African Union

AFRICA CDC
Centres for Disease Control and Prevention
Safeguarding Africa's Health

ASLM
AFRICAN SOCIETY FOR LABORATORY MEDICINE

Guidance on
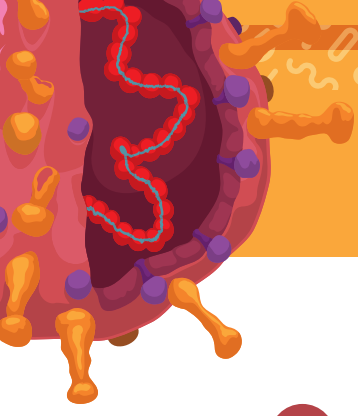**QUALITY ASSURANCE** for

COVID-19

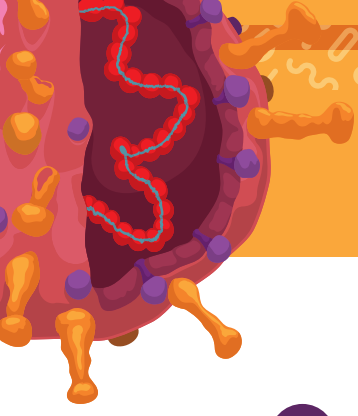## MOLECULAR LABORATORY TESTING

ACKNOWLEDGEMENTS

1 INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first identified in Wuhan City, Hubei Province, China in December 2019, is now a major public health problem across the globe. The pandemic continues evolving in Africa since the first case in Egypt was reported on 14 February 2020. As of 22 June 2020, more than 307 479 cases have been detected in all Member States of the African Union and more than 8 149 people have died of the disease. While there are variations among countries, the overall numbers of reported cases have been increasing exponentially in recent weeks. 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 or quarantine of COVID-19 patients to prevent local spread of the virus, and more broadly informs interventional measures.

> SARS-CoV-2 infections in humans are clinically characterised by fever and pneumonia-like symptoms including coughs and expectoration. In sub-Saharan Africa, where many febrile illnesses are prevalent, symptomatic COVID-19 is likely to be misdiagnosed with other infectious causes of fever. The risk of misdiagnosis and the presence of asymptomatic and mild COVID-19 make laboratory testing a crucial tool for case confirmation. COVID-19 testing is being significantly expanded on the continent. The Partnership to Accelerate COVID-19 Testing initiative launched by Africa Centres for Disease Control and Prevention (CDC) and the Africa Union is helping Member States to expand laboratory testing capacity as a key strategy to contain or slow the progression of the pandemic. As testing is decentralised to sub-national, veterinary, academic, and research laboratories, assuring the quality of testing and enforcing biosafety practices is critical.

Molecular assays conducted on nasopharyngeal swabs or other upper respiratory tract specimens are the most commonly used and reliable tests for the diagnosis of COVID-19. A variety of 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. Most molecular assays have achieved 100% specificity, since the primers are designed specifically for the target gene sequences of SARS-CoV-2. However, sensitivity can be affected by specimen quality, sampling time to symptom onset, testing errors, or other technical deficiencies.

False-negative SARS-CoV-2 polymerase chain reaction (PCR) 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 in all COVID-19 testing laboratory networks.

2 PURPOSE

The purpose of this guidance document is to help Member States and partners as they set up comprehensive quality assurance measures for COVID-19 testing laboratory networks. The guidance emphasises the use of standardized registration formats as a quality tool, quality control (QC), enrollment of laboratories in external quality assessment (EQA) schemes and use of EQA performance data for continuous quality improvement of COVID-19 testing laboratories.
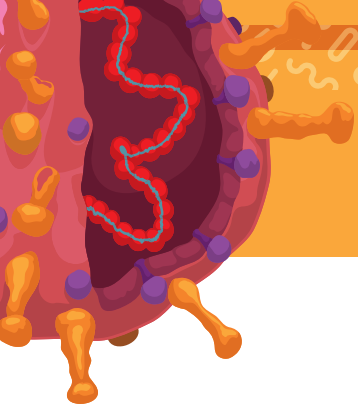
3 QUALITY ASSURANCE

Quality assurance (QA) is the part of the quality management system that focuses on providing confidence for the fulfillment of quality requirements. QA implemented through quality management systems is important for any testing service from PCR tests conducted in complex laboratories to point-of-care testing conducted in community health centres. QA is a system designed to continuously improve the reliability and efficiency of laboratory testing services. It can be implemented to monitor the quality of COVID-19 testing laboratories to minimise error rates that may arise in all stages of laboratory testing processes (pre-analytical, analytical, and post-analytical).

> Laboratories involved in the molecular diagnosis of SARS-CoV-2 should implement three main components of QA, namely:
> - Quality Control (QC)
> - External Quality Assessment (EQA)
> - Quality Improvement (QI)

3.1 QUALITY CONTROL

QC is a material or 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. Hence, QC is a multi-step process with certain checkpoints throughout the testing process: pre-analytical, analytical, and post-analytical stages (Annexes 1–3). In general, 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.
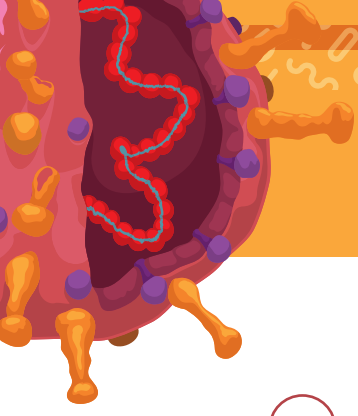
In addition to the QC checks indicated in Annexes 1–3, internal QC (IQC) should be performed routinely as per the recommendation of the test manufacturer for particular molecular assays. For example, some assays have built-in or test kit controls, and using external QC may not be recommended. However, the laboratory must ensure that the extraction and amplification PCR processes are properly checked for quality using IQC in each test run. QCs that are commonly employed for PCR testing include:

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.

No template control (NTC): Checks contamination during specimen extraction and/or plate set up. 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. NTC contains nuclease-free water.

Positive template control(s): 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 QCs 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. Several third-party commercial companies supply standard controls for the extraction and amplification steps of SARS-CoV-2 PCR testing (Annex 4). 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. Nuclease-free water or viral transport medium can be used as the negative control. 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.

3.2 EXTERNAL QUALITY ASSESSMENT

EQA 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. EQA 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.
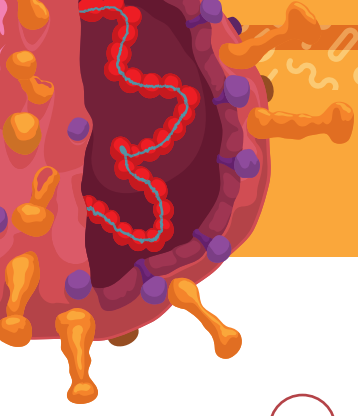
Proficiency testing: An external PT provider sends a set of SARS-CoV-2 positive and negative simulated clinical samples for testing in different laboratories and the results of all laboratories are analysed, compared, and reported back to the participating laboratories. The positive panels contain different genetic lineages of SARS-CoV-2. Several PT providers have started distributing panels for molecular SARS-CoV-2 tests. COVID-19 testing laboratories can be enrolled as part of the influenza laboratory network for free or at a cost not exceeding $420 USD, but cost varies by country (Annex 4). Laboratories should select PT providers with a track record in delivering PT panels within their region. All COVID-19 testing laboratories should participate in proficiency testing every three months (quarterly).

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. Similarly, sub-national COVID-19 testing laboratories should send retesting samples to their national COVID-19 reference laboratory.

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:

- Patient management
- Sample collection procedures
- Standardised testing policies
- Documentation and maintenance of records
- Biosafety adherence
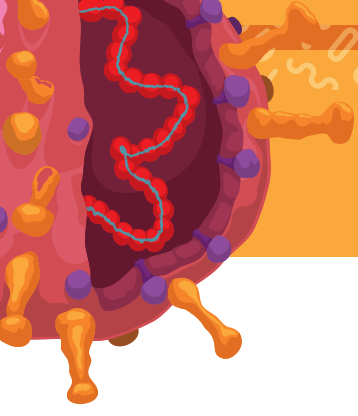- Quality control procedures
- Staff competency

Onsite evaluation should be conducted at least annually, but preferably every three to six months. However, an immediate supervisory visit can be organized, 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 tool. (*https://apps.who.int/iris/bitstream/handle/10665/70874/WHO_HSE_GCR_LYO_2012.2_eng.pdf?sequence=3&isAllowed=y*) or nationally customised assessment checklist).

3.3 QUALITY IMPROVEMENT

QI is a process by which the components of SARS-CoV-2 testing services are analyzed to identify areas requiring improvement, to plan and undertake improvements, and to evaluate the effectiveness of improvements. QI is also recognized 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.
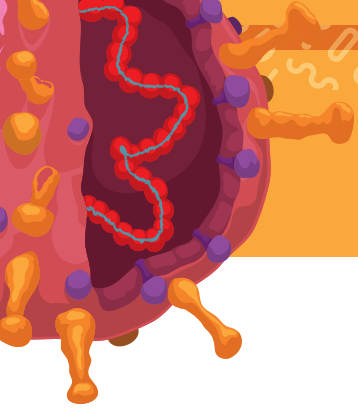
The ultimate target of QI is to take corrective action against the identified problem, remove its root cause, and reduce or eliminate its recurrence. Implementing preventive action reduces the likelihood of recurrence.

ELEMENTS OF QUALITY CONTROL - ADMINISTRATIVE

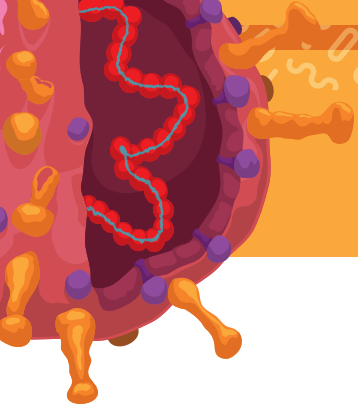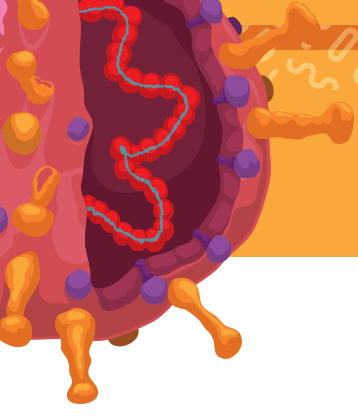| Quality Control	Recommendations
Workplace	• SARS-CoV-2 molecular testing should be performed in a secure, dedicated workspace • Manual PCR requires a three-room set-up (extraction, addition, and detection) • Laboratories should be organised to allow efficient testing workflow
Staff Competency	• Staff 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 • Staff should take part in regular competency assessments, and if required, consider retraining. e.g., online course on COVID-19 diagnostics and testing *https://www.futurelearn.com/courses/covid-19-diagnostics-and-testing*
Standard operating procedures (SOPs)	• 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	• 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	• Laboratories should collect and analyze data monthly
	• Data should be collected on key performance indicators:
	    ○ Sample rejection rate
	    ○ Number of samples tested by sample category
	    ○ Number of positive, negative and invalid test results
	    ○ Turn-around time
	    ○ Number of failed IQC results
	    ○ EQA/PT performance (pass/fail or % score)

Equipment	• 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

ELEMENTS OF QUALITY CONTROL - ADMINISTRATIVE

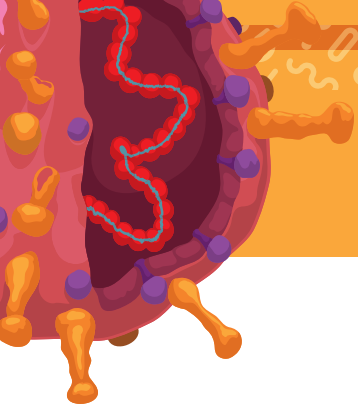| Quality Control	Recommendations
Supplies	• Procure diagnostic kits as per the WHO Emergency Use Assessment and Listing (*https://www.who.int/diagnostics_laboratory/eual/emergency/en/* and/or that have granted Emergency Use Authorization from national authorities • Prioritise diagnostic kits with high performance characteristics in independently evaluated data using a large sample size (e.g., *https://www.finddx.org/covid-19/pipeline/*) • 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.)
Biosafety	• 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 (BSL-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 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)

ELEMENTS OF QUALITY CONTROL - SAMPLE MANAGEMENT

Quality Control	Recommendations
Collection	• All samples collected from COVID-19 suspect patients should be considered as potentially infectious. Hence, biosafety must be emphasised when handling and collecting samples. • Use appropriate PPE (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. More info about developing a request form is available at: *https://extranet.who.int/lqsi/content/develop-request-form-laboratory-testing* • 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. **Please note that expectorated sputum is ONLY for patients with a productive cough. Sputum induction is NOT recommended.** • 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
Transport	• 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. VTM can be locally prepared; see US CDC protocol (*https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf*). 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 (*https://www.un3373.com/category-biological-substances/category-b/*). If shipping is within national borders, comply with national regulations. For overnight shipment, use shipment in an ice pack (temp 2–8°C).
Laboratory	• 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

ELEMENTS OF QUALITY CONTROL - SARS-COV-2 MOLECULAR TESTING

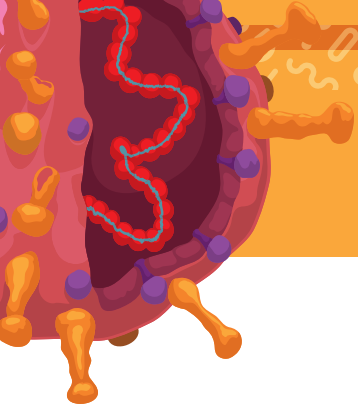| Quality Control	Recommendations
Assay validation or verification	• Validation or verification should be conducted to ensure test performance for intended use as indicated by the manufacturer. However, under emergency conditions, validation and verification studies may be limited. • Five positive and 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	• 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	• 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	• 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	• 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 *https://www.who.int/who-documents-detail/who-reference-laboratories-providing-confirmatory-testing-for-covid-19*
Reporting	• 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.

SELECTED, CURRENTLY AVAILABLE SARS-COV-2 PANEL PROVIDERS FOR QUALITY CONTROL AND PROFICIENCY TESTING

QC PROVIDERS

Provider	Website
ZeptoMetrix	https://www.zeptometrix.com/informationcenter/resources/zeptometrix-coronavirus-products
SeraCare	https://www.seracare.com/
European Virus Archives-Global	https://www.european-virus-archive.com/detection-kit/2019-ncov-e-gene-stabilized-rna-positive-control-shipping-room-temperature
Bio-Rad	https://www.bio-rad.com/featured/en/coronavirus-covid-19-assay-development-vaccine-research.html
National Institute for Biological Standards and Control (NIBSC)	https://www.nibsc.org/
LGC	https://www.lgcstandards.com/GB/en/About-LGC-Standards
Twist Bioscience	https://www.twistbioscience.com/
Randox	https://www.randox.com/coronavirus-randox-2/

EQA PROVIDERS

Provider	Website
QCMD	https://www.randox.com/coronavirus-qcmd/
INSTAND	https://www.instand-ev.de/en/eqas/eqa-program.html
WHO Health, Emergencies and Global Influenza program	https://www.who.int/influenza/gisrs_laboratory/external_quality_assessment_project/en/
ECDC/EVD-LabNet/ERLI-Net	https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-influenza-lab-quality-control
Boca Biolistics	https://www.bocabiolab.com/
National Institute for Biological Standards and Control (NIBSC)	https://www.nibsc.org/
SmartSpot Quality	https://smartspotq.com/

RESOURCES

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- In-house developed molecular assays: *https://www.who.int/docs/default-source/coronaviruse/whoinhouseassays.pdf?sfvrsn=de3a76aa_2&download=true*

- *https://www.afro.who.int/health-topics/coronavirus-covid-19*