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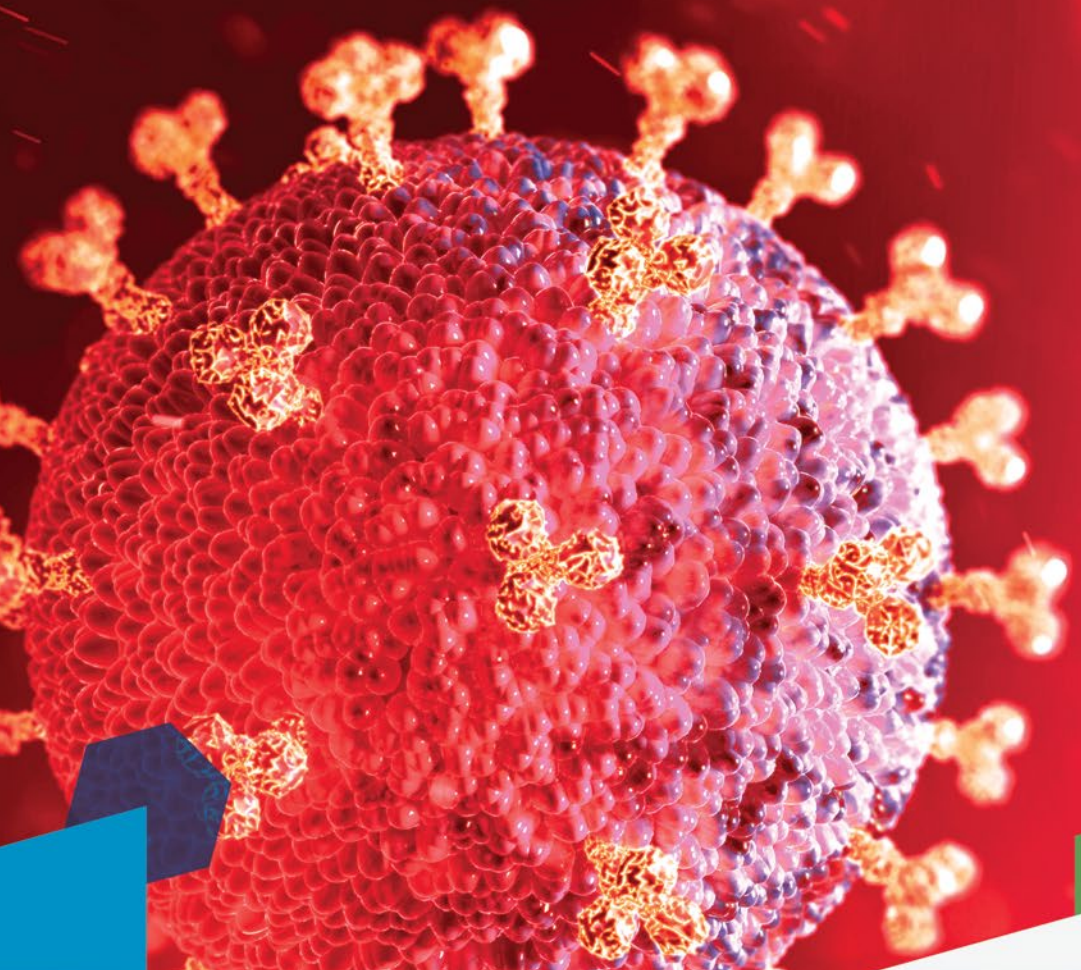
SARS-CoV-2 ANTIGEN RAPID TESTING FOR DIAGNOSIS OF COVID-19

Quality Assurance Framework



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On behalf of the African Society for Laboratory Medicine, all our partners and on my own behalf, we pledge to continue to support roll out, dissemination and implementation of this SARS-CoV-2 antigen RDT quality assurance framework to enhance the strategic response to the COVID-19 pandemic.

Nqobile Ndlovu

Chief Executive Officer

AFRICAN SOCIETY FOR LABORATORY MEDICINE (ASLM)

EXECUTIVE SUMMARY

The outbreak of the coronavirus disease-2019 (COVID-19) pandemic caused by the SARS-CoV-2 is one of the greatest public health dilemmas in our lives and so far during the 21st century. It has infected and affected millions of people globally. As part of the global response to curb this pandemic, the World Health Organization (WHO) recommends early and timely identification of infected individuals through laboratory diagnosis. The objective is to rapidly isolate those infected so as to break the spread of infection and also treat and manage these individuals. This makes access to laboratory services more important than ever in the public health space.

To perform laboratory SARS-CoV-2 testing in an individual for diagnosis of COVID-19, WHO highly recommends molecular testing based detection of specific viral sequences by nucleic acid amplification tests (NAATs). These NAATs platforms are too costly to acquire, operate and maintain their availability in many community settings. To cope with the upsurge in the urgent demand for COVID-19 diagnosis, the WHO recommended the use of alternative antigen rapid diagnostic tests in September 2020.

Given that quality test results are the cornerstone for a successful response to this pandemic, there is an urgent need to develop tools to support SARS-CoV-19 antigen testing to ensure quality is maintained through the testing process. To achieve this, there is a need to develop a quality assurance framework and training materials to guide implementation, rollout and evaluation of SARS-CoV-2 antigen testing. This framework describes the necessary core components for quality assurance in clinical laboratory testing, and critical activities to be implemented including roles and responsibilities of various stakeholders to ensure its successful implementation. It includes the core activities for quality assurance of SARS-CoV-2 testing that include planning, implementing, evaluation, and improvement of the testing program.

This quality assurance framework is highly recommended for country Ministries of Health, COVID-19 testing laboratories and personnel, public health experts, program managers involved in COVID-19 response activities, laboratory staff, authorized community healthcare workers, development partners and agencies funding the COVID-19 pandemic response. We look forward to supporting and working with all stakeholders in the implementation of all recommended core components of this framework for quality SARS-CoV-2 diagnosis to consolidate efforts towards COVID-19 responses.

Nqobile Ndlovu
Chief Executive Officer
AFRICAN SOCIETY FOR LABORATORY MEDICINE (ASLM)

List of Abbreviations and Acronyms

Ag ..... Antigen

COVID-19 ..... Coronavirus disease 19

EQA ..... External Quality Assessment

Fig ..... Figure

HR ..... Human resource(s)

ILC ..... Inter-laboratory comparison

IPs ..... Implementing Partners

IQC ..... Internal Quality Control

M&E ..... Monitoring and Evaluation

MEAL ..... Monitoring, Evaluation, Accountability and Learning

MoH ..... Ministry of Health

NAATs ..... Nucleic Acid Amplification Tests

PPE ..... Personnel Protection Equipment

PT ..... Proficiency Test

QA ..... Quality assurance

RDT ..... Rapid diagnostic test

rRT-PCR ..... Real-time Polymerized Chain Reaction

SARS-CoV-2 ..... Severe acute respiratory syndrome coronavirus 2

TAT ..... Turnaround time

WHO ..... World Health Organization

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1. Introduction

1.1 Background

On December 31, 2019, a novel coronavirus was first identified in the city of Wuhan, China, and in February 2020, the new coronavirus was given the official name severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and confirmed to cause the Coronavirus disease-19 (COVID-19). From Wuhan, COVID-19 rapidly spread to many parts of the world and on March 11, 2020, the World Health Organization (WHO) declared it a pandemic. As part of the response strategies to curb the spread of COVID-19, the WHO recognizes laboratory testing is a cornerstone of the management of the COVID-19 pandemic because it allows for the detection of cases to inform care and for the isolation of infected individuals to interrupt disease transmission (WHO, 2020).

The laboratory diagnosis of COVID-19 is based on confirmation of SARS-CoV-2 infection in the suspected person. WHO highly recommends molecular testing based detection of specific viral sequences by nucleic acid amplification tests (NAATs) using platforms such as real-time reverse-transcription polymerase chain reaction (rRT-PCR). However, molecular platforms by design are quite expensive to maintain in terms of acquisition costs, operational costs which make them prohibitive in low-income settings. With the emergency unprecedented high demand for COVID-19 testing, therefore, NAATs protocols have not matched the testing needs if the efforts to control this pandemic are to be fully effective (WHO, 2020). In response to the urgent need for COVID-19 diagnosis amidst the scarce molecular NAATs and/or rRT-PCR capacities, there has been rapid development of alternative SARS-CoV-2 rapid antibody or serological and antigen-based (Ag) diagnostic kits. The rapid diagnostic antibody/serological kits are designed to detect human antibodies produced in response to the SARS-CoV-2 infection while the antigen-based rapid diagnostic test kits detect specific proteins of the SARS-CoV-2. Both give results in a short time, often between 15–30 minutes. This quality assurance framework provides general guidance to stakeholders on the establishment, implementation, monitoring and evaluation of SARS-CoV-2 Ag RDT for COVID-19 diagnosis.

1.2 Antigen rapid diagnostic tests for SARS-CoV-2 detection

Ag RDTs detect the specific antigens (nucleoprotein) of SARS-CoV-2 if present in sufficient concentrations in samples including nasopharyngeal swabs, oropharyngeal swabs, sputum, and any other respiratory secretion obtained from an infected individual. Most Ag RDTs are based on the principle of lateral flow immunoassay and are known to give test results within a few minutes ranging from 15–30 minutes depending on the manufacturers' specifications. Although the SARS-CoV-2 Ag RDTs give test results faster than the RT-PCR assays, they have lower sensitivity compared to the RT-PCR but specificity is consistently reported to be high (Dinnes J, 2020). However, despite their limitations, SARS-CoV-2 Ag RDTs provide a critical opportunity for a quick response to the pandemic through rapid screening, detection, and timely management of COVID-19 patients and rapid surveillance of the disease.

1.3 General recommendations for use of antigen detecting RDTs for SARS-CoV-2

The WHO interim guidance on antigen detection in the diagnosis of SARS-CoV-2 infection using rapid immunoassays (WHO, 2020) was published in September 2020 and outlined a set of recommendations to be considered if Ag RDTs are used for the diagnosis of COVID-19.

This guidance highlights the importance of deployment of Ag RDTs in areas where molecular-based testing capacities (NAATs/RT-PCR) are not readily available and recommends a threshold performance of at least sensitivity of ≥80% and specificity of ≥97%.

In December 2020, Africa CDC released recommendations specific for African Union Member States, focused on guiding introduction both for diagnosis in populations with known risk exposure as well as for screening in general population with unknown or low exposure risk.

Further information on the general recommendations, including appropriate scenarios for SARS-CoV-2 Ag RDT implementation, testing algorithms, and considerations for deployment can be found in the **WHO Antigen Guidance** and in the **Africa CDC guidance**.

To appropriately implement COVID Ag RDTs, the following factors should be considered:

- Only COVID-19 Ag RDTs that meet the minimum performance requirements of sensitivity > 80% and specificity of > 97% in relation to NAAT as set in the WHO TPP should be used
- Introduction of high specificity tests (>99%) is preferred in all settings, but is of particular importance in low prevalence settings and in general screening applications
- COVID-19 Ag RDT should be deployed as first-line test in contexts where NAAT is not available or where turnaround times are too long for clinical utility (e.g. >24 hours)
- COVID-19 Ag RDT with high specificity (>99%) can be deployed in any setting, but may be of increased importance in settings where the consequences of a false positive is impactful, either due to needs for epidemic management, or due to economic consequences
- Proper interpretation of antigen results within these use cases is important for both clinical management of cases and for assessing the SARS-CoV-2 epidemic
- The accuracy of results depends largely on the context within which the results are interpreted. Therefore, management of results within a given setting should consider the tolerance and consequences of misdiagnosis, either false positive or false negative.

Africa CDC recommendations for SARS-COV-2 RDT use is summarized in the matrix below:

Scenario	COVID-19 Diagnosis in populations with known risk exposure	COVID-19 diagnosis in general population with unknown or low exposure risk
Goal	To manage the epidemic, in addition to provide clinical care where needed	To allow for opening of economic/ social activities safely, while minimizing the risk of new outbreaks
Types of populations	• Individuals with COVID-19 symptoms • Frontline healthcare workers and essential workers (symptomatic and asymptomatic) • High risk populations in areas with confirmed/suspected outbreak (includes the elderly, people with comorbidities, and populations in closed-settings such as prisons, care homes, etc) • Contacts of confirmed cases (symptomatic and asymptomatic)	• Travelers crossing borders/ points of entry • Teachers, students and administrative staff at educational institutions • Factory workers, government employees, and private sector employees at workplaces • In-patients at Hospitals not admitted due to COVID-19 (e.g. elective surgeries, other illnesses, etc.) • Other general populations (e.g. random community screening, surveillance)

1.4 Purpose of this quality assurance framework

The purpose of this quality assurance framework is to provide general technical guidance on the establishment, implementation, monitoring, and evaluation of SARS-CoV-2 Ag RDT quality assurance programs to effectively and efficiently detect, control and minimize errors in the performance of COVID-19 laboratory testing processes.

1.5 Objectives of this quality assurance framework for SARS-CoV-2 Ag RDT

- To guide stakeholders involved in COVID-19 testing on how to establish, implement, monitor and evaluate quality assurance programs for SARS-CoV-2 Ag RDT
- To facilitate the implementation of regulatory requirements for point-of-care assays for SARS-CoV-2 Ag RDT roll-out
- To guide COVID-19 testing laboratories on internal quality control and external quality assessment of SARS-CoV-2 Ag RDTs
- To guide African Union Member States and partners to establish alternative external quality assessment (EQA) schemes for COVID-19 testing laboratory networks where proficiency testing panels are not readily available

1.6 Target audience

This quality assurance framework targets a wide range of stakeholders who are directly or indirectly involved in the COVID-19 pandemic response. These include:

- COVID-19 testing personnel who include
  - Medical laboratory professionals directly involved in COVID-19 laboratory testing
  - Non-laboratory staff such as nurses, clinicians, and pharmacists but are directly involved in COVID-19 laboratory testing. They must have been trained and deemed competent to use SARS-CoV-2 Ag RDT
  - Other testing personnel who are trained, deemed competent and authorized by established protocols
- Program personnel managing the COVID-19 pandemic response
- Ministry of Health representatives
- Government agencies & departments engaged in the COVID-19 response
- Public health experts, epidemiologists and researchers who are engaged in surveillance activities of the pandemic
- Donor agencies funding the COVID-19 pandemic response
- Implementing partners supporting the response to the pandemic

2. Quality Assurance for SARS-CoV-2 Antigen RDT

2.1 Quality Assurance

Quality assurance is a process where all processes of laboratory testing are monitored to ensure that the quality of laboratory test results is guaranteed. Quality assurance aims to detect, control and minimize errors in the laboratory testing processes. The laboratory testing processes where quality assurance is implemented are the pre-examination, examination and post-examination processes. The three processes are augmented by administrative/ supportive processes, namely safety, human resources, equipment and device management, documentation, reagents and consumable inventory management, data and records management to form a Quality Management System (Fig 1).

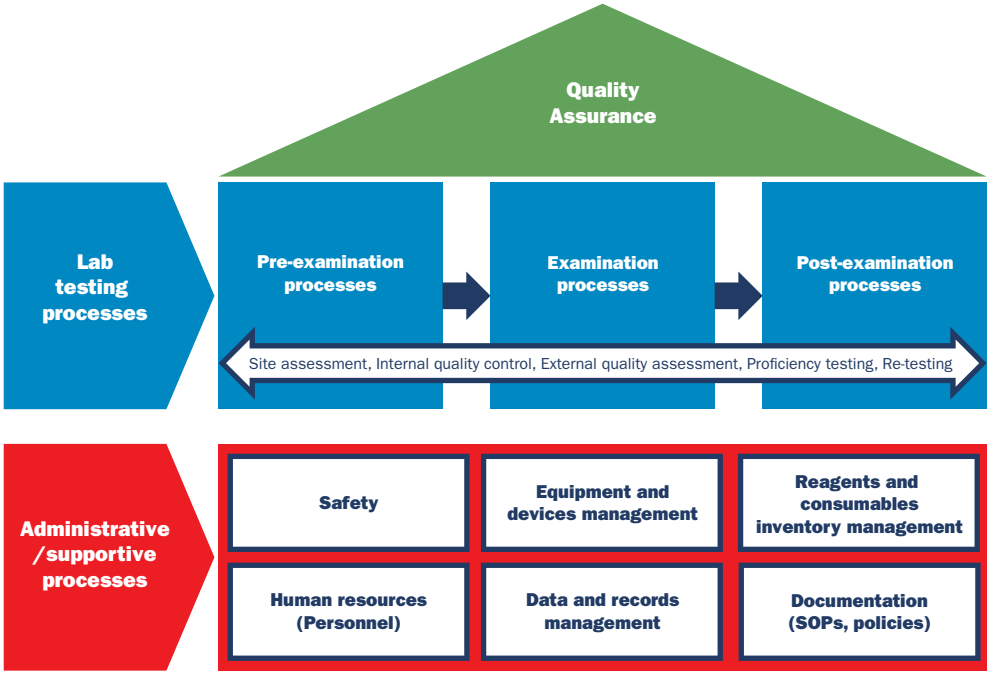

Figure 1: Quality Assurance in relation to the main testing laboratory and administrative/ supportive processes

The goal of quality assurance implementation in SARS-CoV-2 Ag RDT is to reduce the risks and ensure that timely, accurate and reliable test results are released by the laboratory. Any wrong laboratory test results compromise the response efforts to the COVID-19 pandemic. For example, people with a false negative result are told not to self-isolate, so they infect more people. However, a correct positive test result allows swift isolation and treatment of the infected person, reducing the risk of community transmission. Quality assurance in the laboratory encompasses three core components described below.

2.2 Core Components of Quality Assurance

There are three main quality assurance components (Internal Quality Control, External Quality Assessment, and Continuous Quality Improvement) that need to be considered during the diagnosis of COVID-19 using SARS-CoV-2 Ag RDTs. Each of these components needs to be appropriately addressed (Fig. 2).

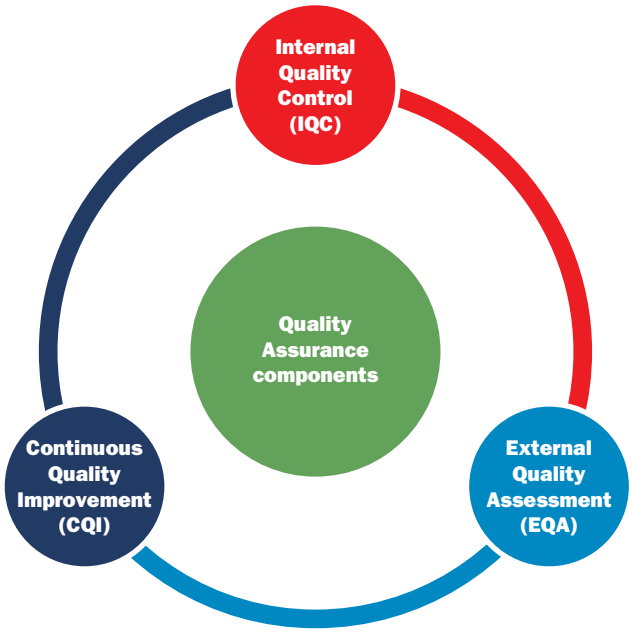

Figure 2: The three components of Quality Assurance

2.2.1 Internal Quality Control for SARS-CoV-2 Ag RDT

Internal Quality Control (IQC) refers to the measures which are included in each test laboratory procedure to ascertain and verify that the test assays (device, equipment, and associated reagents & supplies) are working as they should yield accurate and reliable test results. For SARS-CoV-2 Ag RDTs, the controls come in the form of:

- In-built internal procedural controls that validate the test sample has travelled through the intended reaction area (often in lateral flow designs)
- IQC involves the use of quality control materials (provided by the assay manufacturer or from a third party) that react with the examining system in the same manner as patient samples

The laboratory and the testing personnel are the primary players to ensure that IQC is performed correctly and consistently.

IQC encompasses actions that are geared to:

- Detecting errors that would otherwise invalidate the medical usefulness of the laboratory results
- Allows detection of routine laboratory system problems and ensures the quality of laboratory test results

2.2.1.1. Guidance on implementation of IQC for SARS-CoV-2 Ag RDT

- Establish standard operating procedures (documentation) for performing SARS-CoV-2 Ag RDT, including IQC
- Adhere to the manufacturer's instructions. Each SARS-CoV-2 manufacturer has inserts with instructions on how IQC is performed.
- Use IQC materials provided by the manufacturer (if provided). For RDTs with inbuilt IQCs, ensure that the control results are timely and accurately read before reporting patients results.
- Alternatively, use third party quality control materials such as proficiency testing panels, patients' samples already tested with results known from a verified method/assay. It is highly recommended that key stakeholders supporting the SARS-CoV-2 Ag RDT rollout look for available third party providers for IQC materials, which may include PT accredited and authorized PT providers.
- Define and document the frequency of IQC performance. The frequency of performing IQC when using SARS-CoV-2 Ag RDT is determined by:
  - The instructions from the Ag RDT manufacturer and/or the COVID-19 testing laboratory
  - In cases where the manufacturer does not mention the frequency of performing IQC, it is highly recommended to perform IQC daily at a minimum
  - The frequency may increase depending on the testing volume of the COVID-19 laboratory

- The testing laboratory or facility should define a criterion for accepting or rejecting quality control results by establishing, documenting and communicating the quality control rules
- All IQC results must be reviewed and approved by the appropriate competent and authorized testing personnel before tests can be performed
- Ensure that all Ag RDT quality control and in-built control requirements (as stated by the kit manufacturer) are met before any COVID-19 patient result is released
- Record each event of SARS-CoV-2 IQC performed. Both successful and unsuccessful IQC results must be promptly recorded.
- The testing laboratory or facility should define a regular frequency to conduct trend analysis of SARS-CoV-2 IQC data to detect systemic errors and detect any failed quality control runs
- Verification of new batches or lots of SARS-CoV-2 Ag RDT kits is mandatory prior to using them for COVID-19 diagnosis. This may include basic verification processes such as lot-to-lot testing of the RDTs (where applicable) or the use of characterized specimens to check and confirm the performance of the kits prior to their use on patients' samples.
- In cases of failed IQC prior to testing patients' samples, the laboratory must define and document in the IQC SOP the necessary steps to be taken (namely, root cause analysis, corrective action processes, and preventive actions required). At a minimum, the preventive actions should be designated and authorized persons such as the Laboratory Director, or Laboratory Manager of Quality Officer or Clinic/Facility Manager, etc however named, or delegated officer.

2.2.2 External Quality Assessment for SARS-CoV-2 RDT

External quality assessment (EQA) refers to a defined set of activities performed through an external source to objectively evaluate the performance and operation of a testing laboratory. For RDTs, both in a clinical laboratory or non-laboratory setting such as community settings, this is done through various approaches, namely proficiency testing (PT), on-site assessment, re-testing, and sample exchange. These approaches should be implemented in a COVID-19 testing laboratory using the SARS-CoV-2 Ag RDTs and are generally applicable to all COVID-19 laboratories that use other testing platforms like NAATs or antibody RDTs.

The use of PT is applicable in circumstances where there are schemes with authorized PT providers. However, this is often more costly and may not readily be available. Therefore, re-testing and sample exchange approaches are recommended alternative approaches to the implementation of EQA for COVID-19 Ag RDT and general SARS-CoV-2 testing.

2.2.2.1. Proficiency testing:

This is where SARS-CoV-2 samples from an external source often known as a proficiency test (PT) provider are tested by the testing laboratory or facility and the respective results submitted back to the PT provider for evaluation and comparison with other results from other laboratories, facilities, or testers:

- The testing laboratory is required to integrate PT samples into its routine workflow in a way that follows, as closely as possible, the routine handling processes of patient samples for COVID-19 testing
- The performance of the testing laboratory is compared with other laboratories in the EQA scheme. Reports of results are then sent back to the testing laboratory from the COVID-19 reference laboratory/PT provider. Failing a PT event indicates possible problems in the laboratory quality management system (LQMS) that require investigation and corrective action.
- The testing laboratory or facility reviews the results and if performance was unsatisfactory, corrective actions must be taken. If performance is satisfactory, then it is an indication that the processes allow the release of quality COVID-19 test results.
  In both cases, the EQA results should be shared with the staff and all actions performed must be recorded.

2.2.2.2. Retesting

In re-testing, a laboratory or facility performing COVID-19 testing using SARS-CoV-2 Ag RDT submits some of the samples it has tested to other testing laboratories to check if there is an agreement in the results. This can be done as follows:

- The re-testing at the laboratory where the sample is sent should use a similar or better testing assay than that used at the testing laboratory sending the sample. It is recommended that re-testing is done with laboratories at the same and/or higher along with the tier system (i.e peripheral health facility to the district to regional to national reference laboratory tier system).
- As in PT, the results of the testing laboratory and the other laboratories are compared for agreement. The agreement is where the COVID-19 test results of the testing laboratory are the same as that of the laboratory where the sample(s) was/were sent for re-testing. Percentage agreement can be computed as the number of samples in agreement divided by the total number of samples submitted for re-testing expressed as a percentage (%). The desired agreement is 100%. If there is an agreement, then it is an indication that the laboratory processes allow the release of quality COVID-19 test results. If there is no agreement, root cause analysis must be conducted, corrective and preventive actions must be taken. No agreement is where the COVID-19 test results of the testing laboratory are different from that of the laboratory where the sample(s) was/were sent for re-testing.
- The testing laboratory is required to define the frequency of re-testing. A minimum re-testing frequency of monthly is recommended for low volume sites yielding <500 tests, while for high volume testing sites yielding ≥500 tests per week, a weekly re-testing frequency is recommended.
- The laboratory must also define the proportion of the positive and negative samples to be submitted for re-testing and a specified regular frequency. At a minimum, at least 5% of the positive and 10% of the negative samples are recommended to be submitted for re-testing. In areas where the prevalence is very low (≤2%), it is recommended that all the positive samples be sent for re-testing.
- All unsatisfactory re-testing results must be adequately documented, recorded, investigated and appropriate corrective and preventive actions taken

NOTE: In cases where the SARS-CoV-2 Ag RDTs are used in non-laboratory or non-clinical settings, it is recommended that the facility to which this non-laboratory/non-clinical settings is attached MUST make all necessary arrangements to at least ensure that some samples tested by those non-laboratory/non-clinical settings are re-tested at laboratory or testing facility. This ensures traceability of COVID-19 test results to the parent laboratory or facility that is coordinating the SARS-CoV-2 Ag rapid diagnostic services. The stakeholders managing this processes must addresses all issues associated with biosafety and biosecurity, diagnostics lot-to-lot testing before the RDTs are deployed in non-laboratory/non-clinical settings.

2.2.2.3. Sample exchanges between peer laboratories

This is a valuable method of monitoring laboratory performance in the context of COVID-19 testing where PT-based EQA is limited by air transport constraints and where a retesting scheme would require additional human and financial resources that are unaffordable for many countries. A sample exchange approach between peer laboratories therefore provides a good alternative to help COVID-19 testing laboratories assess the quality of their performance.

General guidance for sample exchange between peer COVID-19 Ag RDT laboratories or facilities including those using other platforms includes:

- Types of samples to be tested at each peer laboratory will be stated including the frequency of sending samples for testing
- Participating laboratories will provide peer laboratories with clinical samples that they have previously processed for COVID-19 diagnosis. They will also inform on the methods used to test these samples to be exchanged.
- Samples will be anonymized and distributed to peer laboratories with matching clinical information
- Participating laboratories will, for example, send 2–3 samples to the peer laboratory at each round of sample exchange
- Sample packaging and transport will be done in accordance with the local established procedures; at minimum, the standard triple packaging protocols for clinical samples must be adhered to
- Exchanged COVID-19 clinical samples will be received at each laboratory and processed together with regular samples using the available method(s) at the peer laboratory. Where available at the receiving laboratory, the NAATs technique is preferred.
- Results will be shared with the peer laboratory within 2–5 days from receiving the exchanged COVID-19 clinical samples

On-site assessment for SARS-CoV-2 Ag RDT

On-site assessment refers to the use of authorized competent personnel who are subject experts to evaluate the various aspects of the laboratory testing site. In this evaluation, standardized criteria and tools are used. In COVID-19 testing laboratories using SARS-CoV-2 Ag RDTs, the following guidance on on-site assessment is provided:

- Regular on-site assessment must be conducted at least quarterly, but preferably monthly, especially during the initial period of testing and during the first twelve months. However, a more frequent on-site assessment is recommended if volume outputs are high, resources are constrained, or if there are recurrent, frequent or persistent non-conformances identified at the testing laboratory.
- The SARS-Cov-2 Ag RDT assessments should be coordinated by the established national COVID-19 testing lead coordination mechanism through to the regional, district, and peripheral health facility levels

- For clinical laboratory settings:
  - Each SARS-CoV-2 testing site must receive the initial assessment prior to starting any RDT testing, and a follow-up assessment within 1–4 weeks immediately after the RDT is initiated at the testing site
  - Standard approved assessment tools must be used to conduct the assessment. At a minimum, the WHO Laboratory Assessment tool (accessible at **https://www.who.int/ihr/publications/Annex1_en.xls?ua=1**) may be used. This WHO checklist can be customized for COVID-19 SARS-CoV-2 according to the national local context. Where applicable, national standardized assessment tools can be used.
- For non-laboratory settings, appropriate assessments must be conducted using standardized or customized tools to ensure that each laboratory correctly performs SARS-CoV-2 AG RDT testing
- At a minimum, the checklists must evaluate the following components of the SARS-CoV-2 Ag RDT during the on-site assessments of testing in the laboratory and non-laboratory settings:
  - Sample management
  - IQC, EQA, and re-testing records to check and confirm that the laboratory's performance is satisfactory
  - Biosafety and biosecurity aspects
  - Testing personnel competency
  - Logistics and supplies inventory management
  - Documentation of relevant procedures and processes
  - Records management for all testing processes
  - Results management
  - Quality improvement processes (evidence for corrective actions, preventive actions)

2.3 Evaluation of PT, re-testing and sample exchange results analysis and interpretation using Cohen's kappa coefficient statistics

To evaluate the contribution of chance to the agreement between the two sets of results from the sub-national laboratory and the reference laboratory, Cohen's kappa coefficient statistics will be used.

Cohen's kappa coefficient is a statistic that measures inter-rater agreement for categorical items. It is generally considered a more robust measure than simple percent agreement calculation since *k* takes into account the agreement occurring by chance. Cohen's kappa measures the agreement between two raters (laboratories in this case) who each classify N items into C mutually exclusive categories. Cohen's kappa coefficient is defined and given by the following function:

$$k = (p_0 - p_e)/(1 - p_e) = 1 - ((1 - p_o)/(1 - p_e))$$

Where:

p_0 = *relative observed agreement among testers/laboratories*

p_e = *the hypothetical probability of chance agreement*

- p_0 and p_e are computed using the observed data to calculate the probabilities of each observer randomly saying each category. If the laboratories are in complete agreement then $k = 1$. If there is no agreement among the two laboratories other than what would be expected by chance (as given by pe), $k \leq 0$.
- In practice, kappa values above 0.8 can be considered an excellent analytic agreement, and those between 0.6 and 0.8 can be considered to be a reasonable agreement

Example

Suppose the reference laboratory has retested 15 samples coming from a sub-national laboratory. Each sample is either reported as "Positive" or "Negative" for COVID-19 by both laboratories. Suppose the disagreement count data were as follows, data on the diagonal slanting left shows the count of agreements and the data on the diagonal slanting right, disagreements:

		Reference laboratory	
		Positive	**Negative**
Subnational laboratory	**Positive**	2	6
	Negative	4	3

Calculate Cohen's kappa coefficient.

Solution

Note that 2 samples were tested and reported as “Positive”, and 3 samples tested and reported as “Negative” by both laboratories. Thus, the observed proportionate agreement is:

$$p_0 = (2 + 3)/15 = 0.33$$

To calculate pe (the probability of random agreement) we note that:

- The reference laboratory reported 6 “Positive” and 9 “Negative” samples among the samples received. Thus, the likelihood of finding a “Positive” sample in the set of samples received was 40% at the reference laboratory.
- The sub-national laboratory reported 8 “Positive” and 7 “Negative” samples among the samples originally collected and tested. Thus, the likelihood of finding a “Positive” sample in that set of samples was 53.33% at the sub-national laboratory.

Using formula P(Reference lab and Subnational lab) = P(Reference lab) x P(Subnational lab) where P is the probability of an event occurring.

The probability that both of these laboratories would report a “Positive” result randomly is 0.40 x 0.53 = 0.21 and the probability that both of them report a “Negative” result randomly is 0.60 x 0.47 = 0.28. Thus the overall probability of random agreement is pe = 0.21 + 0.28 = 0.49.

So now applying our formula for Cohen’s Kappa we get:

$$k = (p_0 - p_e)/(1 - p_e) = (0.33 - 0.49)/(1 - 0.49) = -0.31$$

This example shows a case of complete disagreement, which in the laboratory setting suggests a systematic reversal of results, perhaps by clerical or programming error.

- Kappa values above 0.8 can be considered an excellent analytic agreement, and those between 0.6 and 0.8 can be considered to be reasonable agreement;
- A reporting form (see Appendix I) should be filled by the reference laboratory to summarize and compare results obtained from sites and those obtained by NRL through retesting. Percentage agreement and kappa values will be reported to support this comparison.

2.4 Quality Assurance planning, implementation, and evaluation

To ensure effective and efficient implementation of the quality assurance functions for SARS-CoV-2 RDT programs (Fig 3), it is recommended to carry out three main phases, namely planning, implementation, and evaluation phases (WHO, 2015). In each of these phases, core and specific activities need to be executed to attain the desired quality assurance goals (Table 1).

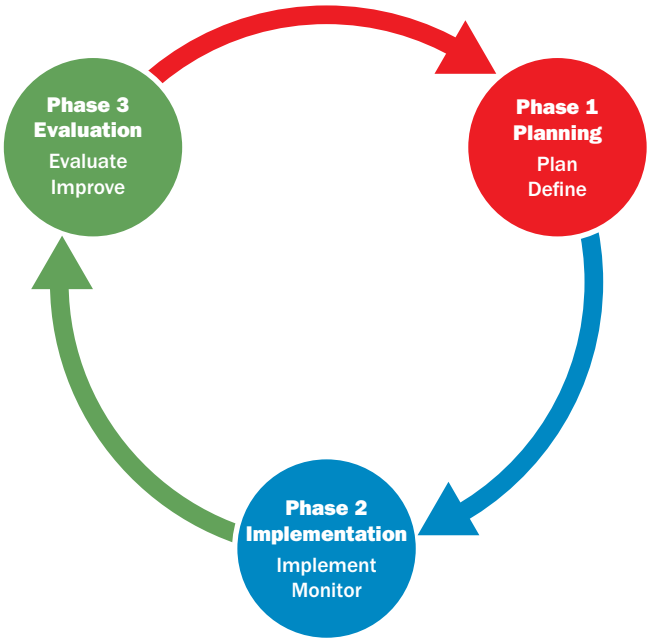

Figure 3: Quality Assurance Cycle

Table 1: Quality assurance planning, implementing and evaluation activities

Core activities	Specific activities
Phase 1: Planning	
Plan & Define	• Draw the full support and engagement of key stakeholders (Ministries of Health, Funding agencies, Implementing Partners) so they appreciate the urgent need for the QA intervention • Formulate required strategic documentation (policies, guidelines, SOPs) • Establish key standards for SARS-CoV-2 QA program including biosafety/biosecurity programs • Institute national coordination mechanisms (e.g appointment of QA desk force or coordinating committee, etc) • Develop the SARS-CoV-2 Ag RDT QA roll out plan (selection of testing sites, decide on testing algorithms, etc) • Mobilize the main resources (finances, human resources, diagnostic supplies, etc) • Select and procure the necessary validated SARS-CoV-2 diagnostic products • Define the duties and responsibilities of all the players/ stakeholders
Phase 2: Implementation	
Implement & Monitor	• Ensure that COVID-19 frontline personnel are appropriately trained and imparted with necessary knowledge and skills for their respective tasks (testing personnel, sample transporters, data clerks, sample collectors, etc) • Train and certify Trainers, Mentors and Supervisors for the QA program • Ensure that all established SOPs, policies and guidelines are adequately adhered to by all responsible personnel, e.g. performance of IQC, participation in EQA, accurate recording of data, etc • Procure (supply, deliver, monitor) diagnostics and associated supplies • Perform validations or verifications of all diagnostics • Conduct planned and/or targeted support supervision/ mentorships for all testing sites and testers
Phase 3: Evaluation	
Evaluate & Improve	• Carry out post-market surveillance of key diagnostics and products for SARS-CoV-2 to determine the value for money and quality • Generate information to guide and inform strategic planning for COVID-19 responses • Formulate platforms for advocacy and feedback as an avenue to sustain quality services delivery • Perform/conduct testing sites' assessments using standardized checklists • Conduct operational research-based field experiences • Use the data, lessons learnt from phases 1 & 2 above for continuous improvement on the efficiency and effectiveness of the SARS-CoV-2 Ag RDT implementation

2.5. Ensuring Quality in Pre-Examination, Examination and Post-Examination processes, including Administrative/Supportive Processes

To ensure quality in SARS-CoV-2 Ag rapid diagnostic testing, quality assurance must be implemented in all the three laboratory processes (pre-examination, examination and post-examination processes) including administrative and supportive processes, (safety, human resources, equipment and device management, documentation, reagents and consumable inventory management, data and records management) to form the Quality Management System. Each of these processes has core activities that must be implemented by the various stakeholders engaged in the COVID-19 response (Table 2).

Table 2. Core activities for implementation of the laboratory processes to ensure quality

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
A. Pre-examination Processes							
Documentation and records • Develop avail and approve all appropriate COVID-19 (national and testing facilities) SOPs, standards, policies and guidelines for patient preparation, sample management, reagents and supplies management • Essential data collection and reporting tools and mechanisms must be established	X	X	X	X			
Patient preparation • Ensure appropriate PPE is donned before taking a COVID-19 sample from a suspected patient • Ensure appropriate documentation (laboratory request form) for the COVID-19 test is correctly and completely filled with all relevant patient's information before taking the sample	X	X	X	X	X	X	X

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
Sample management (collection, transportation and storage) • Ensure adequate communication with the testing facility/lab/point prior to taking or sending/delivering the samples for testing • Ascertain correct sample labeling, full and correct filling of the lab request form • Ensure that the correct sample type and volume is taken • Ensure correct sample collection, packaging, transportation and storage processes are adhered to as per the established laboratory SOPs • Sample transit duration must be within the recommended period (in cases where samples are referred for testing) • In cases where immediate sample testing is not possible, ensure that the samples are appropriately stored at the recommended temperature • Ensure appropriate sample transportation temperature is maintained throughout • Only recommended SARS-CoV-2 Ag RDT sample collection devices and transport medium must be used • Institute protocols at the testing point to ensure correct sample reception and check to confirm sample packaging status to rule out sample spillage • Check/confirm that all the conditions stated above have been fulfilled before accepting a sample	X	X	X	X	X		X

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
Personnel competency • Only trained and competent personnel should be allowed to participate in patient preparation, sample management (i.e. sample collection, sample transportation and sample receipt) • Competency assessment of testing personnel must be regularly assessed through participation/performance in EQA or, witnessing personnel perform testing activities, etc	X	X	X	X	X	X	X
Reagents and supplies management • Perform verification of SARS-CoV-2 Ag RDTs before putting into use • Lot-to-lot testing SARS-CoV-2 Ag RDTs where testing already commenced • Ensure good laboratory supplies inventory management practices are practiced (e.g apply FEFO-FIFO principle)	X	X	X	X			X

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
B. Examination Processes							
• Make sure all the relevant documentation in form of SOPs are available and adhered to • Confirm that correct required reagents/supplies and consumables are available before opening sample • Check and confirm that all the reagents/kits supplies are not expired • Perform IQC/reagent acceptance (verification/lot/batch testing) of the SARS-CoV-2 Ag RDTs before using them for COVID-19 testing • Establish and confirm correct sample labelling, full and correct filling of lab request form before testing a sample • Only trained and authorized individuals who have been deemed competent are allowed to perform COVID-19 testing	X	X	X	X	X		X
Sample processing procedures: • Ensure sample is in recommended COVID-19 sample transportation medium prior to testing it • Always perform IQC as per established lab procedures before testing patients' samples for COVID-19 on any SARS-CoV-2 Ag RDT • It is mandatory to adhere to the manufacturers' procedures (e.g. timing, reading/interpreting test results, etc) • Ensure timely and accurate recording of test results • It is highly recommended to participate in SARS-CoV-2 PT programs		X	X				

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
C. Post-examination Processes							
Documentation and records • SOPs for post-examination processes must be readily available and adhered to. SOPs for review, approval & release of COVID-19 test results, sample disposal, etc	X	X	X	X			X
Records and data management of COVID-19 test results • Ensure appropriate review of processes for COVID-19 test results to confirm that all information reported is accurate • Timely and accurately record all COVID-19 test results in the appropriate laboratory information management system. For each released COVID-19 result, a copy must be retained in the lab. • Ensure that all COVID-19 test results are released and dispatched in accordance with the established protocol of the local regulatory criteria	X	X	X				
Sample storage after testing • SARS-CoV-2 leftover samples after testing must be stored, disposed and managed in-line with standard COVID-19 WHO or established national guidelines • Where applicable/indicated, appropriately store the sample at the recommended temperature • Submission to the national repository for long term storage • Provide a pool from which samples for re-testing will be drawn for inter-laboratory comparison		X	X				

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
D. Administrative/Supportive Processes							
Safety (Biosafety and Biosecurity) • Provide an appropriate environment, infrastructure, facilities and amenities for the provision of SARS-CoV-2 Ag RDT • Install or provide biosafety and biosecurity facilities • Essential PPE must be provided at all times and in adequate quantities	X	X	X	X	X	X	X
Equipment and devices • Required equipment and relevant devices essential for SARS-CoV-2 Ag RDT must be provided	X	X	X	X	X	X	X
Human resources/personnel: • Address workforce competencies by ensuring that all personnel involved in SARS-CoV-2 Ag RDT receive the necessary training • Ensure adequate number of personnel are available	X	X	X	X	X	X	X
Reagents and consumables • Logistical support must be provided by the COVID-19 response authorities by procurement of required SARS-CoV-2 Ag RDT reagents, kits and related supplies • Reagents and consumables verification prior to use • Systems for regular post-market surveillance required	X	X	X	X	X	X	X

Key: **X** (Responsible stakeholders for core activities)

Core activities	Key responsible stakeholders						
	Testing personnel	Testing laboratories	Min. of Health	Gov't Departs. & Agencies	Public Health Experts, Epidemiologists	Donor agencies	IPs
Data and records management systems • Data management facilities must be defined and provided (standard COVID-19 laboratory registers, relevant LIMS, relevant data collection tools, etc)	X	X	X	X	X	X	X
Documentation • Standardized relevant documentation specific for COVID-19 response must be developed and disseminated. These include but are not limited to SOPs, Policies and Guidelines for SARS-CoV-2 Ag RDT utilization • Resources mobilization and advocacy	X	X	X	X	X	X	X

3. Resources Mobilization and Advocacy

Successful implementation of quality assurance for SARS-CoV-2 Ag RDT intervention for COVID-19 response largely depends on adequate availability of resources (essential diagnostic and safety commodities), relevant regulatory mechanisms, monitoring and evaluation system including advocacy. This quality assurance framework, therefore, provides general guidance to mobilize necessary resources, and advocate for stakeholder engagement to ensure the optimal provision, regulation, and utilization of SARS-CoV-2 Ag RDT (Table 3).

3.1 Resources Mobilization

Table 3: Resources mobilization core activities

Required resources	Core responsibilities/activities	Lead stakeholders
SARS-CoV-2 Ag RDT kits	• Procurement of quality diagnostic kits • Validation and/or verification • Transportation and distribution	MoH Reference Labs Development Partners
Biosafety and biosecurity facilities	• Procurement/provision of Biosafety and Biosecurity facilities • Ensure appropriate PPE are procured, provided and are available in adequate quantities • Testing points must have the recommended materials to allow effective implementation of infection prevention and control measures	MoH Development Partners
Vital supplies (PPE, sample collection and transport materials, etc)	• Procurement and distribution of required commodities • Evaluation and monitoring the quality of supplies	WHO, MoH, Government Departments,
Human resources (testing personnel and all staff actively engaged in COVID-19 testing responses	• Adequate and competent HR workforce recruited to support SARS-CoV-2 Ag RDT along the testing cascade *(sample collection-transportation-reception-examination-reporting-results-sample disposal)* • Enhance HR competencies through training and mentorship programs	MoH, IPs, Donor Agencies, Testing laboratories, Others
Strategic guiding documentation & information	• Formulate/develop relevant policy guiding documents for SARS-CoV-2 Ag RDT rollout and implementation through the diagnostic tier • Develop and provide data/information management systems	WHO, MoH, National Reference Labs
Data and information management systems	• Acquire and/or modify existing data/information management systems to support COVID-19 M&E	MoH, National Reference Labs, Testing Labs

3.2 Advocacy

To accelerate uptake and utilization of SARS-CoV-2 Ag RDT, there must be deliberate efforts to advocate a wide range of stakeholders (Table 4) and make relevant data and information available.

Table 4: Advocacy considerations for SARS-CoV-2 Ag RDT Quality Assurance programs

Advocacy mechanism	Approaches	Stakeholders
Develop a communication strategy to advocate for use of SARS-CoV-2 Ag RDT in line with WHO and relevant guidelines	• High-level recommendations from WHO need to be communicated to country levels through national mechanisms • Develop, review and approve standardized testing algorithm for SAR-CoV-2 and communicate them to all relevant stakeholders • Identify champions and ambassadors to popularize the COVID-19 Ag RDT agenda	• Development Partners such as WHO, ACDC, etc • Ministries of Health • National/Regional Reference Laboratories • Funding agencies
Disseminate all relevant data and information on the performance of SARS-CoV-2 Ag RDT	• Disseminate all relevant COVID-19 Ag RDT diagnostic information to end-users as fast as possible through online platforms such as webinars, ECHO sessions, e-blasts, etc • Make available COVID-19 downloadable materials on gazetted e-platforms • Social media platforms can be used to rapidly disseminate SARS-CoV-2 Ag RDT data and information to a targeted audience	• Development Partners • Ministries of Health • Implementing Partners supporting COVID-19 response
Post-market surveillance	• Regular appraisal of the performance of SARS-CoV-2 Ag RDT by national agencies is recommended • Kits' post-market surveillance can be performed through review of IQC data, EQA performance data, lab test statistics data, or by conducting fully-fledged evaluations where resources allow	• MoH • National medical warehouses • Reference Laboratories • Academia (Universities, Researchers, etc)

4. Monitoring, Evaluation, Accountability, and Learning (MEAL)

Throughout the use of SARS-CoV-2 Ag RDT in the diagnosis of COVID-19, there is a continuous generation of data which is critical for strategic and technical decision-making to guide the response to the pandemic. This makes monitoring, evaluation, accountability, and learning (MEAL) indispensable in SARS-CoV-2 Ag RDT implementation. The data collected provides a core function of generating information for guiding the overall management of the pandemic response (Table 5).

Table 5: MEAL considerations for SARS-CoV-2 Ag RDT implementation

MEAL function	Core activities for consideration	Lead stakeholders
Data management	• Develop and standardize data collection tools for SARS-CoV-2 Ag RDT • Provide data storage facilities to maintain the integrity of the data/information management systems • Collection and recording of routine day-to-day operations at the SARS-CoV-19 testing facility	MoH Development Partners
Data reporting	• Develop reporting tools for SARS-CoV-2 Ag RDT services • Define types of data to be reported • Frequency, levels and channels of data reporting must be defined • Formulate KPI for the SARS-CoV-2 Ag RDT intervention for all testing tier levels	MoH COVID-19 Response Teams
Key Performance Indicators	• Define KPIs for all core activities of the QA program for SARS-CoV-2 Ag RDT **Examples of KPIs for SARS-CoV-2 Ag RDT for consideration are:** **(a) Test outputs/statistics:** • Sample rejection rates • COVID-19 positive or negative rates • Number/proportion (%) of tests performed by category, i.e. segregated by Sex (male/female), by sample type (e.g. oropharyngeal/nasopharyngeal/ nasal/sputum), by sample origin (e.g. region/district/hospital/ward) and as may be deemed necessary by the local requirements, etc • Invalid test rates **(b) System issues** • Stock management, e.g. stock out rates • Performance in QA • Safety issues • Customer feedback (management of complaints) • Test turn around time (TAT)	MoH Testing laboratories

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Appendix I:

Reporting form to be used by reference laboratories (NRL)

Summary of results of External Quality Assessment of COVID-19

Sample type	Number of Samples			Condition of Samples when received	Agreement of results (NRL vs Testing site)	
	Received	Tested	Rejected	Poor/Satisfactory/ Good	Total number of results agreed	Total number of results disagreed

Table 1. Comparison of site results and NRL results

		NRL results		Total
		Negative	Positive	
Site results	Negative			
	Positive			
Total				
Percentage agreement =				
Kappa* =				
Strength of agreement =				

Discussion/Recommendations

Prepared by

Checked by

Glossary

Alternative approaches:
Approaches developed to provide objective evidence for determining the acceptability of examination results (ISO 15189:2012). Whenever possible, this mechanism shall utilize appropriate materials. Examples of such materials may include:

- certified reference materials;
- samples previously examined;
- material from cell or tissue repositories;
- exchange of samples with other laboratories;
- control materials that are tested daily in inter-laboratory comparison programs

Antigen
An antigen is any substance foreign to the body that is capable of evoking or stimulating an immune system response to produce antibodies against it, example of antigen can be viruses, bacteria, proteins, or part of a virus, chemicals, pollen grains, toxins, etc

External Quality Assessment (EQA)
Objective assessment of a test site's operations and performance by an external agency or personnel (WHO/LQSI). Also, the term external quality assessment (EQA) is used to describe a method that allows for the comparison of a laboratory's testing performance with the performance of another laboratory. This comparison can be made with the performance of a peer group of laboratories or with the performance of a reference laboratory

Inter-laboratory comparison
ISO 17043 definitions: Organization performance and evaluation of measurements or tests on the same or similar items by two or more laboratories in accordance with predetermined conditions
ISO 15189 definitions: **Inter-laboratory comparisons:** The laboratory shall participate in an inter-laboratory comparison program(s) (such as an external quality assessment program or proficiency testing program) appropriate to the examination and interpretations of examination results

Nucleic acid amplification tests (NAATs)
These are laboratory diagnostic techniques that use small amounts of genetic material to detect nucleic acid sequences to detect and identify particular species or subspecies of organisms often micro-organisms such as viruses or bacteria. Because this technique deals with small amounts of genetic material, often it involves a step that amplifies the genetic material to make many copies that can then be measure and/detected.

Pandemic
A pandemic is defined as "an epidemic occurring worldwide, or over a very wide area, crossing international boundaries and usually affecting a large number of people"

Proficiency testing
Evaluation of participant performance against pre-established criteria through inter-laboratory comparisons
ISO/IEC Guide 43-1:1997: "Proficiency testing schemes (PTS) are inter-laboratory comparisons that are organized regularly to assess the performance of analytical laboratories and the competence of the analytical personnel".

Rapid diagnostic test (RDT)
A rapid diagnostic test is a medical diagnostic test that is quick and easy to perform and provides the test results within a short time of less than one hour, typically 15–30 minutes