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Guidance on Quality Assurance for COVID-19 Molecular Testing in Nigeria Centre for Disease Control Network Laboratories

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Table of Contents

Acronyms	3
List of figures	3
1 Executive Summary	4
1.1 Negative consequences of laboratory errors	5
2 Objectives.....	7
2.1 Primary Objective	7
2.2 Secondary Objectives	8
3 Quality Control/Quality Assurance in the Laboratory	8
3.1 Quality Control	8
3.1.1 Extraction positive control	9
3.1.2 No template control.....	9
3.1.3 Positive template control.....	9
3.2 External Quality Assessment.....	10
3.2.1 Proficiency testing	11
3.2.2 Retesting	11
3.2.3 Onsite evaluation	11
3.3 Quality improvement	12
4 Annexes	13
Annex 1: Elements of Quality Assurance - Administrative	13
Annex 2: Elements of Quality Assurance - Sample Management	14
Annex 3: Elements of Quality Assurance – SAS-COV-2 Molecular Testing	15
5 Bibliography	16



List of abbreviations

COVID-19	Coronavirus disease-2019
EQA	External Quality Assurance
FMoH	Federal Ministry of Health Internal Control
IC	Medical Laboratory Science Council of Nigeria
MLSCN	National External Quality Assessment Laboratory
NEQAL	Nigeria Centre for Disease Control
NCDC	Negative extraction control
NEC	Nasopharyngeal
NS	Negative template control
NTC	Oropharyngeal
OP	Positive extraction control
PEC	Personal protective equipment
PPE	Positive template control
PTC	Quality Assurance
QA	Quality Control
QC	Quality improvement
QI RT-rtPCR	Reverse Transcription real-time Polymerase Chain Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome type 2
WHO	World Health Organization

List of figures

Figure 1: Path of workflow for laboratory processes.....	6
Figure 2: Model for laboratory Quality Management System.....	7



1 Introduction

The corona virus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in Wuhan, China in December 2019. The disease poses a major public health challenge globally and continues to spread in Africa since the first case in Egypt was reported on 14 February 2020. New evidence shows that the virus is highly contagious with a doubling time of 2.3–3.3 days, and spreads much faster than initially thought (doubling time of 6–7 days). Therefore, early detection of SARS-CoV-2 in infected people plays a critical role in limiting the transmission of COVID-19, ensures the isolation of COVID-19 patients to prevent community spread of the virus, and as well inform public health interventional measures.

In Nigeria, over 64,000 cases have been confirmed in all states of the Federation, with 1,163 deaths as at November 15th 2020. In order to rapidly contain the pandemic, the Presidential Task force on COVID-19 (PTF-COVID-19) and the Federal Ministry of Health (FMOH) has prioritised testing as one of the key interventions to the COVID-19 response; as increased testing, matched by swift isolation of confirmed cases and contact tracing reduces disease spread. The Nigeria Centre for Disease Control (NCDC) is the country's national public health institute, with the mandate to lead the preparedness, detection and response to infectious disease outbreaks and public health emergencies. As at November 15th 2020, 89 laboratories provide national testing capacity with an approximate combined testing output of approximately 2,500 samples daily.

Molecular testing conducted on nasopharyngeal (NS), oropharyngeal (OS) swabs or other upper respiratory tract specimens are the most commonly used and reliable tests for the diagnosis of COVID-19. A variety of ribonucleic acid (RNA) gene targets are used by different molecular assays, commonly targeting one or more of the envelope (env), nucleocapsid (N), spike (S), RNA-dependent RNA polymerase (RdRp), and the first open reading frame (ORF1) genes of SARS-CoV-2. Majority of these assays have achieved 100% specificity, since the oligonucleotides are designed specifically for the target gene sequences of SARS-CoV-2. However, sensitivity and specificity can be affected by specimen quality, sampling time to symptom onset, testing errors, or other technical deficiencies including equipment malfunctioning.

False-negative SARS-CoV-2 reverse transcription real-time polymerase chain reaction (RT-rtPCR) test results have been documented in a few positive cases after having two



consecutive negative PCR tests within a 24-hour period. This could be due to technical errors from sampling to testing. Both false- positive and false-negative results have negative implications for disease containment efforts. Therefore, it is critical to implement quality assurance measures (key critical elements to identify and minimise the risk of laboratory errors) in all testing laboratories in the COVID-19 network in Nigeria.

1.1 Negative consequences of laboratory errors

This broad concept is not exclusive to SARS-CoV-2 diagnostic testing but also applies to any kind of laboratory testing. Inaccurate COVID-19 diagnostic results can have significant consequences at the patient care or public health level.

At the patient care level, errors can lead to:

- o Failure to provide proper treatment to the patient
- o Unnecessary treatment, treatment complications, or additional expenses
- o Delay in correct diagnosis
- o Additional and unnecessary diagnostic testing

At a public health level, laboratory errors on identification can impact negatively on public health decisions in the following areas:

- o Delay in determining when the epidemic threshold has been reached and implementing needed public health decisions and measures
- o Inadequate recommendations of national control measure or treatment algorithms
- o Inappropriate choice of antibiotics or vaccines

These consequences result in increased cost in time, wasted resources and technical efforts, and poor patient outcomes in terms of morbidity and mortality.

To achieve the highest level of accuracy and reliability for COVID-19 testing in Nigeria, standard quality control/quality assurance (QC/QA) testing procedures and conditions must be practiced in laboratories on an everyday basis. A quality management system, which oversees the entire system, is very important for achieving optimal laboratory performance. Laboratory processes can be grouped into pre-examination, examination, and post- examination categories (Figure 1).

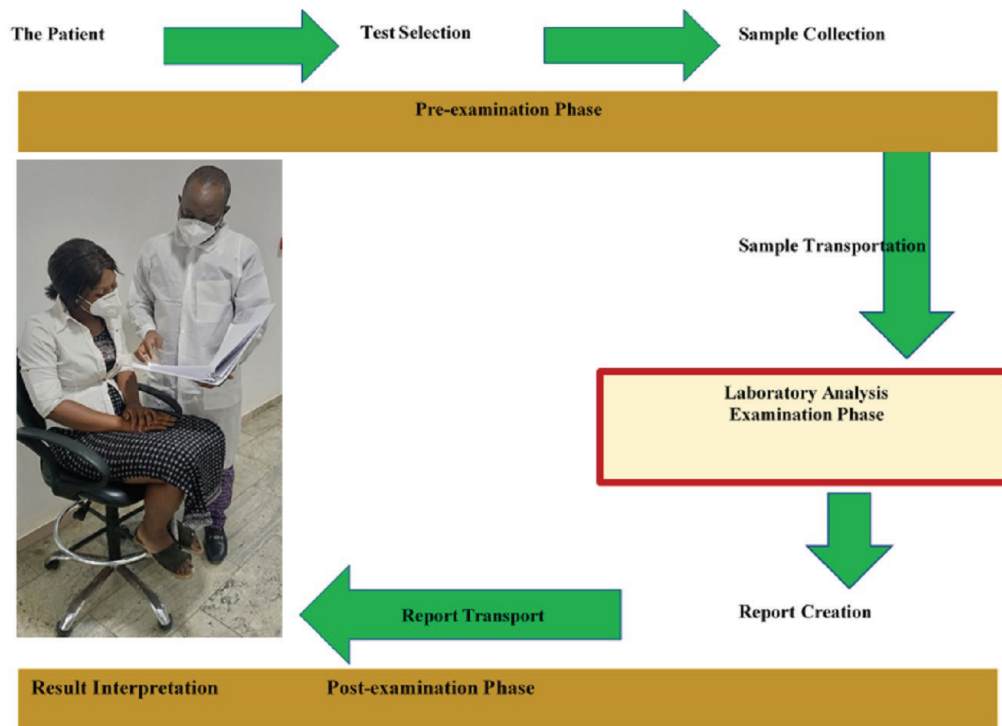


Figure 1: Path of workflow for laboratory processes

Quality management measures should be applied during the entire path of workflow that begins with the patient and ends in interpreting and reporting results. The quality management system is not only concerned with monitoring QC/QA programmes, but should also include administrative considerations that may indirectly influence the quality, efficiency and productivity of the laboratory operation. A widely used quality management system model organises all of the laboratory activities into twelve quality system essentials, which are a set of coordinated activities that serve as building blocks for quality management and include: Organisation and Supervision, Personnel, Equipment, Purchasing and Inventory Management, Documents and Records, Process Control, Information Management, Occurrence Management, Assessment, Customer Service, Process improvement and Facilities/Safety (Figure 2).

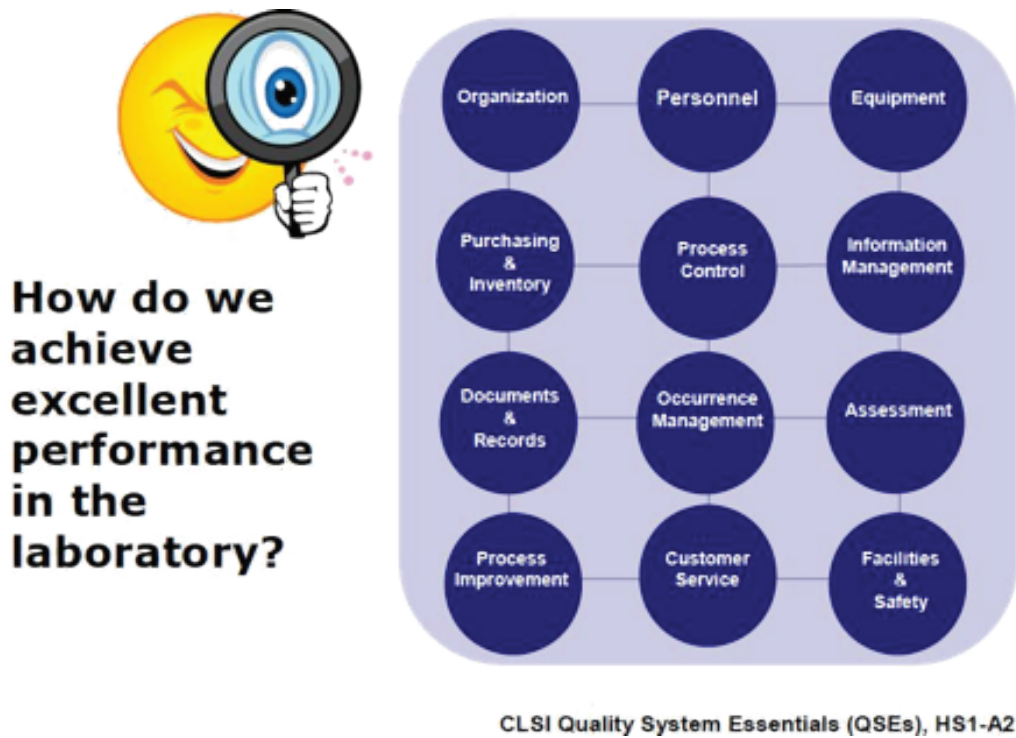


Figure 2: Model for laboratory Quality Management System

2 Objectives

The main purpose of this guidance document is to help testing laboratories in the COVID-19 network to implement key critical elements and systematic activities focused on providing confidence that laboratory quality requirements are fulfilled for COVID-19 diagnosis. The guidance emphasises the use of QC, Internal Quality Control (IQC), enrollment of laboratories in external quality assessment (EQA) schemes and use of EQA performance data for continuous quality improvement of COVID-19 diagnosis.

2.1 Primary Objectives

- o To implement measures that must be included in each assay to verify that the test is working properly
- o To ensure that the final results reported by a COVID-19 testing laboratory are as correct and accurate as possible



2.2 Secondary Objectives

To emphasise the use of in-house IQC and EQA for continuous quality improvement for SARS-CoV-2 testing in NCDC network laboratories.

3 Quality Control/Quality Assurance in the Laboratory

Quality Control/Quality Assurance can be defined as the set of planned and systematic activities focused on providing confidence that quality requirements will be fulfilled. In a medical laboratory, quality can be defined as accuracy, reliability, and timeliness of the reported test results. While QC refers to those measures that must be included in each assay to verify that the test is working properly, QA is the overall programme that ensures that the final results reported by the laboratory are as correct and accurate as possible. Quality Assurance

implemented through quality management systems is important for any testing service conducted in complex laboratories to point-of-care testing to continuously improve the reliability and efficiency of laboratory performance. It can be implemented to monitor the quality of COVID-19 testing laboratories in order to minimise error rates that may arise in all stages of laboratory processes (pre-examination, examination, and post-examination).

Quality Assurance in the laboratory encompasses four major areas: 1) Equipment, 2) Reagents/Kits, 3) Staff training and competency and 4) Facilities and Safety (Annexes 1-3). Systems should be in place to monitor and manage these areas as they directly impact the quality of results obtained by the laboratory. Three main components of QA should be implemented by

laboratories involved in the molecular diagnosis of SARS-CoV-2. These include: 1) Quality Control; 2) External Quality Assessment and 3) Quality Improvement.

3.1 Quality Control

Quality Control is a mechanism that monitors the analytical performance of the test when used with or as part of a test system. It may monitor the entire test system or only one aspect of the test. It is a process of systematic internal monitoring of the performance of bench work in COVID-19 testing laboratories, including instrument checks and verifying new lots of test kits. QC validates the competency of testing laboratories by assessing sample quality and monitoring test procedures, test kits, and instruments against established criteria. It also includes the review of PCR results and documentation of the validity of testing methods. Thus, QC is a multi-step process with certain checkpoints throughout the testing process: pre-examination, examination, and post-examination stages (Annexes 1–3). Overall, QC should



be performed regularly to detect, evaluate, and correct errors due to test system failure, environmental conditions, or operator performance before reporting test results. In addition to the QC checks indicated in Annexes 1–3, IQC should be performed routinely as by recommendation of the test manufacturer for particular molecular assays. For example, some assays have built-in or test kit controls, and using external QC may not be recommended. However, the laboratory must ensure that the extraction and amplification PCR processes are properly evaluated for quality using IQC in each test run. QCs that are commonly employed for SARS-CoV-2 RT-rtPCR testing include:

3.1.1 Extraction positive control

Used as an RNA extraction control to demonstrate successful recovery of RNA and the integrity of the extraction reagent. Extraction controls should be extracted and processed with each sample extraction run. They contain noninfectious cultured human cell (A549) material.

3.1.2 No template control

Checks contamination during specimen extraction (negative extraction control-NEC) and/or plate set up (negative template control-NTC). If any NTC reactions are defined positive, sample contamination may have occurred and the test must be repeated with strict adherence to the testing procedures. It also indicates whether PCR reagents have been compromised to determine the cycle threshold. Nuclease-free water or viral transport medium containing only internal control can also be used as no template control.

3.1.3 Negative control

Controls for specificity of the amplification reaction. Contains all reagents and a nucleic acid template closely related to the target sequence but lacking the target's signature.

3.1.4 Positive template control

Indicates the limit of detection and robustness of the assay. Positive template controls are in vitro-transcribed SARS-CoV-2 RNA, either gene fragment or whole-genome.



This control should be handled with caution in a dedicated nucleic acid handling area to prevent possible cross-contamination.

External positive controls can be obtained for the assay independently from the manufacturer or from QC providers, including national or World Health Organization (WHO) reference laboratories or commercial entities. Commercial QCs are preferred; however, laboratories can use patient samples with known viral RNA concentration, preferably samples with low cycling threshold (Ct) values (25–30) for the target sequences of SARS-CoV-2, as the positive template control.

3.1.5 Internal Control

Internal Control (IC), provides assurance that clinical specimens are successfully amplified and detected. Unless inhibitory specimens are identified, negative amplification test results do not necessarily indicate absence of infection. Inhibitory specimens can be identified by monitoring amplification of a second target nucleic acid, which is an internal control (IC). Obtaining a positive signal from the second target demonstrates successful amplification, thereby validating a negative result for the primary target. The IC can be of endogenous or exogenous origin.

3.1.6 QC failures

QC failures, for example, when a positive control turns out negative or a negative control turns out positive, invalidates the test results. The test must be repeated either from stored or newly collected samples after investigating and fixing the cause of the QC failure, such as contamination or degradation of samples, or expired reagents.

Additional Internal Quality Control measures that are easily implementable include:

- Repeat testing of selected previously tested and appropriately stored samples with CT values that are low, borderline, or negative, on a routine basis.
- Proficiency Testing of Laboratory Scientists on an annual or semiannual basis – this is deliberately administered by the Laboratory Manager or Supervisor by giving selected samples to Lab Scientists to run and the outcome is compared to the expected result and used for individual proficiency testing validation and documented in the staff file.
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- Amplicons recovery test – this is added internal quality to monitor presence of possible contaminants in the lab. This is done by swabbing target surfaces in the lab and testing to identify presence or contaminants. The frequency is determined by the volume of samples tested by the lab per month – it could be quarterly for very high throughput labs or at least annually.

3.2 External Quality Assessment

External Quality Assessment is a process that allows COVID-19 testing laboratories to assess their performance by comparing their results with results from other laboratories within the network (testing and reference laboratories) via panel testing and retesting. EQA also includes the onsite evaluation to review the quality of the laboratory performance and usually evaluates testing competency, the performance of the laboratories, reliability of the testing methods, and accuracy of the results reports, including follow-up for unacceptable EQA results with corrective action. One or more of the following three EQA methods can be applied for COVID-19 molecular testing laboratories, coordinated by National External Quality Assessment Laboratory (NEQAL) hosted in Zaria, Nigeria and NCDC National Reference Laboratory.

3.2.1 Proficiency testing

An external proficiency testing (PT) provider sends a set of SARS-CoV-2 positive and negative simulated clinical samples for testing in different laboratories and the results of all laboratories are analysed, compared, and reported back to the participating laboratories. The positive panels contain different genetic lineages of SARS-CoV-2. The Medical Laboratory Science Council of Nigeria (MLSCN) through NEQAL shall enroll and distribute PT panels for molecular SARS-CoV-2 tests to Nigerian laboratories in keeping with its regulatory and statutory functions of maintenance of good standard of medical laboratory practice as enshrined in section 19 (d) of the enabling ACT 11 of 2003. COVID-19 testing laboratories can be enrolled as part of the influenza laboratory network for free or at a cost not exceeding \$420 USD. NEQAL as an internationally accredited PT provider with a track record in delivering PT panels in Nigeria will be used. All COVID-19 testing laboratories should participate in proficiency testing every six months (bi-annually).



3.2.2 Retesting

Samples that have been tested at one laboratory are retested at another laboratory, allowing for inter-laboratory comparison. A laboratory's first positive COVID-19 sample should be sent to another testing laboratory, preferably a national or a WHO reference laboratory. In the absence of PT, national COVID-19 laboratories should send five positive and ten negative samples, systematically selected, to WHO reference laboratories for retesting bi-annually. Similarly, sub-national COVID-19 testing laboratories should send retesting samples to their national COVID-19 reference laboratory. This activity shall however, be coordinated in conjunction with MLSCN's NEQAL.

3.2.3 Onsite evaluation

Onsite evaluations should be performed by experienced subject matter experts, who observe and assess the quality management systems of the COVID-19 testing laboratories across the three testing phases. Onsite evaluation includes: 1) Patient management; 2) Sample collection procedures; 3) Standardized testing policies; 4) Documentation and maintenance of records; 5) Biosafety adherence; 6) Quality control procedures; 7) Staff competency. Onsite evaluation should be conducted at least annually, but preferably every three to six months. However, an immediate supervisory visit can be organised, if deemed necessary (e.g., EQA failures). A periodic onsite evaluation may not be feasible during the COVID-19 pandemic. However, onsite assessment should be conducted when selecting laboratories for COVID-19 testing. Use WHO Laboratory Assessment or nationally customized assessment checklist.

3.3 Quality improvement

Quality improvement (QI) for COVID-19 diagnosis is a process by which the components of SARS-CoV-2 testing services are analysed to identify areas requiring improvement, to plan and undertake improvements, and to evaluate the effectiveness of improvements. QI is also recognised as process improvement and involves continuous monitoring, identifying defects, and remedial action, such as refresher training, to prevent recurrence of problems. Data collection, data analysis, and creative problem solving are the key components of this process. It may require data from audits, participation in EQA schemes, and onsite evaluation to improve testing processes.



The ultimate target of QI is to take corrective action against the identified problem, remove its root cause, and reduce or eliminate its recurrence. Implementing preventive action reduces the likelihood of recurrence.

3.4 Implementation approach

The NCDC NRL in collaboration with MLSCN's NEQAL will coordinate and lead the implementation of QA/QC with support and partnership from other strategic Partners/Stakeholders as follows:

- Monitoring the implementation of IQC by PCR Labs in the Network
- Development of panels for proficiency testing implementation coordinated by NRL
- Distribution of panels to target labs and collation and analysis of results by NRL
- Facilitation of corrective actions as necessary to be coordinated by NRL
- Onsite monitoring based on schedules
- Enrollment of labs into National and or International Accreditation in coordination with the MLSCN and SLPTA/SLMTA programmes



4 Annexes

Annex 1: Elements of Quality Assurance - Administrative

Quality Control	Recommendations
Workplace	<ul style="list-style-type: none"> SARS-CoV-2 molecular testing should be performed in a secure, dedicated workspace Manual PCR requires a three-room set-up (extraction, addition, and detection) arranged in a unidirectional workflow Laboratories should be organised to allow efficient testing workflow
Staff Training and Competency	<ul style="list-style-type: none"> Staff should have technical knowledge and skills appropriate for laboratory work Staff should receive training on relevant technical and safety practices for SARS- CoV-2 molecular testing Job descriptions should be available for all staff Staff should take part in regular competency assessments, and if required, consider retraining. e.g., online course on COVID-19 diagnostics and testing Competency may be monitored by EQA panels or testing of testing of known patient specimens Annual competency must be monitored and witnessed Competency records must be kept in personnel files. If required re-training must be documented Staff must comply with approved SOPs Continuous training and professional development should be supported by management
Standard operating procedures (SOPs)	<ul style="list-style-type: none"> Laboratory should have SOPs for COVID-19 molecular testing SOPs should comply with current WHO recommendations or national guidelines SOPs should be kept up to date and written exactly as practiced in the laboratory SOPs and manuals should be located in the laboratory for easy access for all staff
Laboratory register	<ul style="list-style-type: none"> All tests performed should be recorded in a standard format in the laboratory register Use an approved register format in COVID-19 testing laboratories throughout the country network Laboratory registers should be located in the laboratory work area at all times and stored in a secure location Test results should be written directly into the register or electronic registry rather than transcribed from a worksheet
Data collection	<ul style="list-style-type: none"> Laboratories should collect and analyze data monthly Data should be collected on key performance indicators: <ul style="list-style-type: none"> Sample rejection rate Number of samples tested by sample category Number of samples tested by sample category Turn-around time Number of failed IQC results EQA/PT performance (pass/fail or % score)
Equipment	<ul style="list-style-type: none"> All laboratory equipment must be maintained in safe and working condition Laboratory records should show supplier, date of purchase, serial number, and cost of each piece of equipment Instrument manuals should be located near the equipment Staff should be trained on the use and maintenance of PCR instruments Equipment should be serviced as per the recommendation of the manufacturer, and service records should be kept in the laboratory
Supplies	<ul style="list-style-type: none"> Procure diagnostic kits as per the WHO Emergency Use Assessment and Listing and/or that have granted Emergency Use Authorization from national authorities Prioritize diagnostic kits with high performance characteristics in independently evaluated data using a large sample size Select suppliers that have local distributors or supply network in-country Carefully consider ancillary items during forecasting and procurement (e.g., extraction buffers, sample collection materials, etc.) Reagents or kits must be stored correctly and cold chain maintained where necessary Date of receipt, initials and when first opened should be recorded Stock should be monitored and managed with the principle of first in first out Expired reagents must be clearly marked and should be discarded OR marked for research use or training use only Documentation/worksheets must show batch/lot numbers and expiration dates SOPs must be written for test methods in use



<p>Facilities and Safety</p>	<ul style="list-style-type: none"> ○ Initial sample processing (before inactivation) should take place in a validated biological safety cabinet (BSC) or primary containment device ○ Non-propagative tests (sequencing and Nucleic acid amplification tests) should be conducted at a facility using procedures equivalent to Biosafety Level 2 ○ For GeneXpert, non-propagative tests can be performed on a bench without using a BSC, when the risk assessment so dictates, and proper precautions are in place. This includes wearing of appropriate personal protective equipment (PPE) and working in a well-ventilated area. ○ Laboratories should conduct risk assessment for intended testing and subsequently, based on the findings, decide on safety control measures to put in place (e.g., personal protective equipment) ○ Laboratory infrastructure- a safe work and laboratory environment must be provided ○ Adequate security should be in place- no unauthorized individuals should be allowed access to the laboratory or to customer or patient records or test results ○ Containment- all biohazards should be secured ○ Waste management- approved SOPs and policies should be in place to ensure safe disposal of all biohazardous and chemical wastes produced by the laboratory
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Annex 2: Elements of Quality Assurance - Sample Management

Quality Control	Recommendations
<p>Sample Collection</p>	<ul style="list-style-type: none"> ○ All samples collected from COVID-19 suspect patients should be considered as potentially infectious. In this regard, biosafety must be emphasized when handling and collecting samples. ○ Use appropriate personal protective equipment (gloves, solid front or wrap-around gown, face masks or respirators, if available) ○ Test request forms should capture all required information from the person being tested, for proper handling, reporting, and clinical care. ○ Upper respiratory samples (nasopharyngeal swab, oropharyngeal (throat) swab, or nasopharyngeal aspirate, or nasal wash) and lower respiratory samples (bronchoalveolar lavage, endotracheal aspirate, and expectorated sputum) are recommended for COVID-19 testing. ○ Use Dacron or polyester-flocked swabs for sample collection. Calcium alginate swabs and cotton swabs with wooden shafts are not recommended. ○ Label sample tubes with patient details, date and time of collection. Please note that expectorated sputum is ONLY for patients with a productive cough. Sputum induction is NOT recommended.
<p>Sample Transport</p>	<ul style="list-style-type: none"> ○ Laboratories that are unable to meet biosafety requirements should consider referring samples to reference laboratories ○ All samples should be stored at 2–8°C for up to 48 hours after collection. For handling or shipping after 48 hours, storage at -70°C is recommended. ○ Use viral transport medium (VTM), if a delay is unavoidable. Minimum essential media, sterile phosphate buffered saline, or 0.9% saline can be used as alternatives to VTM for SARS-CoV2 tests. ○ Samples should be transported as UN3373, ‘Biological Substance Category B’ using triple packaging and comply with national regulations. For overnight shipment, use shipment in an ice pack (temp 2–8°C).
<p>Laboratory</p>	<ul style="list-style-type: none"> ○ Samples must be handled efficiently to ensure prompt and accurate reporting of results ○ Details of submitted samples should be recorded on the laboratory register or entered into an electronic laboratory information system before tests are carried out ○ Samples should be evaluated as per the acceptance criteria, such as leakage, inadequate sample volume, and sample integrity, and reasons recorded if samples are rejected



Annex 2: Elements of Quality Assurance - Sample Management

Quality Control	Recommendations
Assay validation or verification	<ul style="list-style-type: none"> Validation or verification should be conducted to ensure test performance for intended use as indicated by the manufacturer. However, under emergency conditions, validation and verification studies may be limited. Five positive and 10 negative samples should be referred to WHO reference laboratories for confirmatory testing Alternatively, new or less experienced laboratories should be mentored by reference or more experienced laboratories for their initial test results confirmation and performance improvement Lot-to-lot verification should be conducted for a newly received lot or batch of test kits. Each lot should be tested using well-characterised samples to verify performance against existing lots in use.
Reagent	<ul style="list-style-type: none"> Reagent reconstitution should be done in a PCR hood or BSC following the product insert and brought to the right temperature conditions before use (use cold blocks or ice) Do not substitute or mix reagents from different kit lots or other manufacturers Minimise freeze-thaw cycles Maintain primer/probe integrity. After suspension and dilution, aliquot immediately into volume enough for one run. Do not use expired reagents
Sample processing	<ul style="list-style-type: none"> RNA extraction must be performed in a BSL-2 or equivalent facility Sample must be allowed to thaw completely before use Purulent or clotty sputum should be treated with dithiothreitol before aliquoting Test tubes should be labeled with sample details to enable traceability. Always use a new aerosol-barrier or positive-displacement pipette tip for each sample.
Testing	<ul style="list-style-type: none"> SARS-CoV-2 molecular testing procedures should be readily available (manuals, SOPs, job aids) Testing should be performed as per the SOPs of the laboratory Leftover samples should be stored serially at -70 OC for retesting by an EQA program
Interpretation	<ul style="list-style-type: none"> Test result interpretation should follow the testing algorithm of the country or available guidance Discordant results should be resolved by repeat testing on a newly collected sample and possibly by sequencing Any unexpected result should be reported and related samples sent to WHO reference laboratories for confirmation
Reporting	<ul style="list-style-type: none"> Test results should be reviewed independently by a laboratory supervisor to confirm accuracy before release. Independent review involves confirming patient details with the test result and validity test by control results.



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