

LabCoP Cookbook of best practices

RECIPE #5: QUALITY MANAGEMENT SYSTEM CONSIDERATIONS AND GUIDANCE FOR COVID-19 MOLECULAR TESTING LABORATORIES



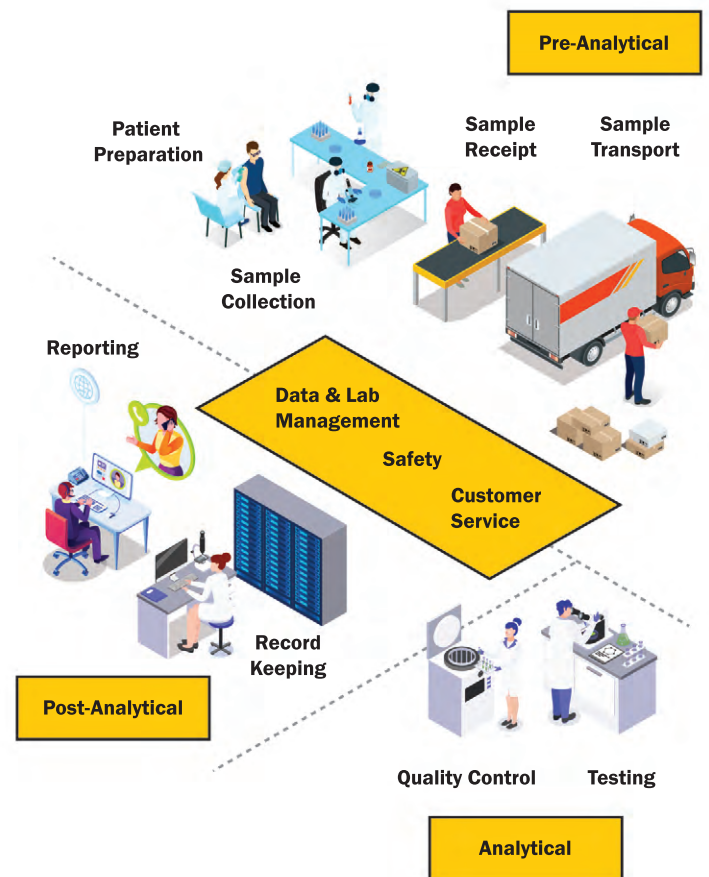
1 BACKGROUND

Laboratory diagnosis is an essential element of communicable disease surveillance, both for routine confirmation of infections and for the rapid identification of the cause of outbreaks and epidemics. A wrong diagnosis can have serious and expensive consequences, such as inappropriate treatment or wastage of vaccines and test kits. In many instances, only the laboratory can provide definitive identification and characterisation of an infectious agent, and in the event of an outbreak, this is the public health laboratory's key role. The coronavirus disease 2019 (COVID-19) pandemic is rapidly expanding in Africa. To help countries respond, the Africa Centres for Disease Control and Prevention (ACDC) recommends that Member States tailor their responses to the African context. COVID-19 diagnosis is currently based on nucleic acid-based amplification testing (NAAT), and serologic test development is in the pipeline.

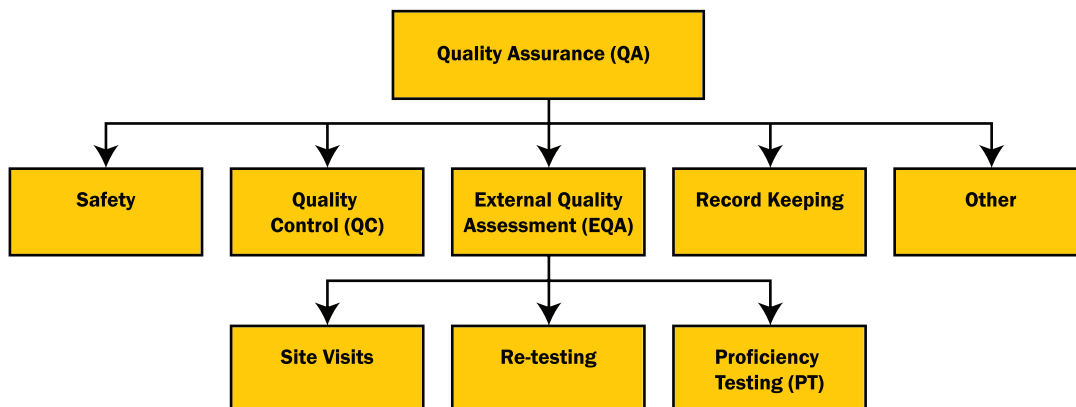
Implementation of a strong quality management system (QMS) consistent with standards for laboratory accreditation are key to improving patient care. To ensure that laboratories provide accurate and reliable results and reduce the risk of errors, implementing a QMS is important. However, in resource-constrained settings, the majority of laboratories are not accredited to international standards and may only be partially implementing elements of a QMS. Introducing a new test, particularly under outbreak conditions, may therefore come with a high risk of errors. Quality assurance (QA) is a part of the QMS that focuses on providing confidence for the fulfillment of quality requirements. It's a system designed to continuously improve the reliability and efficiency of laboratory testing services. Important elements of a quality management system include documentation, standard operating procedures, quality control samples, and external quality assessment scheme.

This LabCoP recipe describes the critical elements that laboratories should put in place to rapidly identify and minimise the risk of laboratory errors in implementing laboratory testing for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, and define the minimum QA requirements for NAAT testing (screening, confirmation and surveillance). These elements may apply to outbreaks similar to COVID-19. This recipe covers critical elements in the entire testing cascade from the pre-analytical to the analytical and post-analytical phases.

LABORATORY WORKFLOW



QUALITY ASSURANCE ACTIVITIES



CONSIDERATIONS FOR FACILITY BIOSAFETY

- Initial processing of all specimen including those for sequencing and NAAT should take place in an appropriately maintained and validated biological safety cabinet or primary containment device. The minimum recommended level of safety to handle samples and/or perform testing containing SARS-CoV-2 is biosafety level 2 (BSL-2).^{1,2}
- Appropriate disinfectants with proven activity against enveloped viruses must be used for the recommended contact time, dilution and within the expiry date after the working solution is prepared.
- All technical procedures should be performed in a way that minimises the generation of aerosols and droplets.
- Appropriate personal protective equipment (PPE) as determined by a detailed risk assessment, should be worn by all laboratory personnel handling these specimens.
- Laboratories not able to meet the above biosafety recommendations should consider transferring specimens to national, regional or international referral laboratories with SARS-CoV-2 detection capacity that can meet the biosafety requirements.

CONSIDERATIONS FOR STAFF REQUIREMENTS

- Laboratory personnel should be qualified and certified to work in the laboratory and should be trained in the relevant technical and safety procedures (Table 1).³ Ideally, they should be familiar with polymerase chain reaction (PCR) processes. A training log should be included as part of the laboratory documentation. The laboratory should be at least BSL-2 and maintain a unidirectional workflow.
- The best approach is to repurpose trained staff for the detection of SARS-CoV-2 wherever possible.
- For molecular testing, staff trained on molecular testing for tuberculosis, HIV and flu surveillance have the correct training for SARS-CoV-2 detection and need a refresher on the standard operating procedures (SOPs) for COVID-19 diagnosis.
- For serology testing, staff engaged in routine serological tests for other viral pathogens such as measles, yellow fever, influenza, etc. have the necessary skills for COVID-19 diagnosis.
- Ensure that the staff know roles and responsibilities for sample collection, inactivation, processing and result reporting. A brief refresher training should be provided and should cover sample collection, packaging and shipment (referral), sample inactivation, testing, interpreting and reporting.
- All accrediting agencies require tests (commercial and in-house) to be validated or verified prior to implementation. An additional refresher training for test validation is useful for in-house testing. In-house tests will require more expertise therefore it may be better to adopt commercially available tests.

Component	Mitigation measures
Quality training	Organisation's/laboratory's code of ethics, quality management system, problem-solving approach, quality objectives, and quality control program
	Staff members' roles in each of the above to include: Receiving training; maintaining competence; following procedures as written; reporting complaints and nonconformance; practicing good customer service skills; collecting data for quality indicators and monitoring; participating in quality improvement initiatives
Safety training	Laboratory's general safety, universal precautions, hazard communication, spill containment and clean-up, fire/disaster preparedness, accident reporting system, bioterrorism preparedness
	Work area-specific safety, special safety precautions, disposal of hazard waste, personal protective equipment, chemical hygiene plan
Job-related training	Work processes (i.e., workflow) and related procedures (i.e., task instructions)
	Recording of all required information

Table 1. Example of laboratory training program.

I. PRE-ANALYTICAL PHASE

The pre-analytical phase refers to all of the steps required to deliver specimens from the patient to the analytical assay. This phase includes specimen collection and handling issues that occur prior to the time the specimen is received in the laboratory. Important errors can occur during the pre-analytical phase with specimen handling and identification. Therefore, the pre-analytical phase must have rigorous control measures to avoid unwittingly allowing problems or errors to travel further 'downstream'. The key considerations under this phase include:

SPECIMEN COLLECTION

- All specimens collected for laboratory investigations are regarded as potentially infectious, hence biosafety must be emphasised.
- Test request forms that capture all information needed for proper handling and reporting for both patient management and surveillance must be used. At a minimum, the form should include patient details (name, address, telephone number, birth date, gender, etc.), requester details, type of primary sample, examination(s), requested clinical information relevant to the laboratory, date, time and place of sample collection and date and time of receipt of the sample at the laboratory.⁴
- The recommended sample types include upper and lower respiratory specimens (Table 2). The upper respiratory specimens include nasopharyngeal and oropharyngeal specimens collected using Dacron or polyester flocked swabs. Calcium alginate swabs and cotton swabs with wooden shafts are not recommended. Wash specimen in ambulatory patients is also acceptable. Lower respiratory specimens include sputum if produced without induction. Others include endotracheal aspirate or bronchoalveolar lavage in patients with more severe respiratory disease collected in sterile container. Blood specimen can be collected for serology. Stool and urine are currently not recommended. Collect sufficient specimen (2-3 ml in case of blood, sputum/aspirate) to allow repeat tests in case of test failure, archiving, etc.⁵
- Specimen collection tubes must be correctly labelled with patient details, and date and time of sample collection.
- Use of appropriate PPE (e.g., gloves, solid front or wrap-around gown, face masks, respirators (if available)). Do not attempt to collect specimen without appropriate PPE.

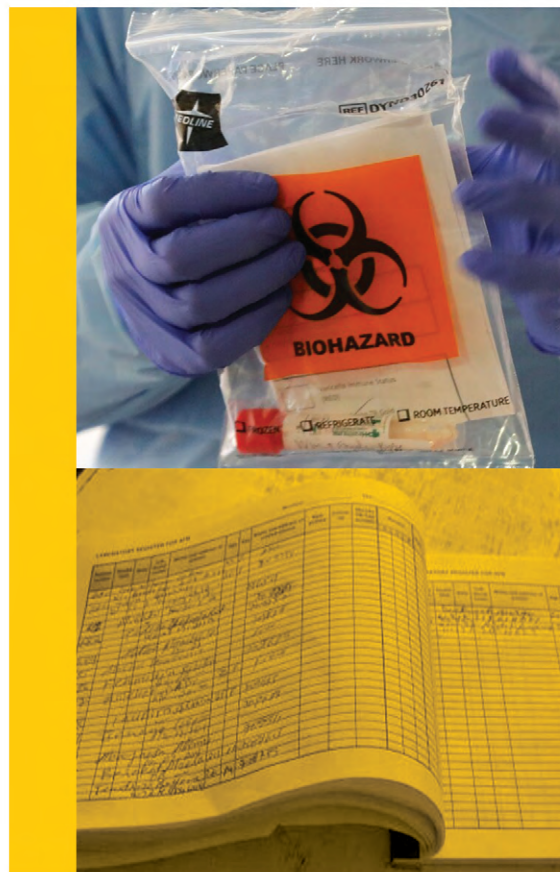


Table 2: Summary of specimen types and collection methods.

Specimen collection location	Specimen type	Collection methods
Upper respiratory	Swab, wash	Nasopharyngeal and oropharyngeal swab such as Dacron or polyester flocked swabs. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended.
Lower respiratory	Sputum, endotracheal aspirate or bronchoalveolar lavage	Sterile, leak-proof, screw-cap sputum collection cup or sterile dry container
Venus	Blood	Anti-coagulated tubes
Lower gastrointestinal tract	Stool, urine	Not recommended

SAMPLE PACKING AND SHIPMENT

- Provide instructions on where to ship the samples, based on national recommendations. If those do not exist, ask the reference laboratory designated in your country or contact ACDC. Do not send samples without confirmation that they can be accepted and processed.
- Specimens which can be delivered promptly to the laboratory within 48 hours of collection must be stored and shipped at 2-8 °C in a cool box. When there is likelihood of a delay (beyond 48 hours) in specimens reaching the laboratory, the use of viral transport medium (VTM) is strongly recommended (VTM can be locally prepared).⁵ Specimens must be frozen immediately to -20 °C or ideally -70 °C and shipped on dry ice. It is important to avoid repeated freezing and thawing in order to maintain specimen integrity.⁶ A recent study demonstrated that minimum essential media, sterile phosphate buffered saline, or 0.9% saline as alternatives to VTM for SARS-CoV-2 testing.⁷
- Monitor temperature with a thermometer or physically check amount of dry ice on reception.
- Triple packaging is necessary for transportation to prevent spillage. It consists of:
 - Leak-proof primary container
 - Rigid, leak-proof, watertight secondary packaging with absorbent material
 - Rigid outer packaging to protect the specimens during shipment.Triple packaging should be available in clinics and in laboratories. The World Health Organization (WHO) has developed guidelines for safe transport of infectious substances.⁸



- Laboratory personnel should record all specimens received in a laboratory accession book, worksheet, computer or comparable system, as well as the date and time of receipt of samples (turn-around-time), as well as the identity of the receiving officer.
- Establish clear criteria for acceptance/rejection at the testing laboratory (e.g., checks on sample integrity, broken/leaking sample tube, specimen reaching laboratory beyond 72 hours, inadequate sample volume depending on the sample type, etc.). The reason for sample rejection must be communicated back to the requesting clinic as part of corrective action and to initiate recollection of specimen from the patient.
- **Reference:** Specimens should be packed according to the *International Air Transport Association (IATA)*. SARS-CoV-2 specimens should be packed in compliance with the *UN regulations UN3373 for biological substances, Category B*.

PROCUREMENT OF EQUIPMENT, TEST KITS, REAGENTS AND PPE

- Laboratories able to procure diagnostics are advised to only procure those with emergency use authorisation from the *United States Food and Drug Administration* or those in the *WHO's emergency use list*.
- Preference should be given to test kits that have been independently evaluated (e.g., *Foundation for Innovative New Diagnostics*) with data using a large sample size.
- Procurement of supplies is best done through existing distributors or supply networks within the country. Due consideration should be given to forecasting and procurement of ancillary reagents (e.g. extraction buffers and sample collection materials).

II. ANALYTICAL PHASE

The analytical phase is usually considered to be the ‘actual’ laboratory testing, or the diagnostic procedures, processes and products that ultimately provide results. SOPs for PCR and sequencing must be in place. The key process under this phase include:

IN-HOUSE ASSAY VALIDATION AND VERIFICATION

- Prior to introducing a new test, your laboratory must confirm that the new test performs as intended.
- Prior to the start of testing, a validation or verification (consisting of known positive and negative samples) should be performed to ensure the test performs as intended.
- **Validation** is the in-depth, rigorous collection and analysis that establishes test performance criteria for a new or modified test. Validation is performed by the test developer/manufacturer or test modifier and often required for regulatory approval of a test.
- **Verification** is the initial and on-going confirmation that test performance meets the criteria established during validation. The initial verification is performed before the new test is offered. Verification is also required to meet accreditation criteria.
- An assay that has been fully validated, and the information is readily available from the product insert or online, only needs verification by the user. For non-standard methods, standard methods used outside of their normal scope, modified methods (e.g. commercial kit used with specimen type not recommended by manufacturer, ‘off-label’ / ‘off-license’ / ‘in-house’ use), all need to be validated.
- Under emergency conditions, validation and verification studies may have to be limited. Laboratories may take advantage of the WHO recommendation of confirming the first five positive specimens and first 10 negative specimens (collected from patients that fit the case definition) by referring them to one of the WHO reference laboratories providing confirmatory testing for COVID-19.
- Instructions and information from suppliers on test performance should be used to guide introduction and implementation of the test as part of the national testing algorithms.
- Alternatively, less experienced laboratories can be mentored by laboratories with more experience with this pathogen to have their initial test results confirmed and improve their performance.



REAGENT PREPARATION

- Commercial kits include all reagents required for the test. For kits supplied by Africa CDC, laboratory personnel must check the kits for any additional requirements, including extraction reagents.
- Testing reagents procured from commercial sources (e.g., QIAGEN, BGI Genomics, DAAN Gene, etc.) should be reconstituted in a biosafety cabinet or laminar flow hood in accordance with the product insert or SOP, and must be brought to the right temperature conditions before use (use cold blocks or ice).
- Freeze-thaw cycles should be minimised. Reagents should be labelled with the date received and opened including the initials of the laboratory personnel. Always check the expiration date prior to use. Do not use expired reagents.
- Do not substitute or mix reagents from different test kit lots or from other manufacturers. Change aerosol barrier pipette tips between all manual liquid transfers.



- Maintain separate areas for assay setup and handling of nucleic acids.
- Primer and probe quality is of huge concern. Thus, primers and probe must be handled with extra precautions (e.g., after resuspension and dilution, aliquot samples immediately into enough for one run).



SAMPLE PROCESSING

- RNA extraction must be done in a biosafety cabinet in a BSL-2 or equivalent facility. Specimens should be aliquoted into separate tubes to avoid repeated freeze-thaw cycles. Samples must be allowed to thaw completely before use in the procedure (use cold blocks or ice). Purulent or clotty sputum should be treated with dithiothreitol prior to aliquoting.⁹
- Test tubes must be clearly labelled with specimen details to enable traceability. Use pipettes with aerosol-barrier or positive-displacement tips to handle specimens. Always use a new pipette tip for each specimen.

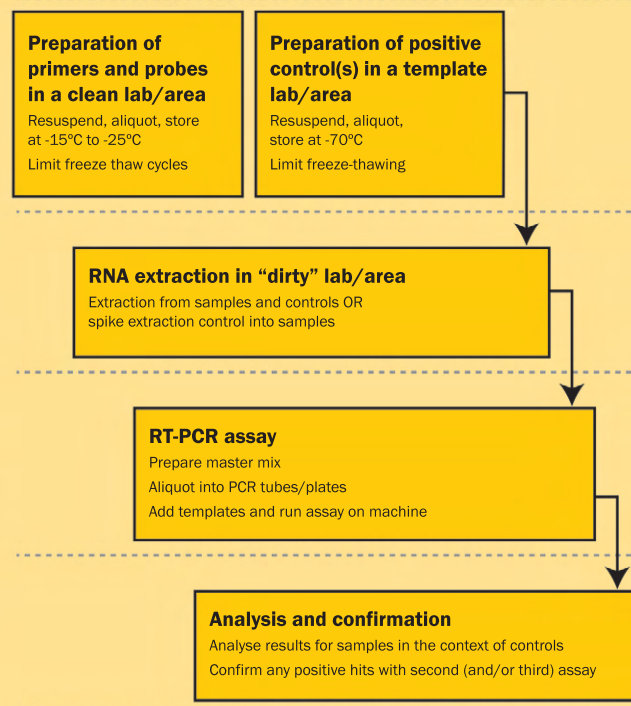


Figure 1: COVID-19 PCR workflow.

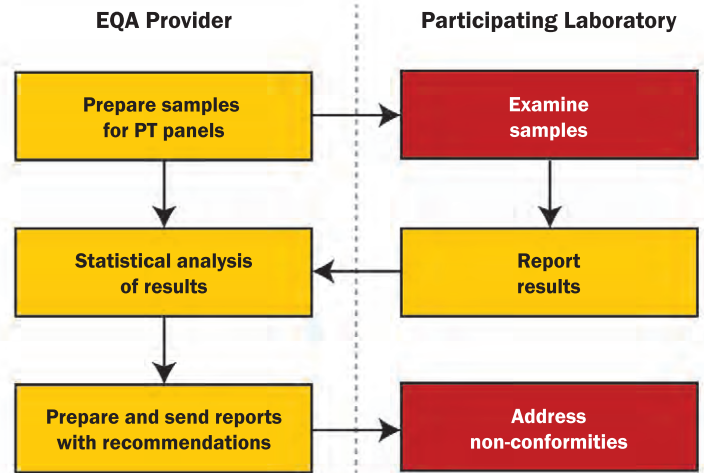
QUALITY CONTROLS

- Quality control (QC) involves examination of control materials or known substances at the same time and in the same manner as patient specimens to monitor the accuracy and precision of the complete analytical process. QC must be included in each run and should cover each critical step of the PCR analysis as shown in Figure 1.
- The QC includes the following components:
 - o **Extraction negative control:** indicates whether contamination was introduced from the extraction phase.
 - o **Extraction positive control:** provides an indication of the quality of the extracted template and whether the PCR was in anyway inhibited.
 - o **No template control:** indicates whether contamination was introduced from the PCR phase. Also indicates whether PCR reagents have been compromised and is used to determine threshold levels.
 - o **Positive template control(s):** uses synthetic SARS-CoV-2 RNA/DNA (either gene fragment or whole genome) to indicate the limit of detection and robustness of the assay.
- A number of third-party commercial companies supply QC kits for the extraction and amplification steps of SARS-CoV-2 testing, including those listed below. Prices range from \$50-\$550 USD for 100 tests:
 - o *ZeptoMetrix*
 - o *SeraCare*
 - o *European Virus Archives-Global*
 - o *Bio-Rad*
- Commercial QCs are preferred, but in the absence of commercial controls, laboratories can use the following:
 - o **Negative control:** Water or universal transport media or viral transport media
 - o **Positive control:** A patient sample with a known (and preferably low 25-30) Ct value (virus concentration) for human gene target (e.g., RNase P) or non-human, non-SARS-CoV-2 extraction control (e.g. Equine Arteritis virus).
- A failure of any one of these controls (e.g., the positive control turns out to be negative) invalidates the test result and the assay must be repeated either from a stored or newly collected sample after investigating and fixing the cause of the failure (e.g., contamination or degradation of the sample or expired reagents). If patient results were already issued, they should be re-called immediately, given an explanation of the reason, and the patient re-tested urgently.
- **New lot QC, or lot-to-lot verification** describes the process in which newly received lots/batches of test kits or test components are tested using a panel of samples to confirm that their performance is acceptable relative to the existing lot in use.

EXTERNAL QUALITY ASSESSMENT

EQA allows a laboratory's testing performance to be compared to the performance of a peer group of laboratories, national reference or WHO reference laboratories. The three different methods for EQA programs are described below.

Proficiency testing (PT): an external provider sends a blinded, well-characterised panel at intervals (usually quarterly) to a set of laboratories for analysis. The blinded panel is treated like a patient sample during testing, and the results are analysed, compared and feedback reports generated as shown in Figure 2. Laboratories should choose providers experienced in delivering EQA in their region.



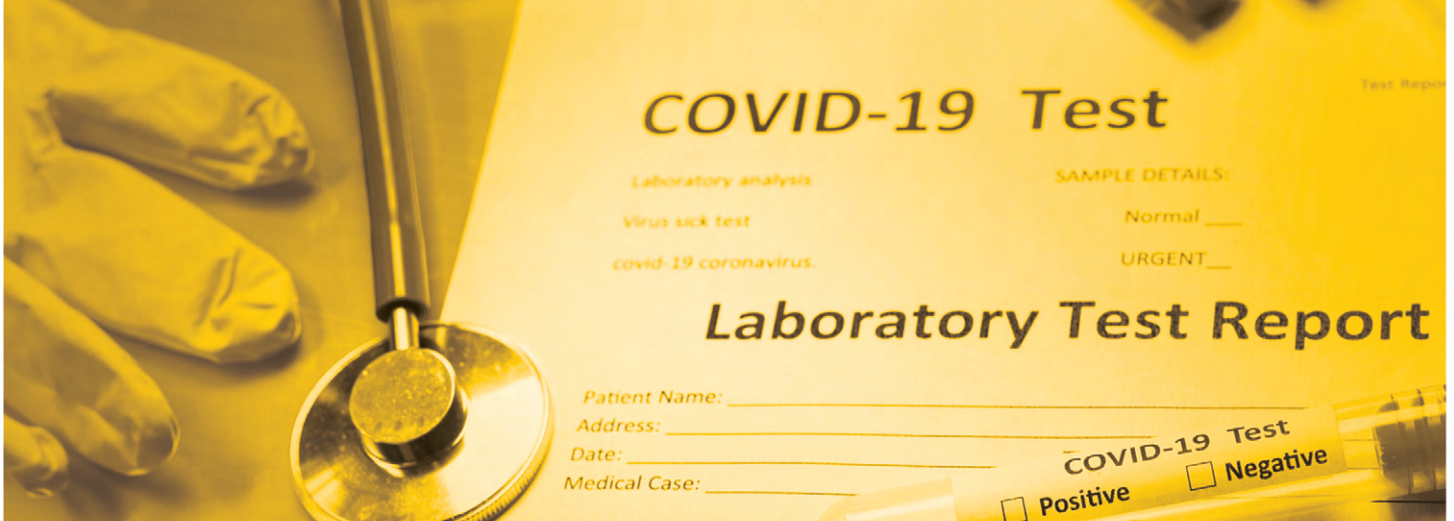
- Some examples of COVID-19 PT are shown below.:
 - o QCMD (<https://www.randox.com/coronavirus-qcmd/>)
 - o INSTAND (<https://www.instand-ev.de/en/eqas/eqa-program.html>)
 - o WHO Health, Emergencies and Global Influenza program (https://www.who.int/influenza/gjsrs_laboratory/external_quality_assessment_project/en)
 - o ECDC/EVD-LabNet/ERLI-Net (<https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-influenza-lab-quality-control>)
 - o Thistle QA (<http://www.thistle.co.za/coronavirus-proficiency-testing/>)
- Laboratories can enroll for free as part of the influenza laboratory network, or at a cost not exceeding \$420 USD, but this may vary by country. Laboratories should choose providers experienced in delivering EQA panels within their region.

Rechecking or retesting: samples tested by one laboratory are retested by another laboratory (inter-laboratory comparison). WHO recommends that the specimens of the first five positive cases and the first 10 negative cases that meet the COVID-19 case definition for testing should be shipped for confirmation to the national reference or international referral laboratory for COVID-19. After that, the laboratory can test for SARS-CoV-2 independently but should still collaborate with national reference laboratories or WHO referral laboratories for troubleshooting. Rechecking can be employed in the absence of a PT program.

On-site evaluation: usually done in addition to PT or rechecking and may be done when it is difficult to conduct traditional PT or rechecking/retesting. An evaluator (e.g. staff from national reference laboratory) will visit the laboratory to check if the laboratory is meeting quality requirements, retest and verify few test results and provide advice to correct any faulty procedures. On-site visits are also important to motivate staff and provide refresher training if needed. Due to the current situation, air transportation is limited, and it may not be feasible to get PT or conduct on-site evaluations. Therefore, countries are strongly advised to use the rechecking/retesting method as an option for an EQA program (sending samples to the national reference or WHO reference laboratories)³ and to consider remote mentoring/supervision of laboratories by the national reference laboratory using web conferencing systems such as Zoom.

- Some challenges associated with implementing QC and possible solutions are summarised in the table below.

Challenges	Mitigation measure
Unavailability of controls	Positive control: use of a confirmed positive patient sample. Negative control: use water/universal transport media/viral transport media.
Most methods are under development hence no validation data	Use methods with emergency use listing by WHO. Check https://www.who.int/diagnostics_laboratory/EUL/en/ and third party evaluated methods and perform method verification to the extent possible.
Unavailability of EQA schemes	Develop inter-laboratory comparison and send positive samples to national reference and/or WHO reference laboratories.



III. THE POST-ANALYTICAL PHASE

The post-analytical phase is the final phase of the total testing process, and involves evaluation of laboratory test results; release of test results in a timely manner to appropriate individuals, particularly critical results; and modification, annotation or revocation of results as necessary to support clinical decision-making. The key processes under this phase include:

TEST RESULT INTERPRETATION AND USE FOR PATIENT MANAGEMENT

- Result interpretation should follow available guidance or algorithms.
- If the result is discordant, the patient should be resampled and possibly the sample sequenced.
- Any surprising result should be sent for confirmation at an international reference laboratory.

TEST RESULT REPORTING

- Test results must be reviewed independently by a laboratory supervisor to confirm accuracy before they are released to the requesting clinic. This involves confirming that the patient details are correct and matching the test requisition and validity of the test indicated by the control results.

NOTIFICATION FOR DISEASE SURVEILLANCE

- Laboratories should follow national reporting requirements. All tests whether positive or negative should immediately be reported to national authorities.

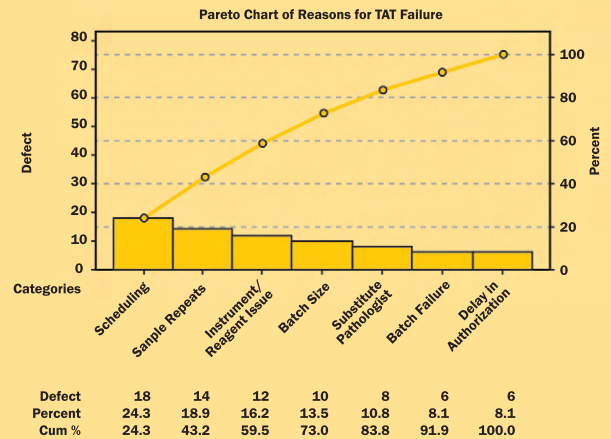


IV. KEY PERFORMANCE INDICATORS (KPI)

KPI are used to monitor the routine performance of the whole testing process and should be analysed and reported on a regular basis (at least monthly). KPIs should include the following:

- Number of specimens tested, by specimen type
- Number (%) of positive, negative and invalid test results
- Specimen rejection rate
- Number (%) of failed internal quality control results
- EQA/PT performance (pass/fail or % score)
- Turnaround time (TAT), between specimen collection and result reporting (total TAT), and within-laboratory TAT (% results reported within target TAT, average and range of TAT)

PARETO ANALYSIS OF TAT ISSUE SOURCES IN THE MOLECULAR DEPARTMENT



Analysis of key performance input variables directly linked to improving TAT in a molecular lab. Credit: Sawney CG and Woody J. *Continuous Improvement in the Molecular Department. Medical Lab Management. 2019; 8:3*

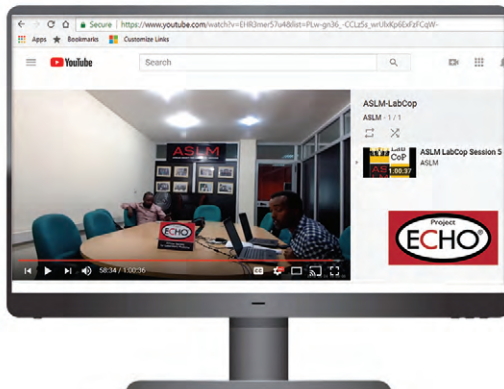


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