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REVIEW ARTICLE

Setting up a PCR Laboratory for the Detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)

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SUMMARY

Background: **Urgent clinical and public health have the challenge of massive testing for the detection of SARS-CoV-2 to provide the information about individual infection status and patient management. All these efforts are significant for government officials to evaluate the spread of a new disease and trace the contacts of infected persons.**

Methods: **The emergence of SARS-CoV-2 has heightened the need for healthcare systems to set up new clinical laboratories for the rapid and effective diagnosis of the coronavirus disease 2019 (COVID-19) to prevent further transmission.**

Results: **With regard to the antibody testing, the molecular assay for COVID-19 in proper respiratory specimens becomes an especially important tool in the setting of an acute illness [1,2]. Because of the strong demand for improving molecular testing capability in urgent clinical and public health within a short time, the molecular laboratories (including the mobile cabin PCR laboratories) have sprung up across the world.**

Conclusions: **Though a long way from curbing the pandemic, the appearance of experienced PCR laboratories armed with most sensitive and specific molecular assays will reduce SARS-CoV-2 spread in human population. We believe the article will provide some reference opinions on the forthcoming new PCR laboratories.**

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KEY WORDS

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INTRODUCTION

The aim of building PCR laboratories is to prevent the breakdown of the health system countering SARS-CoV-2 onslaught. Real-time PCR was successfully used for the detection of SARS-CoV-2 in the proficient diagnostic laboratory without having virus material available in the early days of the outbreak [3]. Extensive routine molecular testing needs to turn into reality in every country to stop the progression of the pandemic. Inevitably, the new molecular laboratories have new questions and challenges regarding to the detection of this novel virus. Accurate SARS-Cov-2 diagnostics rely on quality management, involving person, place, process, materials, quality service, and so on. Here, the authors describe the general demand for the molecular laborato-

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ry according to the molecular assays for detecting SARS-CoV-2 in the never-before-experienced and rapidly changing situation.

Setting up a new PCR laboratory

We must consider more important points: (i) financial support, (ii) primary need, (iii) personnel reserves, (iv) the reasonable physical separation with different pressure, (v) comfortable working environment, (vi) adjacent to sampling room or fever clinic, (vii) convenient supply (water, electricity, and heating), (viii) safety system (fire and electrical emergencies), (ix) the management of waste, and so on.

Physical separation of the PCR laboratory

Using the visual signage displayed throughout the laboratory to emphasize the different physical areas. Creating one-directional paths and workflows is also essential. As shown in Figure 1, (i) Pre-PCR Area: The area is specific for reagent preparation only. No handing of specimens or positive controls can take place within the area. Generally, the reagents being brought to room temperature before reagent preparation would reduce occasional non-specific signal. (ii) Sample Preparation Area: There is a risk of creating aerosols during the sample preparation, especially performing the confirmed specimens and weakly positive controls. (iii) Post-PCR Area: Because the PCR product can be a most important source of contamination, the reaction plates should remain sealed throughout before being discarded. (iv) Autoclave Area: Sterilizing infectious materials (category B infectious substances) are autoclaved at 121°C for 20 minutes based on guidelines for biosafety level 2 laboratories. Learning from successful experience, the laboratory utilizing autoclaves should also validate their management protocols and rapid/standard biological indicators to ensure that the infectious materials are properly decontaminated [4]. In addition, the transfer chambers in each area are convenient for regent/consumable items delivery between the different area in a certain order. The special reserved transfer chamber for specimens delivery in the sample preparation area will provide great convenience for the whole testing process.

Essential equipment

In practice, the choice of instrument depends largely on the strength of financial support and primary need. (i) Extraction and Amplification platforms: If conditions permit, it is better to choose the familiar and advanced platforms. A fully automated system would be suited for providing a large number of urgent clinical specimens in the hospital setting because of random-access workflow concept and rapid time-to-result [5]. Then the simpler method would give reliable results because of the less sample manipulation, so a sensitive and reliable RNA-extraction-free direct method for RT-PCR is perhaps in high demand for the SARS-CoV-2 detection in frequent testing of personnel, reducing the cost and time [6]. (ii) Air handling: the air pressures are individually adjusted from the Pre-PCR Area, Sample Preparation Area and Post-PCR Area. In the Pre-PCR Area, a slight positive pressure should be established. In the Sample Preparation Area and Post-PCR Area, different gradient negative pressure should be confirmed. (iii) Class II biosafety cabinet: The process of handling specimens must be done in the cabinet. UV exposure is also a must before and after operating the cabinet. (iv) UV irradiation: The lock-out mechanism could illuminate the UV light when the last workers close and lock the exit door [7]. The elimination of UV-generated ozone and the enforced assessment schedule on the UV bulbs performance must be performed in the laboratory. Meanwhile, mobile ultraviolet lamps are used to irradiate the area outside the range of the UV lamps on the ceiling, including the contaminated ground and equipment. (v) Refrigeration utilities: Reasonable quantity and adequate space are necessary. (vi) Other equipment: High efficiency air particle filter, waste treatment unit, various types of pipettes with unique identification, and so on. Monitoring and calibration must be performed periodically for all the equipment shown in Figure 1.

Personnel training and risk assessment

The biosafety classification of COVID was fully defined. As shown in Figure 2, personal training and risk assessment complement each other. The laboratory staff must review related laboratory biosafety practices, update these practices with new guidelines as they become available. For example, interim guidelines related with the novel coronavirus (2019-nCoV) were provided by the CDC and the World Health Organization. Before the collecting, handing and testing of specimens from patients under investigation for COVID-19, the staff need to enhance the study of biological risk assessment (risk characterization, risk mitigation strategies and workforce) and the basic core process (training, equipment, inventory control and communication) [8]. Overall, manipulation of specimens perhaps containing SARS-CoV-2 should be processed in a BSL-2 laboratory while using BSL-3 practices (certified BSC, essential physical containment devices, face protection, gloves, shoe cover, protective clothing, and N95 respirator). The staff should follow good microbiological practices and procedures accompanied with regular review and refresher training. The laboratory staff should establish a systemic biosafety risk assessment of their activities, according to laboratory biosafety interim guidance related to the novel coronavirus (2019-nCoV) issued by World Health Organization. (i) Gather hazard identification: biological agents and other potential hazards, lab procedures; the types of equipment, the condition of facility, relevant human factors, and so on. (ii) Evaluate the risks: the likelihood of an exposure occurring (likely, possible, and unlikely) and the severity of the consequences (severe, moderate, and negligible). (iii) Develop a risk control strategy: the resources available for risk control (applicability, availability, sustainability, and manage-

ment support). (iv) Select and implement risk control measures: evaluate the remaining risk after risk control measures (when and where) are in place, and have a mechanism of laboratory communication. (v) Review risks and risk control measure: establish a periodic review about the changes (personnel, lab activities, biological agents, equipment/facilities, new knowledge/lessons, feedback, incidents, and so on).

***In vitro* diagnostic (IVD) assays**

More IVD assays for COVID-19 caused by SARS-Cov-2 have been developed by researchers and companies worldwide [9,10]. (i) Different gene targets are *E* and *RdRp* for WHO, *N1* and *N2* for U.S. CDC, *ORF1ab* and *N* for China CDC, *N* for Thailand, and so on. (ii) More molecular targets should be included in the assays for SARS-CoV-2 detection, reducing the cross-reaction possibility, the ideal design should include at least one specific region and one conserved region [11]. (iii) The performance of IVD assays must be evaluated before being employed for the detection of SARS-CoV-2 in the laboratory, including sensitivity, specificity, repeatability, limit of detection, and so on. (iv) Any surprising results need confirmation from the local Centers for Disease Control.

Specimen types

The specimens should be obtained in a correct and safe manner because the quality of the specimens affect the testing accuracy. (i) Proper upper specimens (nasopharyngeal and oropharyngeal swabs) stored in virus transport media are recommended for the timely diagnosis of SARS-CoV-2, other lower respiratory tract specimens could be used for the detection and monitoring the severe patients [12]. (ii) Aside from individual testing, sample pooling strategies can also be applied depending on various practical needs [13]. (iii) Specimens from different sites may improve the testing sensitivity and reduce false negative reports, including pharyngeal swabs, sputum, nasal swabs, bronchoalveolar lavage fluid, fibrobronchoscope brush biopsy, feces, and blood [14].

Packaging, storage, and shipment

(i) Specimens are placed in a multilayer container to minimize the potential for spill or breakage. (ii) Specimens stored in pH-controlled media would be more stable than those stored in the non-pH-controlled buffer [15]. (iii) According to laboratory medicine guidelines introduced by the public health administration of US/ European/Korea/China, the appropriate shipping and storage temperature for near and far distances is 2°C - 8°C and -70°C, respectively. (iv) Collected specimens should be transferred to lab for the SARS-CoV-2 detection as soon as possible, reducing the false negative results, especially in the specimens with low viral loads.

Sample preparation

(i) Sample reception: the staff must handle the specimens under a class II biological safety Cabinet. The specimens should be stored at 2 - 8°C if short time delay for detection. (ii) Inactivation of viral infectivity: Due to the increase in the limit of detection and decrease in the sensitivity, heat treatment of specimens before extraction is not recommended and chemical inactivation is an alternative method [16,17]. Compared to thermal inactivation, guanidinium thiocyanate solution in the transport media would attenuate the increased threshold cycle value [18]. But the RNA quality and quantity will be improved by the thermal inactivation (60°C for 30 minutes) of the COVID-19 specimens [19]. (iii) Sample testing strategies: It is important for the lab workers to recognize the pros and cons between individual testing and sample pooling method. Though expanding capacity and preserving reagents/resources, one specimen pooling strategy generally would generate the higher likelihood of false-negative results.

Quality assurance

The establishment of internal quality control and external quality assessment are important to assure the detection accuracy [20]. (i) Internal quality control (IQC): It is important for the staff to evaluate the specimen qualities, DNA/RNA extraction and amplification process. The monitoring and analysis of the results (one PCR blank, more than two negative and one appropriate weak positive control) will guarantee the strict and effective IQC. (ii) External quality assessment (EQA): The quality director should pay close attention to and actively participate in the external quality assessment schemes for COVID-19 from various available organizations. The results from EQA would provide invaluable information for the clinical laboratories [5,21,22].

Contamination Avoidance

In the laboratory, PCR contamination opportunities may be increased due to repeated analysis of numerous specimens. It should be emphasized that avoidance is better than cure in the PCR laboratory [23]. (i) PCR products, cloned DNA from previous experiments, sample contamination, and extensive processing before amplification are the main sources of PCR contamination. PCR product should be directly handled according to routine medical waste disposal process, not be opened or autoclaved. (ii) Good laboratory practices with the meticulous attention and UV irradiation remain the most important anti-contamination measure. (iii) Monitoring the occurrence of contamination is also important for the laboratory workers. Multiple random negative/brank controls should be undertaken in PCR process. (iv) If contamination has been detected, remedial measures include discarding all suspected reagents, cleansing/replacing the affected equipment, changing a new reagent with different targets, and so on.

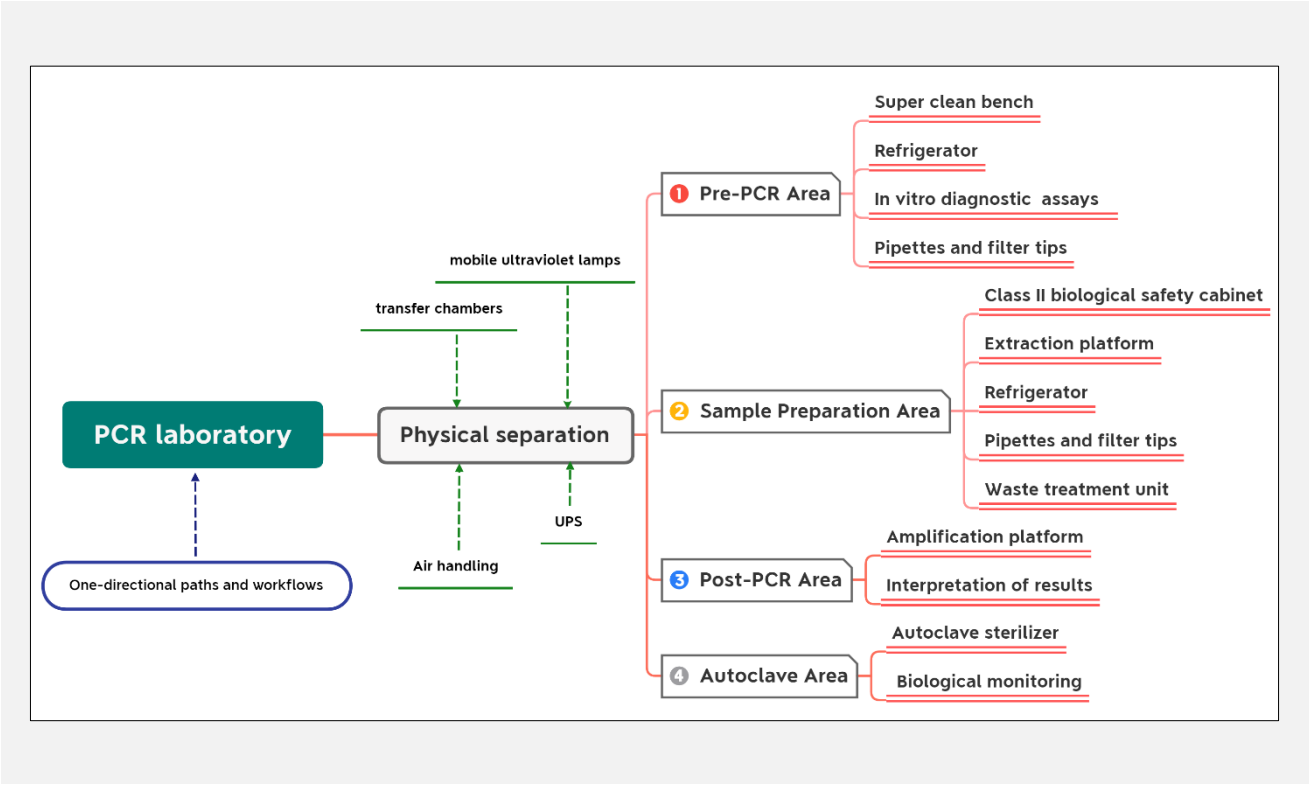

Figure 1. The routine physical separation and essential equipment of the PCR laboratory.

Result interpretation

The RT-PCR results perhaps fluctuate in the patients during treatment, so it is important for the laboratory staff to always interpret results with caution. (i) Negative results: The vast majority of reports should be negative in the current situation except for some areas. (ii) False negative results [24]: The missed diagnosis of patients, discharged infected patients and other problems in transfusion perhaps caused by some factors (personnel operation, timing of sampling, specimen source and quality, sample transport and storage, test kits or apparatus quality, PCR inhibition, virus mutation, lager sample pooling, laboratory quality management, and so on). (iii) Positive results: The result is still positive after the specimen being retested by more than one assay with different genetic locus, generally considered to be laboratory confirmed. Positive for only one target is defined as indeterminant and need to retesting again (two different kits simultaneously). The positive results obtained should be interpreted with caution for the contamination possibility. (iv) False positive results: Most of false positive results are caused by laboratory contamination or improper operation. If false positive results are provided, unnecessary treatment and mental trauma for the patients perhaps emerge. (iv) Suspicious Ct value: An expert interpretation group should be set up to deal with inconclusive results. No guidance on how to deal with the case, so it is recommended that the sample be repurified, or the RNA be reamplified, or to reflexed to the other more sensitive molecular platform [25].

Pay attention to some other questions

(i) An appropriate budget should be available for the facility design. (ii) The lab staff should ensure good communication with the health care professionals about the swabs taken from the oropharynx and/or nasopharynx (the latter more sensitive than the former), and swab materials. (iii) Adequate transport and refrigeration utilities should be equipped in the sampling chamber, avoiding exponentially increasing cases. (iv) Social distancing: minimize interactions by limiting vendors and other external partners and visitors. (v) The adequate and appropriate standard operating procedures in all respects of the laboratory should be prepared for the staff to study thoroughly. (vi) Periodically providing rapid access to SARS-Cov-2 testing for healthcare workers. (vii) Monitoring working circumstances regularly.

Concluding remarks

COVID-19 is still a major threat in the world. Nucleic acid amplification tests performed in laboratories on respiratory specimens are urgently needed and is an important tool for the identification of infected patients. Setting up new laboratories could mitigate the strain on healthcare systems. Biological safety and obtaining quality results are simultaneously important for the lab-

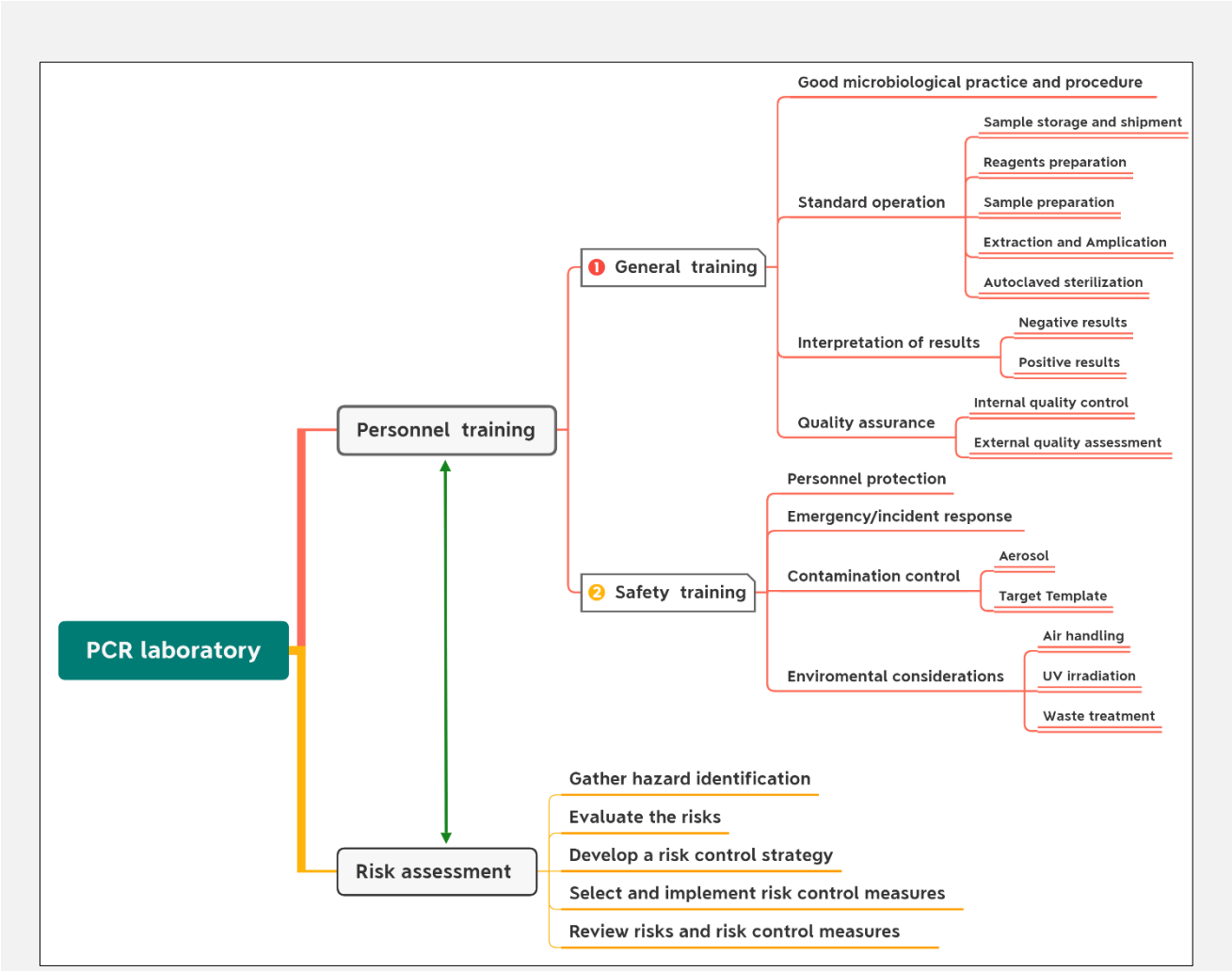

Figure 2. The general training and risk assessment are essential for the PCR laboratory staff.

oratory staff. There are more things to do for setting up a PCR laboratory [26]. Along with automated diagnostic platforms that spring up, unexpected sources of diagnostic errors will emerge. Accurate SARS-Cov-2 diagnostics require advanced assay technology, standardized guidelines, quality assurance, meticulous management, and experienced pathologists/technologists [24]. Despite the many existing problems, we believe that the new molecular laboratory staffs would benefit from the content of the article.

Declaration of Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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