Assuring quality COVID-19 test results: quality control and external quality assurance strategies

Introduction

To ensure that laboratories provide accurate and reliable results and reduce the risk of errors, implementing a quality management system (QMS) is important. However in resource-constrained settings, the majority of laboratories are not accredited to international standards and may only be partially implementing elements of a QMS. Introducing a new test, particularly under outbreak conditions, may therefore come with a high risk of errors, and this step describes the key critical elements that laboratories should put in place to rapidly identify and minimize the risk of laboratory errors. In the absence of quality assurance (QA), use of inaccurate test results can lead to the wrong treatment and management decisions and lapses in surveillance of disease epidemics.

What is QA?

QA refers to the total process implemented by a laboratory which aims to ensure that the final results reported are as accurate and reliable as possible.

Main elements of QA include:

- **Standard operating procedures (SOPs)** should be available and staff trained on their use. SOPs should cover all procedures, from managing incoming specimens to authorizing and issuing test reports.
- **Documentation.** Laboratory forms and registers should be standardized and staff trained to fill out all documentations consistently and fully.
- **Quality control (QC)** which refers to procedures used in each assay to assure a test run is valid and results are reliable.
- **Quality indicator monitoring**, which refers to collection and analysis of that can serve as indicator for correct performance of the total testing process.
- **External Quality assurance**, which aims to analyze the accuracy of the entire testing process from receipt of sample and testing of sample to reporting of results

PCR for COVID -19 is the recommended method to reliably identify COVID-19 cases. This article focuses on QA for the molecular testing for SARS-COV-2 which has better sensitivity and specificity.

What quality control should be performed for COVID-19 testing?

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- 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. Under emergency conditions the validation and verification studies may be limited as discussed in the previous steps.
- Laboratories may take advantage of the WHO recommendation of confirming the first 5 positive specimens and 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 (Refer to WHO Reference laboratories providing confirmatory testing for COVID-19 https://www.who.int/docs/default-source/coronaviruse/who-reference-laboratories-providing-confirmatory-testing-for-covid-19.pdf?sfvrsn=a03a01e6_4 in **See Also** section of this step).

- QC aims to detect, evaluate and correct errors due to test system failure, environmental conditions or operator performance, before patient results are reported.

- QC must be included in each run and should cover each critical steps of the PCR analysis as shown below.

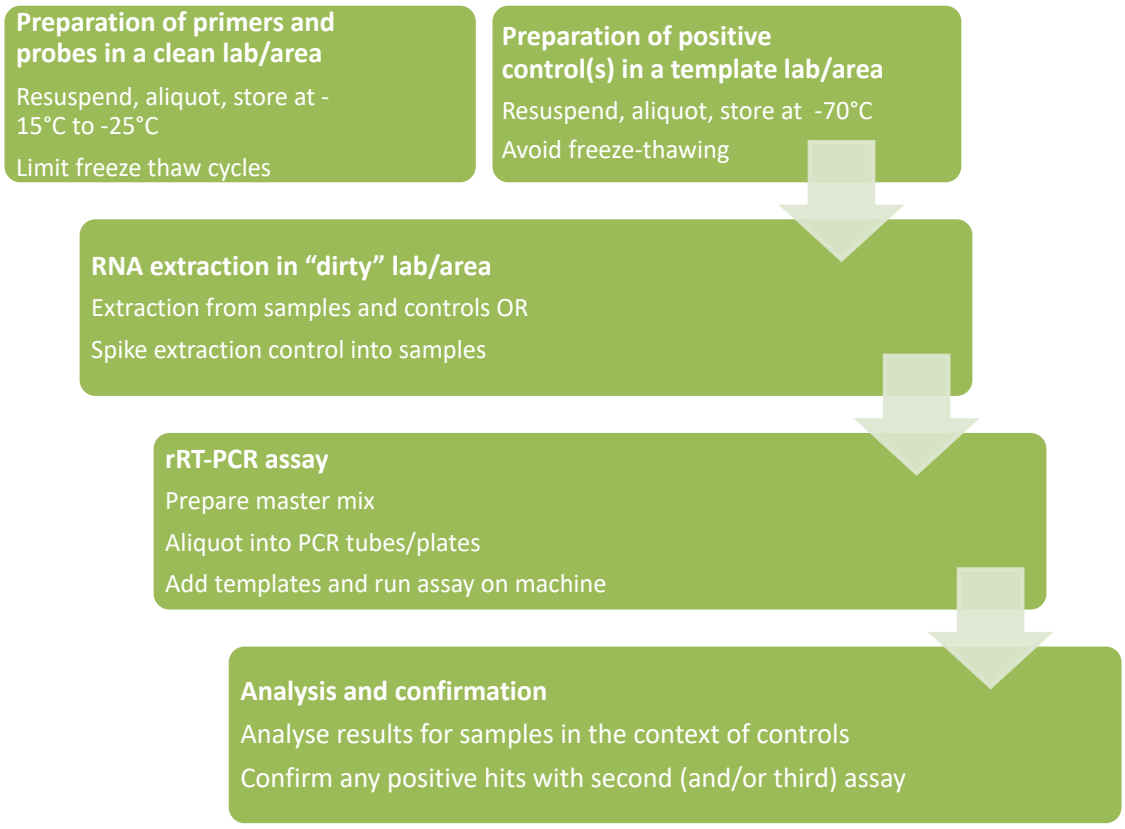

Figure 1: COVID-19 PCR work flow. Key steps in sample/QC processing

- The QCs include;
    - **Extraction negative control:** Indicates whether contamination was introduced from the extraction phase
    - **Extraction positive control:** Provides an indication of the quality of the extracted template and whether the PCR was in anyway inhibited.
    - **No template control:** Indicates whether contamination was introduced in from the PCR phase. Also indicates whether PCR reagents have been compromised and to determine threshold.
    - **Positive template control(s): synthetic SARS-CoV-2 RNA/DNA** (either gene fragment or whole genome): Indication of limit of detection and robustness of the assay.

A number of third-party commercial companies supply full process controls for the extraction and amplification steps of SARS-CoV-2 testing. Some of the current providers include those listed below with prices ranging from 50-550 UD Dollars for 100 tests:

- 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)

Commercial QCs are preferred but absence of commercial controls, laboratories can use the following;

- **Negative control:** Water/universal transport media/viral transport media
- **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
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  A failure of any one of these controls (for instance, the positive control turns out to be negative) invalidates the test result and the assay must be repeated either from 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). In case patient results were already issued, they should be re-called immediately (giving an explanation of the reason) and the patient re-tested urgently.

New lot QC, or lot to Lot Verification - Newly-received lot (or batch) of test kits or test components is tested using a panel of samples to confirm its performance is acceptable relative to the existing lot in use.

Key performance indicators (KPIs)

- KPIs refers to collection and analysis of data at each step of the testing cascade that can serve as indicator for correct performance of the whole testing process.

KPIs include the following, and should be analyzed and reported on a regular basis, at least monthly:

- Number of specimens tested, by specimen type
- Number (%) of positive, negative and invalid test results
- Specimen rejection rate
- Number (%) of failed IQC results
- EQA/PT performance (pass/fail or % score)
- Turnaround time (TAT)

    - Laboratories should monitor the following: % results reported within target TAT, average and range of TAT).

    - Total TAT is the time between specimen collection and result reporting to the clinician. In addition to monitoring total TAT, the laboratory should also measure time from specimen collection to receipt at laboratory, time from receipt at laboratory to result reporting (within laboratory TAT), in order to identify and address bottlenecks at various stages of the diagnostic process.

What options are available for EQA for COVID-19 testing?

- EQA allows the comparison of a laboratory's testing performance to a peer group of laboratories, national reference or WHO reference laboratories. There are three different methods for EQA programs:
    - **Proficiency Testing (PT):** An external provider sends blinded, well characterized panel at intervals (usually quarterly) that will be treated like patient sample during

testing, to a set of laboratories, and the results are analyzed, compared and feedback reports generated.

- Some examples of PT providers who can provide COVID-19 panels include the following;
  - 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/) and
  - ECDC/EVD-LabNet/ERLI-Net (https://www.ecdc.europa.eu/en/about-us/networks/disease-and-laboratory-networks/erlinet-influenza-lab-quality-control).

  Laboratories can enroll for free as part of the influenza laboratory network or at a cost not exceeding US dollar 420 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 (5) positive cases and the first ten (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 [*WHO, 2020a; WHO, 2020b*]. 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. Re-checking can be employed in the absence of a PT program.
- **On-site evaluation:** usually done in addition to PT or re-checking, and particularly when it is difficult to conduct traditional PT or to use the rechecking/retesting method. 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.

Due to COVID19 crisis, air transportation is limited and it may not be feasible to get PT or conduct on-site evaluation. Therefore, countries are strongly advised to use the rechecking/retesting method as an option for EQA program (send samples to the national reference or WHO reference laboratories [*WHO, 2020c*]) and to consider remote mentoring/supervision of laboratories by the national reference laboratory using Zoom or other web conferencing systems.

What are challenges to implementing QA?

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 Listing (EUL) 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 also send positive samples to national reference and/or WHO reference laboratories

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