Review Article

Laboratory Diagnostic Phases of Detection of SARS-CoV-2 by Real-Time Reverse Transcription-Polymerase Chain Reaction

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Abstract

The emergence of coronavirus disease 2019 (COVID-19) has caused global health concerns in the various strata of health-care authorities. The detection of the etiologic agent, SARS-CoV-2 became essential for case identification and prevention of transmission. In this review, literature was retrieved and sifted from different search engines. We highlighted some mainstream pointers vis-à-vis preanalytical, analytical, and postanalytical phases of laboratory detection of SARS-CoV-2 using real-time reverse transcription-polymerase chain reaction (RT-PCR) assay. The quantitative RT-PCR is considered as the gold standard for establishing the diagnosis of COVID-19. However, laboratories must pay attention to all the testing phases from sample collection to final release of results in order to avoid false-negative or false-positive results. It is also necessary to understand possible challenges and conduct risk assessment before the commencement of testing in the designated laboratory.

Keywords: Detection, Nigeria, reverse transcription-quantitative polymerase chain reaction, SARS-CoV-2

Received on: 12-09-20 **Revised completed on:** 13-10-20 **Accepted on:** 22-03-21 **Published on:** 09-08-21

Introduction

A cluster of patients with pneumonia of unknown origin were identified in Wuhan, Hubei Province, China, at the end of 2019.[1] Since then, the infection has spread to become a global pandemic accounting for more than 21 million infections and about 766 thousand deaths as at August 2020.[2] The cause was later identified from the bronchoalveolar lavage fluid of the patients as a previously described β-coronavirus. It was identified by whole-genome sequencing, direct polymerase chain reaction (PCR), and culture.[3] The virus was initially named as the 2019-novel coronavirus (2019-nCoV) and later renamed as SARS-CoV-2. The World Health Organization (WHO) has officially named the disease as coronavirus disease 2019 (COVID-19).[4]

Real-time reverse transcriptase-PCR (RT-PCR) is considered as the gold standard for the confirmation of SARS-CoV-2 infection.[5-7] The assay is characterized by rapid detection, high specificity, and sensitivity for confirmatory diagnosis of COVID-19 suspected cases and asymptomatic contacts.[8] The test is also used as one of the criteria for discharge of isolated patients[5] and as differential diagnosis for cases with indefinite respiratory syndromes.[8] As at the time of writing this review, there were about 50 centers across Nigeria that test for COVID-19 using the RT-PCR platform while about 15 centers utilize Cepheid Xpert® Xpress.[9]

Notwithstanding the acceptability of the test, the danger of prompting false-negative or false-positive results was reported[6,10,11] that may or may not be likely due to some hitches at the level of samples accessioning, inactivation, nucleic acids extraction, reactions mixture preparation, PCR running,

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Access this article online

Quick Response Code:

Website: www.atpjournal.org

DOI: 10.4103/atp.atp_49_20

How to cite this article: Tahir MI, Ahmad AE, Usman Y, Muhammad A, Ige TO, Jimoh O, *et al*. Laboratory diagnostic phases of detection of SARS-CoV-2 by real-time reverse transcription-polymerase chain reaction. Ann Trop Pathol 2021;12:1-8.

results in analysis, and dissemination. Therefore, this review was aimed to highlight some important pointers associated with the preanalytical, analytical, and postanalytical phases of laboratory detection of SARS-CoV-2 by quantitative real-time PCR technique based on our experiences and those of other testing facilities in Nigeria.

Methodology

Literature for writing this review was retrieved from different sources that included PubMed, Institute of Science Information, Google Scholar, and other search engines. The review is written under preanalytical, analytical, and postanalytical phases of laboratory diagnosis.

Preanalytical phase

Sample types and sensitivity of the test

An initial and very important aspect in accurate laboratory diagnosis of COVID-19 is the type or choice of sample. SARS-CoV-2 RNA could be detected in samples from the upper respiratory tract, lower respiratory tract, stool, blood, and urine of infected persons[6] but with varying sensitivity levels.[6,7,12] The triad of the natural history of COVID-19, viral load kinetics in various anatomic sites of the patients, and sampling procedures contribute to false-negative results.[6] In a study conducted by Wang *et al*.,[13] bronchoalveolar lavage fluid (BLF) showed the highest positive rate of 93% from confirmed patients while sputum, nasal swab, fibrobronchoscope brush biopsy, pharyngeal swab, stool, and blood samples had positive rates of 72%, 63%, 46%, 32%, 29%, and 1%, respectively. Another report has indicated the highest positive rate of 48.68% in sputum, whereas nasopharyngeal swab (NPS), anal swab, stool, and blood had positive rates of 38.13%, 10.0%, 9.83%, and 3.03%, respectively.[7] Despite high sensitivity of BLF, it is not workable for routine laboratory diagnosis and disease monitoring as it is an invasive procedure which requires suction tool and more expertise. Therefore, sputum, nasal swab, and throat swab are often used because they are rapid, simple, and safer.[6] Collection of both NPSs and oropharyngeal swabs (OPSs) into a single tube containing viral transport medium increases sensitivity and is highly recommended.[10,12] When NPSs and OPSs were combined within a single aliquot of viral transport medium, the sensitivity of the test was found to be significantly higher than when samples from either swab methods was used alone.[13]

Some studies have demonstrated the applicability of use of saliva samples for both detection and disease monitoring.[14-16] It was found that the sensitivity of SARS-CoV-2 detection from saliva is comparable to NPSs.[15] The adoption of saliva as sample for real-time RT-PCR will reduce the risk of transmission to health-care workers during sample collection procedure when compared with the nasopharyngeal or OPS procedure.[14] When serial viral load monitoring is desired, the use of saliva will ease the discomfort created to patients by repeated sample collection through nasopharyngeal and OPSs.[14,16] In Nigeria for example, the Nigeria Centre for Disease Control (NCDC) has recommended collection of both NPS and OPS in a single viral transportation medium for SARS-CoV-2 RT-PCR detection.[17]

Sample management (collection, transportation, and storage)

Correct sample management from collection, labeling, transportation, and storage where necessary is crucial to maintain the sample integrity for a reliable outcome and good turnaround time. Sample quality mostly depends on the skills of the collector and the material used for the collection and transportation. NPS is expected to be inserted through the nares and parallel to the palate, while OPS should be inserted into the posterior pharynx and tonsillar areas.[12] NPS has a characteristic quality control as it generally reaches the correct area to be tested in the nasal cavity.[18]

Appropriate swabs for sample collection should be made from dacron or polyester[6] while wooden shaft or calcium alginate swabs are discouraged as they may contain PCR inhibitors.[12] After collection, the swabs are placed into the viral transportation medium for immediate transportation to the laboratory under refrigerated conditions.[6,18] Samples transported in saline were reported to be stable for real-time RT-PCR detection; however, during storage, Ct values increase linearly with time.[19]

Samples are expected to be transported in a triple packaging system accompanied by completed laboratory request forms.[10] The primary container should be a leakproof screw-capped plastic with low risk of breakage. The primary receptacle should be wrapped with absorbent material and placed into a bigger secondary container with leakproof protection mechanism like cap with O-ring. The secondary container is transported in a tertiary container which should be well labeled indicating Category B infectious substance.[10] Laboratories should have written and well-circulated document on sample acceptance and rejection criteria.

Detection of SARS-CoV-2 by real-time RT-PCR is recommended to take place as soon as possible after sample collection. A study demonstrated that storage of throat samples from confirmed patients for 1 day at 2°C–8°C resulted in 14.8% decrease change in the result while storage at the same temperature for 2 days resulted in 22.7% decrease change in the result.[20] The cycle threshold (Ct) value increases with storage time and freeze-thaw cycles.[20]

Analytical phase

Primers and probes for amplification of SARS-CoV-2 target regions

Similar to other coronaviruses, SARS-CoV-2 possesses a positive-sense, single-stranded RNA genome of approximately 30,000 bases in length.[21] The SARS-CoV-2 genome comprises of noncoding transcriptional regulatory sequences at both ends of the genome, 16 open reading frames (ORFs) that codes for nonstructural proteins (Nsp1-16) such as enzymes necessary for replication, and sequences responsible for the

four coronaviruses structural proteins-spike (S), envelope (E), membrane (M), and nucleocapsid (N) [Figure 1].[22] While the structural proteins are largely general for coronaviruses, a number of the Nsps are highly specific for SARS-CoV-2 and are exploited for the design of diagnostic methods and for potential chemotherapeutic options.

A good number of real-time RT-PCR assays were developed to target and amplify genomic region of SARS-CoV-2. The gene segments that have been targeted include those of Nsp1 (ORF1), Nsp2, Nsp3, Nsp12 (RdRp), S, and E.[12] However, the preferred targets are conserved and/or abundantly expressed genes such as structural S and N genes and the nonstructural RdRp and replicase ORF 1a/b genes.[23] Protocols for SARS-CoV-2 assays targeting different regions of the virus and from different institutes were made available online by the WHO.[24] Some of the assays are nonspecific that detect both SARS-CoV-2 and other related beta-coronaviruses such as SARS-CoV while others specifically detect SARS-CoV-2.

Assays targeting E gene and RNA-dependent RNA polymerase (RdRp) gene were developed by Corman *et al.* with the best sensitivity while N gene assay had relatively lower sensitivity. The E gene and RdRp gene had limit of detection (LOD) of 5.2 and 3.8 copies per reaction, respectively therefore, they recommended E gene for screening and RdRp for confirmation.[25] However, Chan *et al.*[25] have targeted a different region of the RdRp/Hel and found to be significantly more sensitive and specific than the Corman *et al*.'s[25] RdRp assay.[23] Chu *et al*. targeted ORF1b and N gene where N gene assay was found to be 10 times more sensitive than ORF1b gene assay in detecting clinical samples and consequently, N gene was recommended as a screening assay while ORF1b assay as a confirmatory assay.[26] Scientists from different parts of the world have developed in-house assays targeting different regions of SARS-CoV-2 and made respective recommendations. Two or three of the regions are detected at the same time in most of the developed assays.[12] This will preclude possible cross-reaction with other endemic coronaviruses and potential genetic drift of SARS-CoV-2.[8,18]

A comparative analysis of ten primer-probe sets targeting different regions of SARS-CoV-2 from six different national institutions involved seven N gene and three Orf1 gene sets.[27] The best sets for the sensitive and reliable confirmation of the N and Orf1 genes are presented in Table 1.

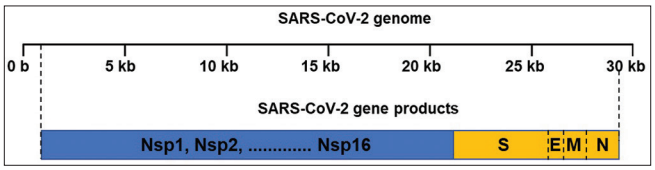

Figure 1: The genome of SARS-CoV-2. The 3'end contains sequences that do not result in protein, followed by those for the expression of 16 nonstructural proteins Nsp1-16 and for the structural proteins S, E, M, and N. The downstream terminal portion also contain noncoding sequences that are involved in genome replication and transcription[22]

Sample inactivation and RNA extraction

Samples transported to the laboratory are received and inactivated before RNA extraction and amplification. Staff receiving and inactivating samples should wear appropriate personal protective equipment (PPE) which include N95 respirator, protective gown, disposable gloves, goggle, etc. Sample inactivation is expected to be handled in a class II or higher biosafety cabinet.[10]

The aim of inactivation is to prevent personnel from infection by high pathogenic viruses and the process should take place without reducing the detection efficiency of the system.[12] Inactivation can be achieved by heat or chemical method. However, a study on SARS-CoV-2-positive throat swab samples inactivated at 56°C for 30 min has resulted to 17.0% decrease change in the positive result when SARS-CoV-2 is detected by the same method.[11] Therefore, heat inactivation should be avoided since the process may degrade the SARS-CoV-2 RNA and consequently produce false-negative results.[18]

Buffer AVL (Qiagen, Hilden, Germany) and Trizol (Life Technologies, Carlsbad, California, USA) have been used for purifying and extracting viral RNA for years.[12] These reagents contain guanidine isothiocyanate which could denature and dissolve protein as such inactivating SARS-CoV-2 and other enveloped viruses. Phenol as a component of Trizol also denatures protein.[12] The buffers included in common commercial extraction platforms contain guanidium and detergents.[18] Total nucleic acid or RNA extraction is performed manually including repeated steps of centrifugations or by using automation systems such as NucliSENS easyMAG (BioMérieux, Marcy-l'Étoile, France) and MagNA Pure 96 (Roche, Penzberg, Germany)[23,25,30] in which centrifugations are not required.

Real-time reverse transcription-polymerase chain reaction procedure

Reverse transcription PCR involves the application of two enzymes in two different steps. In the first step, an RNA-dependent DNA polymerase otherwise known as reverse transcriptase copy RNA into complementary DNA (cDNA), and the second step uses Taq polymerase to amplify the cDNA in a typical PCR.[31] Corman *et al*. have used thermal cycling conditions as 55°C for 10 min for reverse transcription, followed by 95°C for 3 min and then 45 cycles of 95°C for 15s, 58°C for 30s.[25] After cDNA synthesis, the DNA strands are denatured at higher temperature to produce single strands.[25]

During the annealing and polymerization step of the PCR, the amplification primers and detection probes hybridize to the single-stranded DNA templates and allow the polymerase to replicate the template, creating double-stranded DNA. In polymerization, the probe is displaced and hydrolyzed, separating fluorophore (reporter) and quencher and releasing fluorescence. The cycle is repeated for about 40 times.[32] Many researchers have reported assays with comparable thermal cycling conditions with little variations.[23,26,32,33]

Table 1: Information on the best primer-probe sets analyzed in a comparative analysis

Target	Primer-probe set	Primer and probe oligonucleotide name	Reference
RdRp/Orf1 gene	ORF1ab (China)	ORF1ab-F	China CDC[28]
		ORF1ab-R	
		ORF1ab-P	
N gene	2019-nCoV_N2 (USA)	2019-nCoV_N2-F	CDC 2020[27]
		2019-nCoV_N2-R	
		2019-nCoV_N2-P	
	209-nCoV_N3 (USA)	2019-nCoV_N3-F	
		2019-nCoV_N3-R	
		2019-nCoV_N3-P	
	NIID_2019-nCOV_N (Japan)	NIID_2019-nCOV_N_F2	Nao *et al*.[29]
		NIID_2019-nCOV_N_R2	
		NIID_2019-nCOV_N_P2	

ORF – Open reading frames, nCoV – Novel coronavirus, CDC – Centre for Disease Control, RdRp – RNA-dependent RNA polymerase

Graphical profile of a SARS-CoV-2 RT-PCR detection condition run using by a BioRad CFX96 Real-Time System is presented in Figure 2. During the annealing stage of PCR, both primer and probe are attached to the target cDNA. Extension of the primer to form new DNA strand reaches the probe and results to the physical separation of fluorescent reporter from the quencher and thereby causing fluorescence. The fluorescence values are recorded during every cycle and denote the amount of product amplified up to that point in the PCR process.[30]

Some commercial assays such as that produced by Daan Gene Co., Ltd (China) employ multiplex qPCR to detect two (N gene and ORF1ab) or more viral genes and a host housekeeping gene as internal control using different probes in a single reaction tube. Corman *et al*.[25] diagnostic assay detects and discriminates SARS-CoV-2 from other SARS-CoV using E gene and RdRp gene assays.

During the exponential phase of PCR, the amount of PCR product is proportional to the amount of starting template, and therefore, the more template at the beginning of the reaction the fewer cycles it takes to reach point where the fluorescent signal is first detected.[30] Cycle threshold (Ct) is a value during the exponential phase where the concentration of the product is separable from background noise.

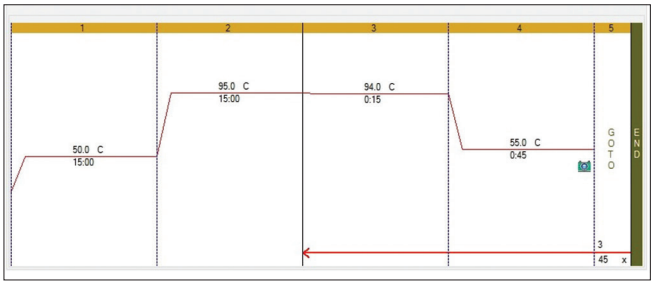

Figure 2: Thermal conditions for an RT-qPCR test run on a BioRad CFX96 qPCR instrument using Daan gene kit. The reverse transcription was performed at 50°C for 15 min and was followed by initial denaturation and RT deactivation step at 95°C for 15 min. PCR cycles continued with denaturation at 94°C for 15 s and annealing/polymerization step at 55°C for 45 s. RT-qPCR – Reverse transcription-quantitative polymerase chain reaction

Quality Controls

Few external controls are recommended to be run alongside the samples to monitor reagent and assay performance. One negative and one positive control are recommended for RNA extraction in addition one water control (no-template control [NTC]) and one positive control for the PCR run.[12] The patient sample can be spiked with a weak positive control (inhibition control) to detect possible PCR inhibitors.[12,30] Negative control shows no amplification or curve may appear after 40 cycles, whereas positive control checks the integrity of reagent with amplification. NTC should have no amplification and it is employed to monitor contamination from environment and/or reagents.

Human specimen control (HSC) consists of noninfectious cultured human cells' material run with the CDC 2019-nCoV rRT-PCR diagnostic panel. It is used as RNA extraction procedural control to check extraction reagent integrity. Successful RNA recovery should yield positive results with the RNase P primer and probe set and negative result with other SARS-CoV-2 primer sets.[31]

Internal control is a control for sample collection, extraction, reverse transcription, amplification, and inhibition to avoid false-negative results. There are many internal controls in use; however, the human RNase *P* gene is recommended because it is present in all types of SARS-CoV-2 infected samples. Other internal controls include plasmid DNA and virus-like particles.[12]

Results Interpretation and Reporting

Laboratory diagnosis of SARS-CoV-2 using RT-qPCR involves multiple controls at different stages of the testing process.

These controls are very essential to peruse before any test result is interpreted and eventually released.

Quality Controls interpretation

No template control

No NTC reaction for all primer and probe sets should display curves that cross the threshold line. If this happens, sample contamination may have occurred and therefore, the run must be invalidated and assay repeated.

Reagent positive control

This is expected to yield amplification with the primer and probe sets of the target regions. Failure of this amplification may indicate compromise in the reagent integrity.

Human specimen control

When the assay is run with HSC, successful RNA recovery is ensured by amplification curve with RNase P primer set.[31]

Internal control

When RNase *P* is used as a marker for internal control, all clinical samples should display fluorescence amplification curves in the RNase *P* reaction that cross the threshold line within 40.00 cycles (<40.00 Ct), hence representing the presence of the human RNase *P* gene.[31] The most valid positive result is when the internal control and the SARS-CoV-2 markers exhibit amplification. If the internal control does not produce a positive result but the two SARS-CoV-2 markers are positive, the result is considered valid. However, if the two SARS-CoV-2 markers and RNase P are negative, the result is invalid for that specimen.[31] The test can be repeated if there is available residual specimen and after the repeat, if the markers remain negative, the result is reported as invalid and new specimen should be collected if possible.[31]

Test result interpretation

All controls should be observed before interpretation of test results. If the controls are not valid, the results cannot be interpreted. The positive result for SARS-CoV-2 is detected when at least two different targets of the virus are amplified where at least one target is specific for the SARS-CoV-2[31] and if only one region is positive, the result needs to be re-tested.[12] Different Ct cutoff values for both positive and negative results are used in different SARS-CoV-2 RT-PCR assays. For example, a multiplex qPCR diagnostic kit amplifies internal control, ORF1ab, and N genes at HEX, FAM, and ROX channels, respectively, and cutoff Ct values for positive are ≤40 for the trio. If only the ORF1ab or N gene is amplified (Ct ≤40), the sample is considered suspected. When neither the ORF1ab nor the N gene is amplified (Ct >40 or no signal), the sample is considered negative.[20] Low Ct values are indicating high viral load and can be used as a signal for possible transmissibility.[12] Figure 3 demonstrates a characteristic positive amplification with Ct <40 for both the SARS-CoV-2 markers and the internal control.

Postanalytical phase

Postanalytical is the final phase of the testing process in which results are evaluated before release. It is critical as errors generated during this phase may render all the efforts made during the preanalytical and analytical phases futile.

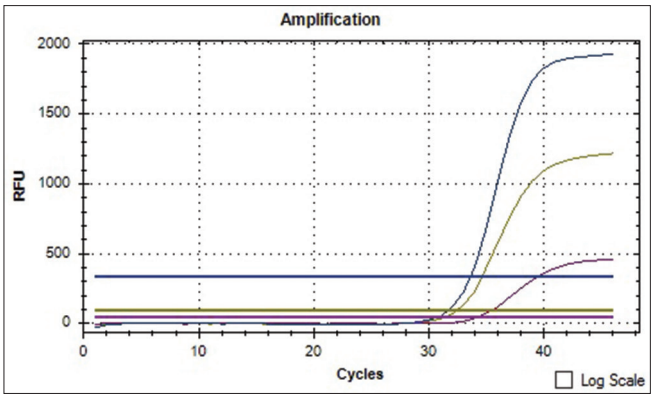

Figure 3: Positive amplification curve of ORF1ab (Ct = 32.37), N gene (Ct = 33.64), and internal control (Ct = 34.22) using VIC, FAM, and Cy5 channels, respectively, run on a BioRad CFX96 qPCR instrument. RFU = Relative fluorescence units. qPCR – Quantitative polymerase chain reaction

Result evaluation, preparation, release, and document archiving

Results are evaluated against the laboratory internal quality controls using standard operating procedure (SOP) by authorized personnel before release. The test result is prepared according to the format adopted by the laboratory. There must be documented procedures for the release of result including who may release the result and to whom.[34] In epidemiological surveillance settings, results are always released to the common national or local public health office through print or electronic means. All copies of results generated are properly archived for easy future retrieval.

The format for reporting results should be sustained and have inherent attribute that will help to avoid transcription errors.[34]

Storage, retention, and disposal of clinical samples

Many laboratories aliquot inactivated samples in duplicates or triplicates for the immediate diagnostic test and possible future research work. The laboratory should have SOPs for identification, collection, retention, indexing, access, storage, maintenance, and safe disposal of samples.[34] Every laboratory should define the length of time that samples are to be retained. Retention time is usually defined by the nature of the sample, the examination, and any applicable requirements.[34]

Biosafety and containment requirements for working with SARS-CoV-2 suspected respiratory samples

Laboratory biosafety is the term used to describe the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to pathogens and toxins or their accidental release.[35,36] The need for implementation of biosafety measures cuts across the three highlighted phases of testing. Every laboratory setting up for SARS-CoV-2 testing should conduct a local risk assessment for each of the steps involved in the sample workflow.

Potential hazards identified should have appropriate risk control measures which must be implemented.[37] Laboratories should have policy on safety such as safety manual and implemented by strict adherence to the safety SOPs. Staff must be well trained on the biosafety of working with the virus and become acquainted with all the biosafety measures. These requirements often pose significant challenges for laboratories in resource-challenged settings.

All guidelines for infection prevention and control for aerosol-producing procedures must be observed during sample collection from the patients. In addition, all requirements for good microbiological practice and procedure should be strictly followed during sample collection and subsequent laboratory analysis.

Health-care personnel collecting the samples should wear complete PPE (N95, overall gown, disposable gloves, safety goggle, face shield, etc.).[9] Biosafety is enhanced when the sample collector is assisted by an associate who is also kitted with the PPE. Proper and sequential doffing of the PPEs after sample collection is important to avoid self-contamination. A triple sample packaging system (the UN3373 P650 packaging instructions) should be adopted for the transportation of samples to the testing laboratory.[9]

In the laboratory, dedicated personnel laboratory clothing, e.g., scrubs which should not be worn outside the laboratory is recommended.[35] Laboratory workers should wear protective equipment, including disposable gloves; solid-front or wrap-around gowns, scrub suits, or coveralls with sleeves that fully cover the forearms; head coverings; shoe covers or dedicated shoes; and eye protection (goggles or face shield).[36] Received samples in the laboratory are expected to be opened only inside a validated biosafety cabinet II (BSC II) or primary containment device and subsequent procedures should be performed therein or in higher biosafety level. Collection transportation and receiving links should be signed for traceability.[13] Samples are inactivated upon receipt before succeeding procedures. Absorbent material should be placed on the benches and BSC to contain spills.[35] The BSC and benches used should be disinfected after procedures.[10,18]

Engineering Controls

A controlled ventilation system maintains inward directional airflow into the laboratory room; exhaust air from the laboratory room is not re-circulated to other areas within the building. Air must be HEPA (high-efficiency particulate air) filtered, if reconditioned and recirculated within the laboratory. When exhaust air from the laboratory is discharged to the outdoors, it must be dispersed away from occupied buildings and air intakes. This air should be discharged through HEPA filters. Dedicated hand-wash sinks should be made available at all sections in the laboratory. All manipulations of infectious or potentially infectious materials must be performed in appropriately maintained and validated BSCs.[37] Partial isolation of contamination source by compartmentalization of the testing units into the sample reception area, inactivation room, extraction room, preparation room, and detection room can help contain the spread of contaminants. The laboratory is designed to allow only unidirectional movement across these units.

Use of Appropriate Disinfectants

Right disinfectants with demonstrated activity against enveloped viruses should be used for decontamination. For example, 0.1% sodium hypochlorite for general surface disinfection and 1% for disinfection of sample spills. Others include 62%–71% ethanol, 0.5% hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds.[36] All generated waste should be properly labeled and efficiently treated within the laboratory and remain in the laboratory till disposed properly. Surfaces in the laboratory should be easy to clean and decontaminated without hitches and equipment should be decontaminated before maintenance, repair, or decommissioning.[35]

Possible causes of false-negative and false-positive results of SARS-CoV-2 RT-PCR testing

Accuracy and precision are crucial toward producing laboratory test results that are reliable. However, cases of false-negative result of SARS-CoV-2 RT-PCR have been reported.[11,38] One of these studies reported 21.4% results that turned positive after two consecutive negative results which may be related to false-negative RT-PCR and prolonged nucleic acid conversion.[38]

False-negative test result will tend to increase as testing is scales up and prevalence of COVID-19 infection rises.[39] False-negative results have their implications as individuals with such result may relax safety measures put in place to reduce the spread of the virus to others. Health-care workers at frontlines with false-negative result may transmit the virus to their families, patients, and colleagues.[39] Confirmed COVID-19 patients in isolation may be discharged while relying on false-negative result and integrate into the society and consequently increase community transmission.[11]

Genetic diversity and rapid evolution caused by natural mutation and active viral recombination of the virus could affect the annealing efficiency of primers and probes to the target regions in the SARS-CoV-2 genome, thereby affecting the test sensitivity and producing false-negative result.[6,8]

Inhibitors of PCR in the sample may cause false-negative results or insufficient viral load[23] in the sample rising from poor sample collection, transportation, or handling.[5,6] However, aRT-PCR assay with higher sensitivity, such as the COVID-19-RdRp/Hel assay, might help to reduce the false-negative rate among these samples.[23] The COVID-19-RdRp/Hel assay detected SARS-CoV-2 RNA in 15.4% additional samples that tested negative by another widely used assay.[23] In addition, thermal inactivation of samples could cause false-negative results in samples with low viral loads.[40] False-negative result may

also arise from the assay as real-time PCR test is not 100% sensitivity.[6]

Poor laboratory practice standard and personnel skill on technical and safety procedures may be attributed to false-negative results.[6] Poor sample handling during the testing process may cause undesirable outcomes that can cause false-negative result. For example, extracted RNA may get degraded and bring about false-negative result. Other causes of false-negative result include transcriptional errors and poor result interpretation.[6]

Clerical and transcriptional errors such as mislabeling during the preanalytical, analytical, and postanalytical phases of testing may give rise to release of false-negative or false-positive results. Reagents and patient samples can be contaminated with positive samples or nucleic acid through the process of testing and release of false-positive results.

Conclusion

With the increasing incidence of COVID-19 around the world, there is proportionate scale-up of testing centers or units to meet the need of the brimming laboratory test requests. Despite that the quantitative RT-PCR is considered as the gold standard for establishing diagnosis of COVID-19. These laboratories must meticulously pay attention to all the testing phases from sample collection to final release of result in order to avoid false-negative or false-positive results. It is also necessary to understand possible awaiting challenges and conduct risk assessment before commencement of testing in the designated laboratory.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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