/or nasal wash/ aspirate specimens/mid-turbinate swab specimens collected from individuals suspected of COVID-19 by their healthcare provider.

Results are for the detection of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in nasopharyngeal swab specimens and/or nasal wash/ aspirate specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. All Laboratories are required to report positive test results to the EOC as per the MoH guidelines.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpert Dx, GeneXpert Infinity and/or GeneXpert Xpress systems. The Xpert Xpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization

2.0 Principle of the Procedure

The Xpert Xpress SARS-CoV-2 test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays. The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized.

The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal swab specimens. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the

amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off, automated sample processing, and real-time RT-PCR for detection of viral RNA.

3.0 Materials provided

The xpert xpress SARS-CoV-2 kit contains sufficient reagents to process 10 specimens or quality control samples. The kit contains the following;

- Xpert xpress SARS-CoV-2 cartridges With integrated reagent tubes — 10
- Bead 1, Bead 2 and Bead 3 (freeze dried) — 1 of each per cartridge
- Lysis Reagent — 1.5ml per cartridge
- Binding Reagent — 1.5ml per cartridge
- Elution Reagent — 3.0ml per cartridge
- Disposable transfer pipettes — 12 per kit
- CD — 1 per kit
- Flyer — 1 per kit

4.0 Storage and Handling

- ✓ Store the Xpert Xpress SARS-CoV-2 cartridges at 2-28°C.
- ✓ Do not open a cartridge lid until you are ready to perform testing.
- ✓ Do not use a cartridge that is wet or has leaked

5.0 Warnings and precautions

- Treat all biological specimens, including used cartridges, as if capable of transmitting infectious agents. Because it is often impossible to know which might be infectious, all biological specimens should be handled using standard precautions.
- Follow safety procedures set by your institution for working with chemicals and handling biological specimens.
- Consult your institution's environmental waste personnel on proper disposal of used cartridges, which may contain amplified material.

Specimens

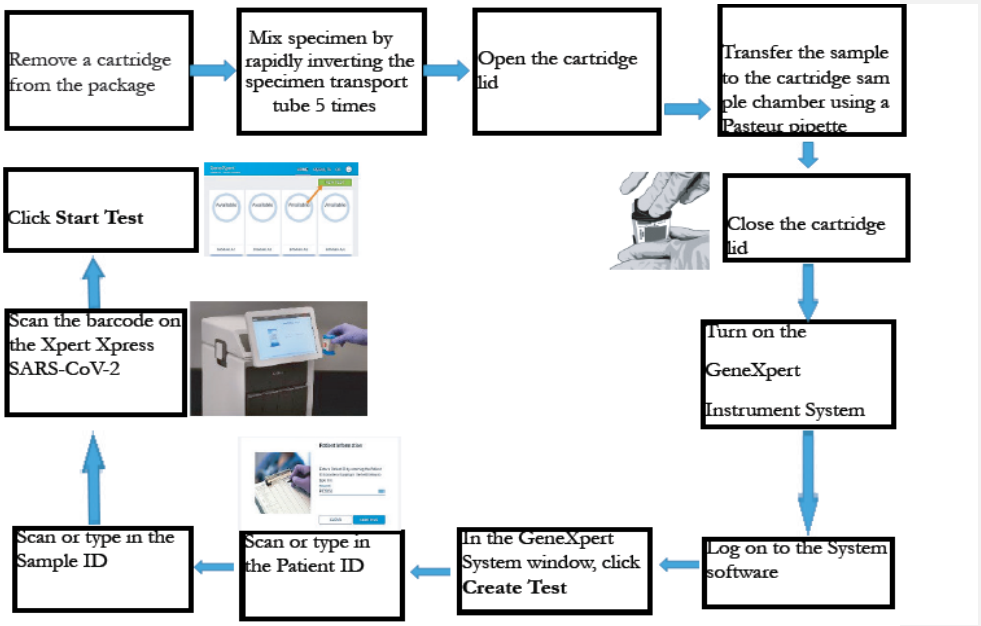
• Maintain proper storage conditions during specimen transport to ensure the integrity of the specimen

Assay/Reagent

Warnings and Precautions

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

6.0 PROCEDURE



7.0 Quality Control

Internal Controls

Each cartridge includes a Sample Processing Control (SPC) and Probe Check Control (PCC).

Sample Processing Control (SPC) – Ensures that the sample was processed correctly. The SPC verifies that sample processing is adequate. Additionally, this control detects sample-associated inhibition of the real-time PCR assay, ensures that the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction, and that the PCR reagents are functional. The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The SPC passes if it meets the validated acceptance criteria.

Probe Check Control (PCC) – Before the start of the PCR reaction, the GeneXpert System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity, and dye stability. The PCC passes if it meets the validated acceptance criteria.

8.0 Interpretation of Results

The results are interpreted automatically by the GeneXpert System and are clearly shown in the **View Results** window. The Xpert Xpress SARS-CoV-2 test provides test results based on the detection of two gene targets according to the algorithms shown in Table 1.

Xpert Xpress SARS-CoV-2 Possible Results Result Text	**N2**	**E**	**SPC**
SARS-CoV-2 POSITIVE	+	+	+/-
SARS-CoV-2 POSITIVE	+	-	+/-
SARS-CoV-2 PRESUMPTIVE POSITIVE	-	+	+/-
SARS-CoV-2 NEGATIVE	-	-	+
INVALID	-	-	-

Result	**Interpretation**
SARS-CoV-2 Positive	SARS-CoV-2 target nucleic acids are detected
SARS-CoV-2 Presumptive positive	SARS-CoV-2 target nucleic acids may be detected. Test should be repeated with a new cartridge. If presumptive positive results are obtained on the repeat test, the sample should be sent to UVRI.
SARS-CoV-2 Negative	SARS-CoV-2 target nucleic acids are not detecetd
Invalid	SPC does not meet acceptable criteria

	Nucleic acids can't be detected
Error	SARS-CoV-2 nucleic acids can't be detected

9.0 Limitations

- Performance of the Xpert Xpress SARS-CoV-2 has only been established in nasopharyngeal swab specimens. Specimen types other than nasopharyngeal swab may give inaccurate results.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if inadequate numbers of organisms are present in the specimen.
- As with any molecular test, mutations within the target regions of Xpert Xpress SARS-CoV-2 could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Xpert Xpress SARS-CoV-2 test but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19.

This test cannot rule out diseases caused by other bacterial or viral pathogens

10.0 Appendices

- None

Commented [EM1]: Add the COVD-19 test algorithm and infographics

Institution Name	**SOP NAME:** **RAPID TESTING USING ABBOTT COVID IGG/IGM RAPID TEST**
SOP Number:	**Effective Date:**

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1.0 Background

The COVID-19 IgG/IgM Rapid Test is an in vitro diagnostic rapid test for the qualitative detection of IgG and IgM antibodies to SARS-CoV-2 in human serum, plasma, venous and fingerstick whole blood. The Abbott COVID-19 IgG/IgM Rapid Test Device is for professional use only and is intended to be used as an aid in the diagnosis of SARS-CoV-2 infection. The product may be used in any laboratory and non-laboratory environment.

The test provides preliminary test results. Negative results will not preclude SARS-CoV-2 infection and they cannot be used as the sole basis for treatment or other management decision.

The test is not intended to be used as a donor screening test for SARS-CoV-2.

2.0 Principle of method

The Panbio™ COVID-19 IgG/IgM Rapid Test Device (Fingerstick Whole Blood/Venous Whole Blood/Serum/Plasma) is an immunochromatographic test for the qualitative detection of IgG and IgM antibodies to SARS-CoV-2. The test contains a membrane strip and a plastic housing. The test device shows the letters C, G and M at the right side of the reading window and the letter S above Specimen well of device where C stands for Control line, G for IgG test line, M for IgM test line and S for Specimen well, respectively. To use the test, the whole blood/serum/plasma is applied into the Specimen well (S) first and then two drops of buffer are applied. The mixture of specimen and buffer migrates along the membrane strip to the reading window. On the nitrocellulose membrane within the reading window anti-human IgG and anti-human IgM antibodies are precoated at the G area and M area separately and a goat anti-rabbit antibody is precoated at the C area. If the specimen is SARS-CoV-2 IgG antibodies positive, the G line will become visible. If the specimen is SARS-CoV-2 IgM antibodies positive, the M line will become visible. If the specimen is SARS-CoV-2 antibodies negative, only the C line will become visible. The Control line (C) must always be visible if the test has been performed correctly.

The visible Control line indicates that the result is valid. If the Control line does not appear, the test result is invalid. When the Control line, IgG test line and/or IgM test line are visible this indicates a positive result. When only the Control line is visible this indicates a negative result.

3.0 Materials
3.1Materials Provided

- ✓ Test Devices Individually Foil Pouched with a Desiccant
- ✓ Buffer (3mL/vial)
- ✓ Specimen Dropper (for fingerstick whole blood only)
- ✓ Instructions for Use

3.2 Materials Required but Not Provided

- ✓ Specimen collection equipment and containers
- ✓ Micropipette
- ✓ Lancet (for fingerstick whole blood only)
- ✓ Cotton wool or gauze pad (for fingerstick whole blood only)
- ✓ Centrifuge
- ✓ Timer
- ✓ Biohazard waste containers for sharps and non-sharps

3.3 Precaution/Kit Storage and Stability

- The test kit should be stored at 2-30°C (storage in refrigerator is permitted). Higher temperatures up to 55°C for a limited period of time (i.e. up to 48 accumulated hours) have no impact on test performance. Do not store the kit in the freezer.
- The test device and buffer are stable until the expiration date printed on the outer package. Do not use it beyond the expiration date.
- The test device must remain in the sealed pouch until use.
- Do not use it if the pouch is damaged or the seal is broken.
- The test device is recommended to be used at room temperature (15-30°C).
- Perform the test as soon as possible after removing the test device from its foil pouch (within one hour).
- After opening of the pouch the test is sensitive to relative humidity above 70%.
- Do not re-use the test device.
- EDTA has been validated and can be used as anticoagulant. Other anticoagulants have not been validated. Do not use specimen treated with other anticoagulants.
- Do not mix buffers from different lot of test kit.
- Do not touch the Specimen well or reading window of the test device directly with the finger as this can cause incorrect results.

4.0 Procedure

4.1 For finger stick whole blood

Fill the specimen dropper and transfer approximately 20 µL of fingerstick whole blood specimen to the Specimen well (S).

→

Subsequently, hold the buffer bottle vertically and add 2 drops of buffer (approximately 60 µL) to the Specimen well (S).

→

Start the timer and wait for 10 minutes

4.2 For venous whole blood

A micropipette may be used for 20 µL of whole blood application to the Specimen well (S)

→

Subsequently, hold the buffer bottle vertically and add 2 drops of buffer (approximately 60 µL) to the Specimen well (S).

→

Start the timer and wait for 10 minutes

4.3 For Serum or Plasma

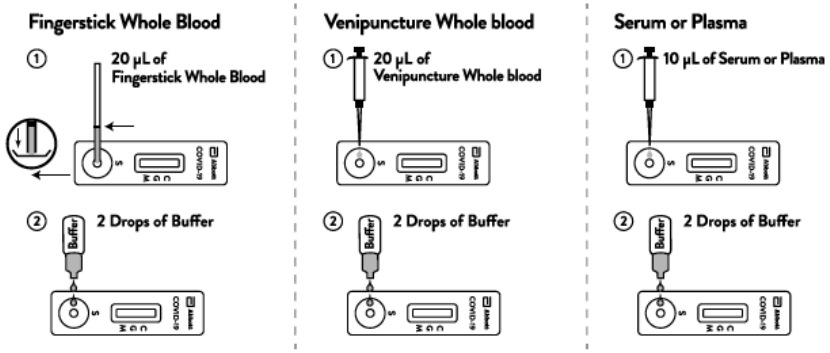
A micropipette may be used for 10 µL of serum or plasma application to the Specimen well (S).

→

Subsequently, hold the buffer bottle vertically and add 2 drops of buffer (approximately 60 µL) to the Specimen well (S).

→

Start the timer and wait for 10 minutes.



5.0 Interpretation of Results

- A red line will appear at the C area of the reading window to show that the test is working properly. This line is the Control line.
- A red line that might appear at the G area of the reading window is the IgG test line.
- A red line that might appear at the M area of the reading window is the IgM test line.

IgG POSITIVE: The presence of both Control line and IgG test line within the reading window indicates an IgG positive result.

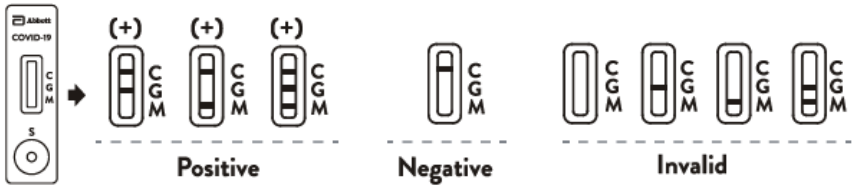
IgM POSITIVE: The presence of both Control line and IgM test line within the reading window indicates an IgM positive result.

IgG and IgM POSITIVE: The presence of Control line and IgM and IgG test line within the reading window indicates a positive result for both, IgG and IgM.

NOTE: The intensity of the color in the IgG and IgM test line regions may vary depending on the concentration of COVID-19 antibodies present in the specimen. Therefore, any visible line at the G area or the M area should be considered positive.

NEGATIVE: The presence of only the Control line and no IgM and IgG test line within the reading window indicates a negative result.

INVALID: No presence of the Control line in the reading window indicates an invalid result. If this occurs, it is recommended to read the IFU again and re-test the specimen with a new test device.



6.0 QUALITY CONTROL

A Control line is visible within the reading window after the test is performed.

The Control line is used in the test as a procedural control.

A visible Control line confirms that the lateral flow of the test is successful but is not the confirmation that the specimen and buffer have been applied properly.

Quality control specimens are not supplied in this kit; however, it is recommended that quality control specimens can be tested as a good laboratory practice.

7.0 LIMITATION

1. The Panbio™ COVID-19 IgG/IgM Rapid Test Device (Fingerstick Whole Blood/Venous Whole Blood/Serum/Plasma) is for in vitro diagnostic use only. This test is used for the detection of antibodies to SARS-CoV-2 in human serum, plasma and fingerstick and venipuncture whole blood. Other body fluids or diluted specimens may not give accurate result and should not be used.
2. For venipuncture whole blood and plasma, EDTA should be used as the anticoagulant. Other anticoagulants have not been validated and may give incorrect results.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the medical doctor.
4. The test detects SARS-CoV-2 antibodies that are formed as part of the body's immune response to the infection caused by this virus rather than detecting the virus itself.
5. A negative result does not eliminate the possibility of a SARS-CoV-2 infection, particularly in those who have been in contact with the virus. Follow-up testing with a molecular diagnostic should be considered to rule out infection in these individuals.
6. The test will show negative results under the following conditions: The titer of the novel coronavirus antibodies in the sample is lower than the minimum detection limit of the test, or the novel coronavirus antibody has not appeared at the time of sample collection.
7. There is no positive correlation between the intensity of a red line at G and/or M area and the titer of antibody in the specimen.
8. Positive results may be due to past or present infection with non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.
9. Reading test results earlier than 10 minutes or later than 20 minutes may give incorrect results

Institution Name	**SOP NAME:** **STANDARD Q COVID-19 ANTIGEN TEST**
SOP Number:	**Effective Date:**

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SOP FOR RAPID TESTING USING STANDARD Q COVID-19 ANTIGEN TEST

1.0 Introduction

Coronavirus is a single-stranded positive-sense RNA virus with an envelope of about 80 to 120 nm in diameter. Its genetic material is the largest of all RNA viruses and is an important pathogen of many domestic animals, pets, and human diseases.

It can cause a variety of acute and chronic diseases. Common signs of a person infected with a coronavirus include respiratory symptoms, fever, cough, shortness of breath. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure, and even death. The 2019 new coronavirus, or "2019-nCoV", was discovered because of Wuhan Viral Pneumonia cases in 2019, and was named by the World Health Organization on January 12, 2020, confirming that it can cause colds and the Middle East Respiratory Syndrome (MERS) and more serious diseases such as acute respiratory syndrome (SARS). This kit is helpful for the auxiliary diagnosis of coronavirus infection.

2.0 Test principle

STANDARD Q COVID-19 Ag Test has two pre-coated lines, "C" Control line, "T" Test line for the COVID-19 antigen on the surface of the nitrocellulose membrane. Both the control line and test line in the result window are not visible before applying any specimens. Mouse monoclonal anti-COVID-19 IgG antibody is coated on the test line region and mouse monoclonal anti-Chicken IgY antibody is coated on the control line region. Mouse monoclonal anti-COVID-19 IgG antibody conjugated with color particles are used as detectors for COVID-19 antigen device. During the test, COVID-19 antigen in the specimen interact with monoclonal anti-COVID-19 IgG antibody conjugated with color particles making antigen-antibody color particle complex. This complex migrates on the membrane via capillary action until the test line, where it will be captured by the mouse monoclonal anti-COVID-19 IgG antibody recombinant protein. A violet test line would be visible in the result window if COVID-19 antigens are present in the specimen. The intensity of violet test line will vary depending upon the amount COVID-19 antigen present in the specimen. If COVID-19 antigens are not present in the specimen, then no color appears in the test line. The control line is used for procedural control, and should always appear if the test procedure is performed properly and the test reagents of the control line are working.

3.0 KIT STORAGE AND STABILITY

Store the kit at room temperature, 2-40°C / 36-104°F, out of direct sunlight. Kit materials are stable until the expiration date printed on the outer box. Do not freeze the kit.

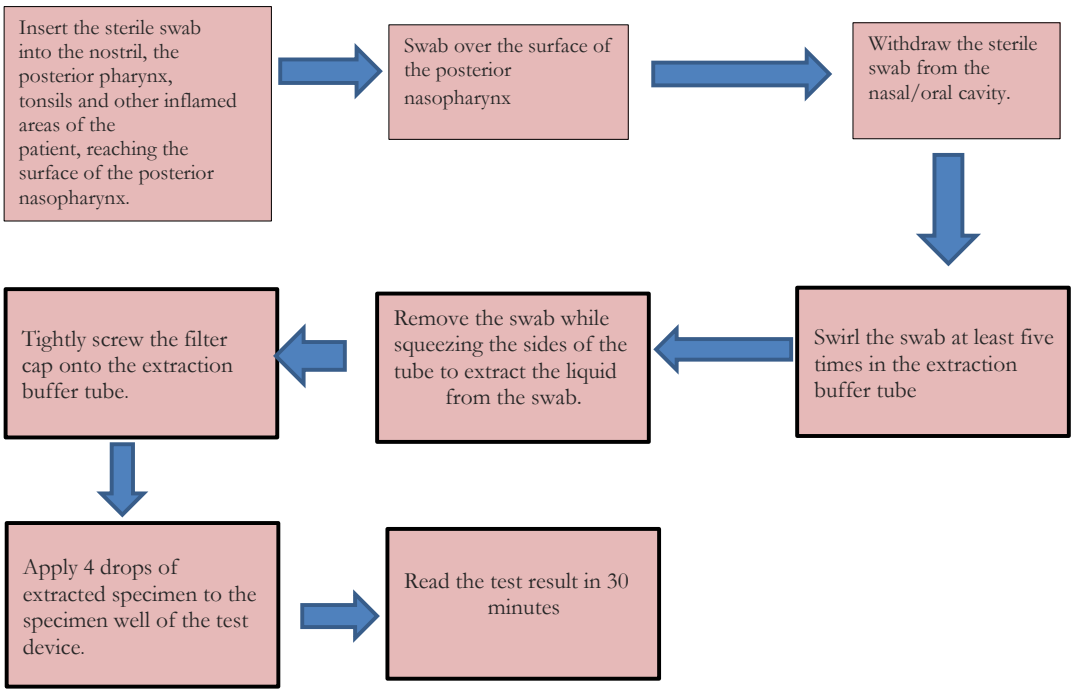
INTENDED USE

This test is for in vitro professional diagnostic use and intended as an aid to early diagnosis of COVID-19 infection in patients with clinical symptoms of COVID-19 infection. It provides only an initial screening test result. More specific alternative diagnosis methods should be performed in order to obtain the confirmation of COVID-19 infection.

Kit contents

1. Test device (individually in a foil pouch with desiccant)
2. Extraction buffer tube
3. Filter cap
4. Sterile swab
5. Instructions for use

4.0 PROCEDURAL STEPS



5.0 INTERPRETATION

- A coloured band will appear in the top section of the result window to show that the test is working properly. This band is control line (C).
- A coloured band will appear in the lower section of the result window. This band is test line of COVID-19 antigen (T).
- Even if the control line is faint, or the test line isn't uniform, the test should be considered to be performed properly and the test result should be interpreted as a positive result.

NOTE:

1. Positive results should be considered in conjunction with the clinical history and other data available to the physician.
2. The presence of any line no matter how faint the result is considered positive.

6.0 WARNINGS AND PRECAUTIONS

1. Do not re-use the test kit.
2. Do not use the test kit if the pouch is damaged or the seal is broken.
3. Do not use the extraction buffer tube of another lot.
4. Do not smoke, drink or eat while handling specimen.
5. Wear personal protective equipment, such as gloves and lab coats when handling kit reagents. Wash hands thoroughly after the tests are done.
6. Clean up spills thoroughly using an appropriate disinfectant.
7. Handle all specimens as if they contain infectious agents.
8. Observe established precautions against microbiological hazards throughout testing procedures.
9. Dispose of all specimens and materials used to perform the test as bio-hazard waste. Laboratory chemical and biohazard wastes must be handled and discarded in accordance with all local, state, and national regulations.
10. Desiccant in foil pouch is to absorb moisture and keep humidity from affecting products. If the moisture indicating desiccant beads change from yellow to green, the test device in the pouch should be discarded.

7.0 LIMITATION OF TEST

1. The test procedure, precautions and interpretation of results for this test must be followed strictly when testing.
2. The test should be used for the detection of COVID-19 antigen in human nasopharyngeal swab or Throat swab samples.

3. Neither the quantitative value nor the rate of COVID-19 antigen concentration can be determined by this qualitative test.
4. Failure to follow the test procedure and interpretation of test results may adversely affect test performance and/or produce invalid results.
5. A negative test result may occur if the level of extracted antigen in a sample is below the sensitivity of the test or if a poor-quality specimen is obtained.
6. For more accuracy of immune status, additional follow-up testing using other laboratory methods is recommended.
7. The test result must always be evaluated with other data available to the physician.

Institution Name	**SOP NAME:** **SAMPLE AND RESULTS MANAGEMENT AT RDT & PCR LABS**
SOP Number:	**Effective Date:**

	Name	Designation	Signature	Date
WRITTEN BY:				
REVIEWED BY:				
AUTHORIZED BY:				

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Change history

Date	**Version**	**Created by**	**Description of change**
May 15, 2020	1.0		Original Document

1.0 Background

Like for other public health emergencies, results management for COVID-19 follows the guidance as outlined by the command structure of the national task force on COVID-19 at the emergency operation center (EOC), Ministry of Health at national level and by the district rapid response teams (DRRTs) at district level.

The guidance on how results are reported to the public shall be done by the EOC and the DRRT to initiate the needed responses. Depending on the evolution of guidance from the EOC, the testing facility shall disseminate the results using the tools and through the channels approved by the NTF and DTF.

2.0 Principles of results management

a) Accuracy of the result

- The SOPs of the respective test method used should be followed during the test to ensure accuracy of the results
- The results for positives shall be verified by at least two senior laboratory officers prior to dissemination to the managing clinician (case management team) or EOC/DRRT.
- All remnant aliquots of positive specimen shall be archived in a temporary biorepository at the testing laboratory. At an appropriate time they will be transferred to the national biorepository for further confirmation and long term storage.

b) Documentation and chain of custody of the results

- All the results (both negative and positive) shall be recorded in a dedicated laboratory record book (not in the general laboratory register where other test data is recorded) or electronic laboratory information system ensuring that it aligns with the sample collection information or case investigation form on the respective test request forms.
- Each individual result (both negative and positive) shall be signed off by the lab manager or approved quality assurance focal person in the laboratory with the date and time of release as a sign of verification. A laboratory results' stamp should be used if available at the facility.
- Only approved laboratory staff shall release the results from the laboratory following the approved chain of custody from requester, EOC/DRRT, to final results owner.
- The return of the results to the EOC and DRRT shall be implemented using the appropriate electronic information systems for results management

c) How to handle samples sent without forms, forms without samples and poorly collected samples

- When a testing lab receives a batch of samples without request forms or poorly filled request forms, it should do the following;
    i. document these incidents in the sample reception log and also document it in the incident and occurrence log
    ii. the incident and occurrence log having these events shall be shared with the EOC and laboratory pillar on weekly basis for remedial action follow-up at the respective sample collection sites.
    iii. add the usual internal laboratory IDs on the specimen to enable effective internal audit for testing and results generation.
    iv. ensure to test and generate results on time following the FIFO procedure
    v. in case a laboratory is totally unable to carry out a test on a specific specimen, a rejection report should be written to the sample collection site highlighting the reason for rejection following the same urgency of results return as other positive results. This report should be considered as a result from the laboratory but shall not be counted among the cumulative total tests done.
    vi. For negative results generated for samples collected with no sample request form or poorly documented sample requests, these should be sent through the normal channels with indication on the report that the sample collection form had missing information.

d) Confidentiality

- The case management, laboratory supervision teams and EOC/DRRT are the primarily justified stakeholders to access COVID 19 results.
- Do not share patient identifiable information unless it is absolutely necessary; e.g. in cases of aggregate data collection, analysis and reporting, patient identifiers should not be shared.
- Access to patient identifiable information should be on a strict need-to-know basis or as pronounced in the study/testing protocol
- Everyone with access to patient identifiable information should be aware of their responsibilities
- There should be controlled use of electronic gadgets in the laboratory. Staff must refrain from using mobile electronic devices (for example, mobile telephones, tablets, laptops, flash drives, memory sticks, cameras, or other portable devices, including those used for DNA/RNA sequencing) when not specifically required for the laboratory procedures being performed.
- No results shall be released from the laboratory except using the approved channels and tools of reporting COVID 19 results. No photos, social media messages shall be used as a way of sending results out of the lab.

e) Urgency

- The reporting of positive results shall be handled as an emergency by the lab; immediately notifying the lab manager and quality assurance focal person who shall likewise relay the verified to EOC/DRRT and case management.
- All samples shall be tested in a first-in-first-out order (FIFO) to ensure minimal delay of results to customers. Samples that are received first should be documented and lined up for earlier testing. The laboratory should ensure to set up a system which documents the time of reception of each specimen.
- All completed results (whether individually produced or in batches) shall immediately be entered into the electronic results management system to enable timely utilization by respective EOC/DRRT and case management teams. For example, if a machine runs a batch of 93 samples, this batch of 93 results shall verified and immediately uploaded into the electronic results dispatch system as other specimen are being loaded into the machine.
- The sample reception desk shall ensure to reconcile the list of sample batches received with the list of results dispatched daily to ensure 100% concurrence. The missing/delayed results shall be reported to the lab manager or quality assurance officer for immediate follow-up. This reconciliation activity shall be done on daily basis to ensure good turnaround time of the results.
- In case of a special arrangement in which specimen received later are required for urgent testing ahead of earlier received specimen, the lab team shall document the reason for fast-track at the sample reception.

f) Intended use of the results

- The intended use of the results shall be guided by the terms of reference during activation of individual laboratories for COVID 19 testing e.g. epidemic response service delivery, rapid assessment surveys or sentinel surveillance. However, the primary purpose of epidemic response is the overarching purpose of results generation for all labs. In this respect, all results, positive and negative, shall be reported to the EOC/DRRT using the approved channels of reporting.
- The use of the generated results for scientific writing and research should follow approved protocols and institutional research bureau (IRB) approval. This is to ensure ethical use and scientific accuracy of the findings generated.
- In order to have a better understanding of how to ethically use of COVID results, it is strongly recommended (but not compulsory) for the laboratory staff and other health workers who intend to do scientific writing with COVID 19 data to do a short training in good clinical

practice (GCP). This can be accessed online and takes an average of one to three hours. It also requires refresher training every three years.

Flow diagram showing the process of results management at a COVID 19 testing laboratory