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Cross-contamination in molecular laboratories: A challenge to COVID-19 testing in Bangladesh

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Abstract

Rapid and accurate laboratory diagnosis of SARS-CoV-2 infection is crucial for the management of COVID-19 patients and control of the spread of the virus. At the start of the COVID-19 pandemic, Bangladesh had only one government molecular laboratory where real-time RT-PCR will be performed to diagnose SARS-CoV-2 infection. With the increasing number of suspected cases requiring confirmation diagnostic testing, there was a requirement to quickly expand capacity for large-scale testing. The government of Bangladesh established over 100 molecular laboratories within one year to test COVID-19. To fulfil the requirement for expanded testing, the government was compelled to recruit laboratory employees with inadequate experience, technical knowledge, and skills in molecular assays, particularly in processing specimens, interpreting results, recognizing errors, and troubleshooting. As a result, the risk of diagnostic errors, such as cross-contamination, is increased, as is that the risk of false-positive results, which might risk the patient's health and undermine the efficacy of public health policies, public health response, surveillance programs, and restrictive measures aimed toward containing the outbreak. This review article aims to explain different sources of cross-contamination in the COVID-19 RT-PCR laboratories and the way to forestall them in efficient and practical ways.

Keywords: SARS-CoV-2; RT-PCR; cross-contaminations; quality control; COVID-19, false positive.

Introduction

The coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 has triggered a global public health emergency. Due to the rapid spread of SARS-CoV-2 infection, there were widespread shortages of Personal Protective Equipment (PPE), diagnostic test kits, and vital patient treatment equipment. The laboratory diagnosis of SARS-CoV-2 infection is an essential aspect of resolving the present pandemic [1]. For detecting SARS-CoV-2 nucleic acids, Reverse transcriptase-polymerase chain reaction (RT-PCR) is currently the most sensitive and specific method. Testing is essential to identify infected people and track down their contacts [2].

In Bangladesh, the first confirmed case of COVID-19 was announced on March 8, 2020 [3]. At the beginning of the pandemic, like many other countries, Bangladesh also had minimal testing facilities to diagnose SARS-CoV-2 infection. Bangladesh boosted its testing capability by setting up more than one hundred COVID-19 dedicated RT-PCR laboratories within a year. As of September 7, 2021, 56 government and 83 private COVID-19 dedicated RT-PCR laboratories are running across the country [4].

The government of Bangladesh put a lot of effort into running these molecular laboratories by the ministry of health and family welfare and by the directorate general of health services. They set up the necessary instruments and test kits, recruit a workforce, including doctors, scientific officers, medical technologists, computer operators, cleaners, etc. The directorate general of health services published several guidelines regarding infection prevention and control, rational use of Personal Protective Equipment (PPE), waste disposal [5]. But the chance of cross-contamination in these laboratories is still a significant concern regarding quality control, especially country like Bangladesh, where most of them are established within a brief period [6]. The purpose of this article is to present the various sources of contamination in COVID-19 RT-PCR laboratories and provide efficient, effective, and feasible solutions to address these issues.

Preanalytical considerations

Nasopharyngeal (NP) and Oropharyngeal (OP) swabs are the recommended specimens for diagnosing SARS-CoV-2 infection by RT-PCR. Sputum, endotracheal aspirate, or bronchoalveolar lavage can be used in the case of ambulatory patients or patients with more severe respiratory disease or lung tissue postmortem [7]. Combined NP/OP swabs may increase the positivity rate but should be done by the availability of swabs [8]. Each sample should be appropriately identified and collect an adequate amount [9]. Many countries are constrained by a lack of PPEs and a scarcity of human resources, while the number of suspected cases requiring confirmation testing increases exponentially [10]. With limited resources and an overburdening workload, adhering to the recommended protocols may be difficult. Still, it should not be overlooked because breaking them can result in immediate cross-contamination, jeopardizing the accuracy and quality of RT-PCR testing as well as a source of laboratory-acquired infections [11].

All PPEs (disposable gown, gloves, cap, shoe cover, protective eyewear, and an N95 respirator mask) must be sterilized and worn in the correct order before specimen collection. When using gloves, make sure they cover a portion of the forearm while remaining under the sleeves to avoid skin exposure. To cover part of the sleeves, the second pair of gloves might be used. PPEs must be worn at all times, including the gown, FFP2 (N95), goggles or face shield, and gloves [12]. Shaving is also recommended for male health workers to ensure that the mask adheres to their faces correctly [13]. The patient must be comfortable with their head resting against a plexiglass partition when the sample is taken. The nasopharyngeal or oropharyngeal swabs are collected and deposited in sterile test tubes. The tubes are then correctly labeled with the patient's personal information [14,15]. Samples should be tightly capped and transported to the corresponding laboratory in biohazard zip-lock bags within a leak-proof icebox. The biohazard label outside the box should be visible [16]. Proper labeling, handling, and storage of obtained samples are necessary to avoid false-positive and false-negative results [12].

To avoid cross-contamination, it's essential to change gloves and clean the workspace between each collection. Suppose it is impossible or practical due to a lack of resources and people on top of a heavy workload. In that case, another option is to disinfect gloved hands with 70% alcohol in a squeeze or spray bottle and then dry with fresh paper towels after each patient. Surfaces of the collecting booth, whether made of plastic or metal or covered with a nonporous cover, should be disinfected as well, especially if patients have come into personal touch with the area. Disinfectants such as sodium hypochlorite or bleach (0.1 percent for general surface disinfection and 1% for sample spill disinfection), 62-71 percent ethanol, 0.5 percent hydrogen peroxide, quaternary ammonium compounds, and phenolic compounds (used according to manufacturer's recommendations) have all been recommended by the WHO. Benzalkonium chloride or 0.02 percent chlorhexidine digluconate can also be utilized, albeit they are less effective. Aside from choosing the proper disinfectant, the contact time, dilution, and shelf life should be considered. Alcohol can also be sprayed, but only after at least 20 seconds of contact with the surface should it be cleaned. A new solution has to prepare each time when using bleach [17]. Regular disinfection is also required for sample collecting boxes or coolers, reusable cold packs, pouches, and racks. However, after disinfection, the technician must clean the surfaces with a sterile water-soaked paper towel followed by a 70 percent alcohol-soaked paper towel to avoid residue build-up and PCR inhibition.

When sampling is completed, PPEs should be removed properly to avoid contact with exterior surfaces. The used suit, shoes, gloves, and mask must all be disposed of in a specific garbage receptacle. Hands are also sanitized with an alcoholic solution or washed with soap and water [13].

Analytical concerns

When detecting unique sequences of the SARS-CoV-2 genome, RT-PCR remains the gold standard [18]. RT-PCR is a labor-intensive and intrinsically complex assay requiring extensive testing knowledge in all aspects, limiting the potential for rapid turnaround time from sample collection to data availability. This bottleneck could result in extended wait times and an exponential increase in testing demand [9]. Laboratory employees are being pushed to operate under extreme pressure in high-throughput environments with an overwhelming workload and inadequate access to personal protective equipment as the number of suspected cases requiring confirmatory diagnostic testing grows [19]. To meet the excess demand, the laboratories have to recruit additional laboratory personnel with limited experience, technical knowledge, and skills in molecular assays, particularly in processing specimens, interpreting results, identifying errors, and troubleshooting [20]. As a result, laboratory medical services become more vulnerable to diagnostic errors, such as cross-contamination. They have a higher risk of producing false-positive results, which can jeopardize the patient's health and undermine the efficacy of public health policies, public health response, surveillance programs, and restrictive measures for containing the outbreak [21]. 15. In the worst-case scenario, a false-positive result may result in wasteful treatment. It may jeopardize the available workforce, mainly if the patient is a public servant obliged to self-isolate. Meanwhile, due to failures in applying restrictive and containment measures and identifying other suspected cases, particularly those exposed to the patient infected with SARS-CoV-2.6, a false-negative result can foster the rapid human-to-human transmission of the virus [22].

The World Health Organization released guidelines on biosafety in laboratories handling COVID-19 samples [23]. The directorate general of health services of Bangladesh also released guidelines on how to operate local COVID-19 testing laboratories [4]. These initiatives establish a standard for ensuring the accuracy of tests and ensuring the safety of laboratory personnel. RT-PCR diagnostic kits have a high rate of false-negative test results. Unnecessary mistakes have to be prevented during the collection and processing of samples [24]. All samples should be processed within a class 2 biological safety cabinet (BSC) in full PPE attire as described above [12].

Due to the complexity of the RT-PCR test procedure, it is vulnerable to cross-contamination. For several testing kits, RNA from the COVID-19 suspected sample needs to be extracted. SARS-CoV-2 RNA can easily be transferred from an infected gloved hand to a working surface or a laboratory environment [25]. Although WHO guidelines recommend excellent microbiological techniques and procedures, it is unclear how frequently laboratory employees should replace gloves. The rules also place a greater emphasis on safeguarding laboratory staff. To avoid cross-contamination, gloves should be changed as often as possible, especially if they have been soiled with solutions containing template RNA. When moving to a new part of the laboratory, not only should gloves be changed, but the complete set of PPE should be changed as well. Materials including pens, tiny equipment, tubes, pipette tips, and other consumables should never be brought from the RT-PCR area to the pre-PCR area. Laboratorians and even cleaning staff should be reminded that laboratory standards necessitate unidirectional workflow. Thus they should regard each space as a separate room to avoid transporting amplicons to amplification product-free regions.

Furthermore, according to the DGHS guideline of Bangladesh, the pre-PCR room must be divided into specimen handling or sample preparation room and reagent preparation room. Positive internal reaction controls are prohibited in the reagent preparation area, which should remain a "template-free" environment. The samples and reagents should be stored in different freezers.

Pipetting patient samples into the PCR plate or strip is another probable cause of cross-contamination. Due to sample misplacement, negative samples can be mistaken as positive. When doing RT-PCR analysis, correct pipetting and double-checking sample placement should always be followed while following aseptic practices (use of PPE, use of sterile materials, sanitizing work environment). Before and after PCR operations, cleaning the work environment, pipettors, freezer handles, and other equipment with the necessary decontaminating solution is also required. Racks should be disinfected for ten minutes before being dried with a clean paper towel. Autoclavable pipettors should be used to prevent cross-contamination. Disinfectants should be used as recommended by the WHO, either every 30 minutes or following COVID-19 sample processing [17]. Disposables are indicated for consumables that have come into touch with infectious material.

Following RT-PCR analysis, post-PCR is a critical step in diagnosing the data. To ensure that the process is free of contamination, no amplification must be detected in the negative controls provided by the test kit, as well as in the elution buffer (or whatever is appropriate depending on the test kit used). In the event of possible contamination, the quality of the water should be verified, and contamination of the instrument should be considered in some circumstances [26]. To avoid these issues, each run should utilize new (unopened) water, and once the kit is opened, the reagents should be prepared in aliquots in sterile containers. Until the samples are deposited in the machine, the proper aseptic method must be followed. It is recommended that the controls not be placed adjacent to each other when inserting samples and controls in the multi-well plate to avoid cross-contamination. In contrast, samples are transported to their allotted wells. To monitor aseptic pipetting, assigning about 3 or more water controls at random in the multi-well plate is also a good idea. Laboratory cross-contamination isn't always the cause of false data. Contamination in the test kits caused a delay in testing in Europe during the early stages of the COVID-19 pandemic [27].

Technicians working in a COVID-19 testing facility may become infected with the virus and unintentionally contaminate the samples they analyze and the laboratory environment. As a result, technicians, particularly those assigned to the PCR room, should wear goggles or a face shield and a disposable surgical hat and mask, which must be disposed of in designated receptacles in the same room before departing. Disposable lab gowns are widely recommended, however in low-resource settings; they may not be practicable. As a result, technicians should not take laboratory gowns home; instead, they should be washed and disinfected by their hospital linen and laundry services.

Conclusion

Laboratory diagnosis of SARS-CoV-2 infection by RT-PCR method plays one of the most crucial parts of the management of COVID-19 patients and control the spread of the virus. The testing facilities for COVID-19 disease are still not sufficient in Bangladesh. The exiting laboratories may face excessive workload if the SARS-CoV-2 infection rate increases. The guidelines published by the World Health Organization and the directorate general of health services should be strictly followed. Particular attention should be given to avoid any cross-contamination during sample collection from suspected COVID-19 cases, changing gloves as often as possible, and changing PPE when moving from one working place to another within the laboratory. Practicing unidirectional workflow and following aseptic technique in every step is vital in maintaining the quality of testing of a molecular diagnostic laboratory.

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