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Commentary

How Iran responded to expanding need for laboratory services for COVID-19?



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ABSTRACT

After the emergence of SARS-CoV-2 in early 2020 in Iran, the rapid response team of Pasteur Institute of Iran was the first lab starting detection and report of suspected human samples. This article is a short summary of all actions from the preparedness for detecting the first cases of COVID-19, expanding the nationwide laboratory service, choosing the suitable laboratory tests and other challenges in laboratory detection during SARS-CoV-2 pandemic in Iran.

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Introduction

Iran has an expanded health care network ranging from rural health houses to large referral hospitals. Medical education has also been integrated with the national health system. Today, 67 (http://dme.behdasht.gov.ir/uploads/Iranmed_Uuniversity.htm) "Medical universities" under the supervision of the Ministry

of Health and Medical Education (MoHME) are responsible for the wellbeing of the people in their areas. This decentralized system has provided degrees of freedom in the way the medical services are provided to the people, while the "health" interventions are usually designed and implemented under central supervision of the MoHME. However, this system sometimes faces challenges to properly address the more complicated problems such as extensive outbreaks of infectious diseases like what happened during the COVID-19 pandemic.

Pasteur Institute of Iran (PII), was established in 1920 and now celebrating its 100th anniversary, has been responsible for health research, production of vaccines and biologicals, training, and sometimes health interventions in its life. It has been responsible for, or involved effectively in, the control of outbreaks of various infectious diseases including Rabies; smallpox, tuberculosis, viral hemorrhagic fevers, cholera and influenza [1–5]. After taking responsibility for their local area by the medical universities in the 1980s, PII was less directly involved in health interventions in those areas. By establishing the Rapid Response Team (RRT) for infectious diseases in 2006, PII participated more in national responses to emerging and re-emerging diseases. This was followed by reviving the Akanlou outpost (Hamedan province) in the west of Iran, which was later assigned as a national research center for emerging and reemerging diseases. In the third millennium, this Institute has been involved in establishing laboratory diagnosis for

Abbreviations: EVAg, European Virus Archive – GLOBAL; TIB, TIB MOLBIOL Company; HKU, Hong Kong University; TUMS, Tehran University of Medical Sciences; SBUMS, Shahid Beheshti University of Medical Sciences; AUMS, Arak University of Medical Sciences; CLN, COVID-19 laboratory network; PII, Pasteur Institute of Iran; RdRp, RNA dependent RNA polymerase; QUMS, Qom University of Medical Sciences.

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emerging deadly pathogens including Crimean-Congo hemorrhagic fever, Ebola, severe acute respiratory syndrome coronavirus (SARS-CoV-1) and Middle East respiratory syndrome coronavirus (MERS-CoV).

Shortly after the emergence of SARS-CoV-2, the causative agent of COVID-19, in China in December 2019 [6], PII started to establish its tests and services for it, which served as the cornerstone of a laboratory network for COVID-19 throughout the country.

This is a report of what was done, the challenges faced, and the strategies to overcome the hurdles of laboratory diagnosis of COVID-19 in Iran.

The beginning: preparedness for detecting the first cases of COVID-19 in Iran

On January 18, 2020, the RRT of PII discussed what measures should be taken to prepare tests for the newly reported coronavirus disease. During the MERS-CoV outbreak in 2015 in addition to MERS-CoV specific primers and probes, RRT prepared a primer set to detect all coronaviruses (PanCorona primers) [7]. Meanwhile, primers and probes for E, RdRp and N genes from guidelines published by Drosten C. et al. and posted on the WHO website on 13 and 17 January 2020 [8], and the sequences published by Hong Kong University [9] were parallelly ordered to two different providers. On January 25, 2020, the first tests were done with the PanCorona primer sets, and the MoHME was informed about the availability of this service. Meanwhile, contacts were made with European Virus Archive - GLOBAL, and the international network of Pasteur Institutes to request for the positive control samples. Since the resources were minimal and the extent of the worldwide and national outbreak was not known at that time, the policy advised by the Iranian CDC for testing the suspected cases was as follows: 1) Symptomatic patients with a history of close contact with a known patient, or 2) Travel history to affected countries, or 3) A person with pneumonia who despite appropriate treatment, had an inappropriate clinical response, and the patient's clinical condition becomes more severe unusually and unexpectedly.

The PanCorona primer set occasionally resulted in non-specific bands in our clinical samples. Therefore, another set of primers was ordered for a nested PCR [10]. This new set, in its original format, was able to identify a vast range of coronaviruses, including the avian coronaviruses. We changed degenerate positions in favor of SARS-CoV-2. Since no SARS-CoV-2 positive control was available at that time, based on bioinformatics analyses, one forward and two reverse primers were selected, and the annealing temperature of the reaction was decreased to 50 °C. Using OC43 and 229E coronaviruses as positive control samples, PCR products with an approximate length of 600bp and 440 bp could be retrieved in the first and second rounds of a semi-nested PCR, respectively. The accuracy of the semi-nested PCR was verified by sequencing the PCR products of the OC43 positive control samples. If we caught a screening positive result with this relaxed semi-nested PCR, we would expect to confirm it by performing a nested PCR (using both forward primers) with an annealing temperature of 55 °C, followed by sequencing of the PCR product. On February 8 and 9, we received our first real-time PCR reagents purchased from PrimerDesign Ltd., UK (COVID-19 genesig® Real-Time PCR assay, detecting RdRp gene) or gifted by WHO (one set from HongKong WHO CC, detecting N and ORF1b-nsp14 genes; one set synthesized by TIB MOLBIOL, Germany, detecting E gene and internal control named as ModularDx Kit SarbecoV E-gene EVA, CAT number40-0776-96X), respectively. The tests were switched to real-time PCR afterward. All samples tested negative with previous methods were then retested with Real-Time PCR, and all were confirmed as negative.

In the meantime, one set of E and RdRp primers and probe ordered at the beginning of our activity was received. Two other labs (Masih Daneshvari Hospital, Shahid Beheshti University of Medical Sciences, and Arak University of Medical Sciences) received their synthesized primers and probes, and a meeting among the three role players was held on February 17, 2020, to compare the E gene detection reagents and test if they can amplify the positive control of TIB MOLBIOL kit, and also try to prepare a national SOP for real-time PCR testing for COVID-19. The SOP was later used as a basis for the expansion of the lab service. RRT of PII had the intention to release its capacity as soon as possible and hand this service to other labs, including the influenza department of PII and similar labs in other healthcare facilities.

On February 18, 2020, the first two cases from Qom (120 km south to Tehran) showed a screening positive result in the National Influenza Center (NIC) at Tehran University of Medical Sciences, the lab which was responsible for testing influenza samples from Qom. Next morning, we shared the HKU RdRp primer-probe set and some RNA samples, and by noon both labs in NIC and PII got positive results by E and ORF1b-nsp14 testing of those samples, which was publicly announced by the MoHME in two hours. Those samples were then amplified by the nested PCR mentioned earlier, followed by overnight Sanger sequencing. The next morning, we had no doubt about being hit by SARS-CoV-2. *"And the evening and the morning were the sixth day"*.

Exodus: the story of expanding the nationwide laboratory service

By the end of February 19, the main question was, what should we do? The extent of the outbreak in Qom city was not clear, and we had no report from other provinces, the testing capacity was around 50 samples per day in our lab, and not much more in 3 other labs in the country, and also the testing reagents (commercial kits and in-house assays) were barely sufficient for 3000 tests. The RRT has a mobile lab, which we were wondering if it is a wise choice to deploy it near Qom city or keep our all capacity in the capital and have a centralized approach to the outbreak. We made a harder decision and decided to enable a molecular lab in Qom University of Medical Sciences and Health Services (QUMS) to test COVID-19 patients on the site. Overnight the formal arrangements were made, and primer-probes and PCR master mix for about 200 tests were sent to QUMS. Another hospital in Tehran (Baghiatallah hospital), which had some suspected cases, also received the materials needed to run the test the same night.

On February 20, PII was assigned by the MoHME to establish and supervise COVID-19 tests and establish a national lab network. A national lab committee was also appointed on the same day to coordinate the activities. Directors and representatives from PII, Health Reference Laboratories, Food and Drug Control Reference Laboratories, Heyat Omanaye Arzi (the office responsible for international procurements of the MoHME), and the Medical Equipment and Supplies office of MoHME were assigned as the permanent members of this committee. The primary estimates of the required capacity for testing, determined by the committee, were about 40000 tests (mostly primer-probes and PCR master mix) for two months, delivering them among 13 medical universities which had active regional molecular diagnostic labs for influenza or HIV. Very soon, this estimate rose up to 100000 tests and 37 labs. Since different labs were routinely using different extraction kits and PCR machines, our initial decision was to leave supplying these requirements to the Medical Universities and just provide primer-probes and PCR master mix through the network. In one month, the number of active labs upscaled to 77 labs and testing capacity grew to 6000 tests per day. During this expansion phase, a new RRT lab was established in PII, which carried out the tests from differ-

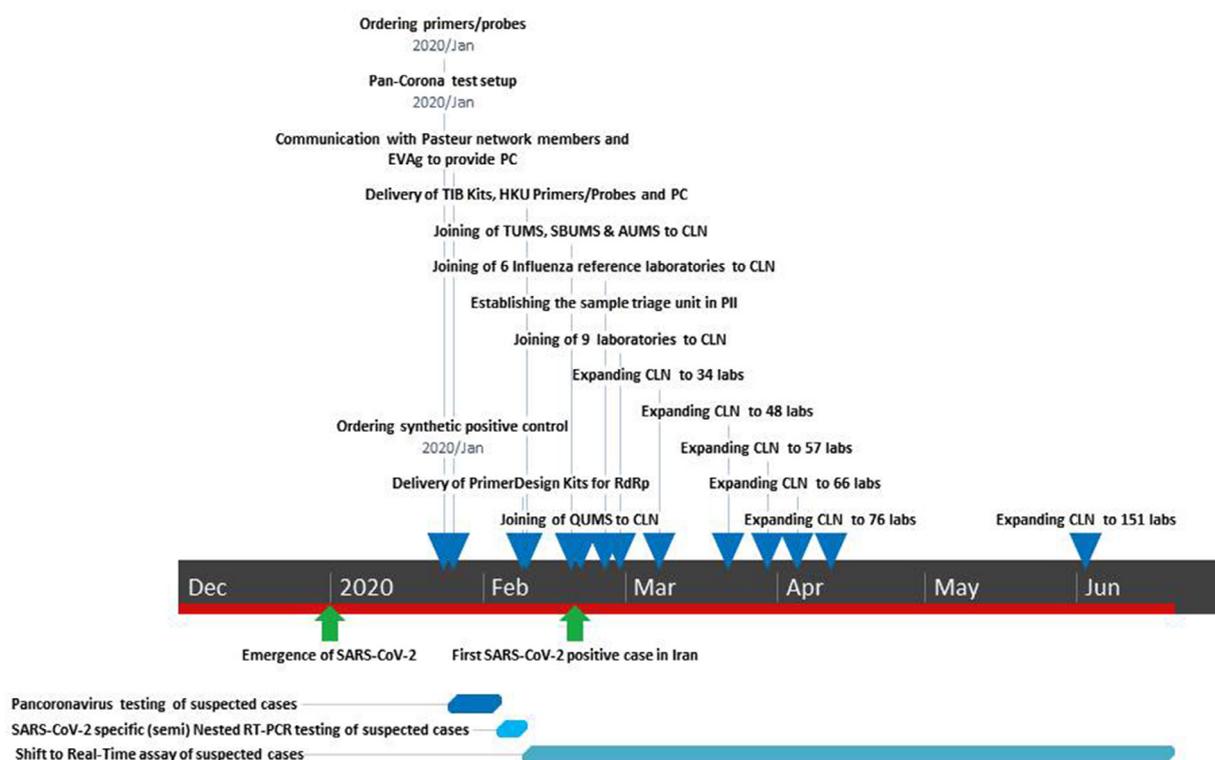


Fig. 1. Timeline representing the progress of COVID-19 laboratory network in Iran.

ent universities which had not established their labs yet. This new lab was able to process 1800 samples per day by March 12. Fig. 1 shows the expansion trend of the labs over time.

The processes of accreditation of new labs by PII and the MoHME were as follows:

- 1-A half a day training of one or two staffs of the candidate lab in PII. The main focus was on workflow and biosafety issues.
- 2-Providing molecular kits for less than 100 tests to set up the lab.
- 3-Receiving the PCR run files of the new labs by PII via email, its evaluation, and phone conference with the lab to check how they interpret the results.
- 4-Issuing the license for the lab to perform COVID-19 molecular test by PII and the Health Reference Laboratories office of the MoHME.
- 5-Providing technical consultation for use or purchasing instruments, master mix, extraction kits, etc.
- 6-Providing molecular kits for the public labs of the network. Private labs had to either make an exclusive service contract with a medical university to receive free kits or purchase their kits from an approved provider in the market.
- 7-Establishing a group in a social network application to enable all labs to share their questions, complaints, and experiences online. The new guidelines were also shared in this group to be discussed before their official release.
- 8-Dispatching external quality control panels to evaluate the proficiency of them.
- 9-Occasional visit of the labs to evaluate their conformity with the standards.

Adaptation of the network to cope with the new requirements in testing

On February 20, 2020, the main purpose of the testing was to identify the extent of the outbreak in the country while ex-

tremely limited testing capacity was available. During the first few days, a centralized approach was inevitable. During the first four days more than 670 samples were received by PII from all affected provinces. The reception of so many samples was our first challenge in PII, which was resolved in 5 days by converting an adjacent building to the triage and reception office. The low RNA extraction capacity, which was manual at the time, was the next major challenge. Installing a Qiacube HT automated extraction machine (Qiagen, Germany) enabled us to process up to 600 samples per day in PII by the end of the first week. During this period, the usual turnaround time of our tests was about 72 hours. In the coming couple of weeks installing two more machines increased the capacity to 2000 tests per day, and a turnaround time of 24-36 hours.

In the first week following the establishment of the lab network, the urgent need of the hospitals was to identify the infected patients to isolate and treat them. Based on data obtained in two weeks after the onset of the outbreak, the medical universities were asked to prepare a testing capacity of 100 tests/day/million people in their areas, resulting in almost 8500 tests/day capacity in the universities, and about 2000 on reserve in PII.

By the end of March, and after a one-week pilot study in Zanjan province, the testing coverage was expanded to suspect cases and their close contact in daycare COVID-19 clinics. Therefore, the testing capacity was planned to increase to 250 tests/day/million in target universities, equal to 20000 tests/day nationally. By the end of April and termination of the partial national lockdown, the main challenge was how to check the health of people before returning to their work. This new challenge may need the expansion of testing capacity to 45000 tests/day or more. We were then involved in evaluating different strategies such as pooling samples, and applying various detection methods and instruments to decrease the number of required tests.

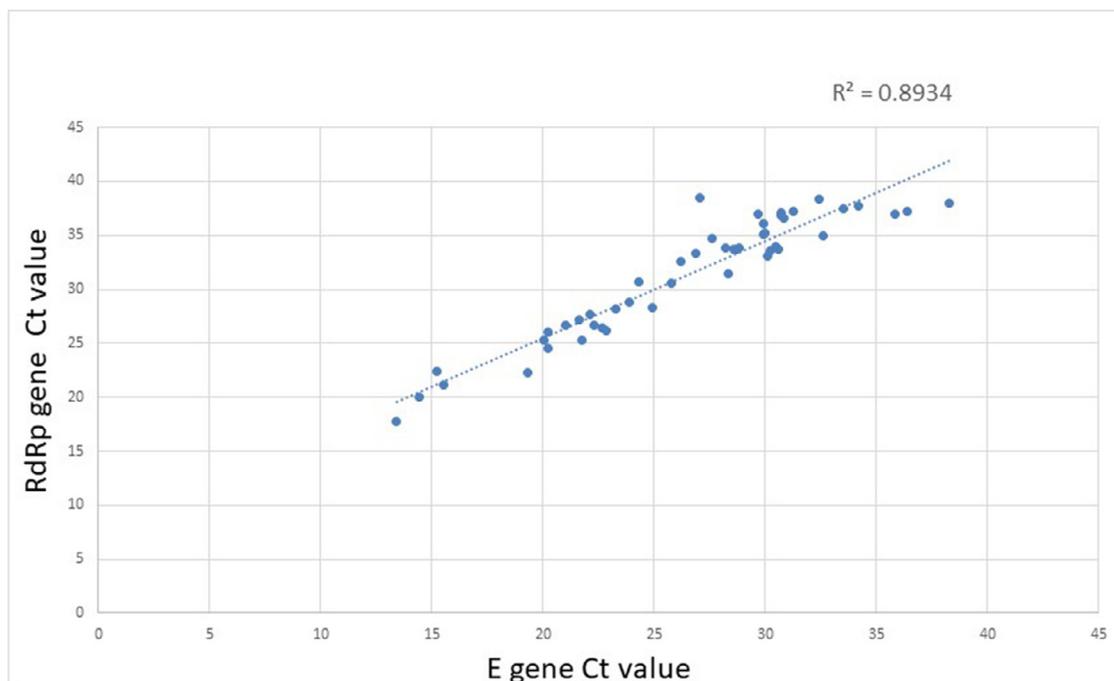


Fig. 2. The correlation between Real Time PCR Ct values of E and RdRp genes of ModularDx Kit SarbecoV E-gene EVA, and ModularDx Kit SARS-CoV-2 (COVID-19) RdRp (TIB MOLBIOL, Germany).

But examine everything carefully; hold fast to that which is good: choosing the right test for the right time

The first considerable batch of real-time PCR tests was donated to Iran by the WHO on February 21, consisting of about 2000 screening test (ModularDx Kit SarbecoV E-gene EVA, TIB MOLBIOL, Germany, cat number40-0776-96X) and 600 confirmatory tests (ModularDx Kit SARS-CoV-2 (COVID-19) RdRp, TIB MOLBIOL, Germany, cat number 53-0777-96)). Since then, we used these kits as our gold standard tests, and other kits (mostly donations) were compared with them. It is noteworthy that in February, almost all available kits in the markets were labeled as RUO, and ensuring their accuracy was the lab's responsibility.

Later, a dedicated team was formed to evaluate the kits in PII. Since such a situation was unprecedented in Iran, an emergency use authorization (EUA) policy was adopted for evaluation of diagnostic kits. For molecular tests, panels of samples with various Ct values (tested with our validated kits) were prepared, and any test which could correctly identify 95% of negative/positive samples was approved as "acceptable under EUA". If an "acceptable" test was in one tube multiplex format (at least two of E, N, S, and Orf1ab genes, and an internal control with different fluorophores), it was also labeled as "suitable for use in the COVID-19 lab network". The reason behind this distinction was the exceedingly high load of tests in this network, which made tests with more than one tube impractical. A similar approach was applied for RNA extraction kits.

The fast expansion of labs in the first weeks of establishment of the network was threatened by not having enough kits available. According to the WHO guidelines at the time, for detecting SARS-CoV-2 in a sample, separate screening and confirmatory PCRs were necessary. It resulted in an additional 25–40 % burden on the laboratories which already suffered from lack of adequate equipment. Although our analysis of first positive cases showed that there was a strong correlation between Ct of E and RdRp genes when tested with TIB MOLBIOL mix sets (Fig. 2), the mean RdRp Ct values was almost numerically cycles higher than the E gene detection results.

This led to negative RdRp results in many cases when the E gene Ct was higher than 32. However, these RdRp-negative samples were positive when tested for N gene. The biological explanation for this phenomenon could be the higher expression of structural proteins (E and N) genes compared to RdRp [11]. Furthermore, since the prevalence of positive cases was very high in hospitalized patients (more than 25% at the time), we concluded that detecting only one viral RNA gene would be enough to provide an acceptable diagnostic value. Approving this recommendation by the national scientific committee of COVID-19 in late-February helped the labs to have less workload and shorter turnaround time. This recommendation was later endorsed by the WHO testing guideline released on March 2 [12].

Despite this timely decision, since April we have preferred testing two targets in virus genome for the following reasons: first, we shifted the tests to suspected cases, their contacts, and the general population, which could lead to a decrease in prevalence among the tested population. This could decrease the predictive value of testing one target. Second, since we were yet uncertain about the probability of mutation in one PCR target position of this virus, it is safer to look for two targets to decrease the chance of false-negative results. Additionally, more validated multiplex tests were available thereafter at affordable prices.

Serologic tests

The high hopes for utilization of rapid serologic tests in controlling the outbreak all around the world soon faded after observing their low sensitivity, and sometimes specificity. By testing some of these kits in April 2020, we came to similar results. Those tests showed the sensitivity of not higher than 50% in outpatients or patients in the early phases of infection (unpublished data). Therefore, the national COVID-19 laboratory committee decided to limit their import, and authorize their usage solely for research purposes. With the availability of ELISA tests by mid-April, we considered its application in return to work permission or issuing immunity passport. However, due to the scarcity of evidence with re-

spect to seroconversion prevalence in the general population, the rise of different antibodies during the course of infection, and the linkage between the antibody (especially anti N protein antibodies, which is the common antigen in most available ELISA kits) and immunity against SARS-CoV-2, the committee decided to let the Iranian food and drug organization (FDO) issue RUO or IUO labels for such kits, providing confirmatory evaluation in PII, and leave IVD labeling till enough evidence for their application is available.

Count it all joy when you meet trials of various kinds: other challenges

Experienced human resources

Among medical laboratories in Iran that were able to provide molecular testing services, very few had the experience of testing tens of samples per day. The closest national experience for such a service was the influenza surveillance system and HIV monitoring program. These two services were operational in parallel networks in 13 medical universities. The first reasonable target of expanding COVID-19 test was the universities who had either of these labs because the setting was somehow prepared, and the staff were trained. However, to answer the increasing number of suspected cases to be tested, we had to go beyond this infrastructure and encouraging research or educational labs to start doing tests. Most of the staff in these latter settings were not trained for health services, nor biosafety issues. The Health Reference Laboratories office of the MoHME posted three questionnaires to the candidate labs, evaluating their infrastructure, biosafety measures and human resources. Then one or two of their staffs were trained first in PII, or later in COVID-19 labs of major medical universities. However, after performing 400,000 tests, it still remained challenging.

Using various instruments

The most common PCR machine in the Iranian health system laboratories is Rotorgene 6000 series machines (Corbette, Australia, and Qiagen, Germany); therefore, the early plan was to check the kits on this machine and then roll it out in the network. Most of these tests were compatible with many four or higher plex PCR machines (ABI 7500; Biorad CFX96, Sansure Slan96, Roche LC96, etc.), though with minor adjustments. However, many research labs who joined the network used machines with less fluorescent channels, like StepOne™ Real-Time PCR System. We had specifically more problems with the latter machine and finally decided to use fewer color channels for interpretation of data obtained from them, consequently different kits were suggested for those labs. In order to quickly check and fix the problems with different machines in the system, we had to install at least one of each brand in the central PII lab.

Switching between kits

In the beginning, we had to rely on donated kits from different sources. Most of these donations were less than 100,000 tests from each brand. So, we had to change the kits every few weeks. Although we did not face any major problem, it was difficult to inform or train all the labs several times. Besides, on one occasion, some packs of one of the donated kits seemed to have a leak of positive control tube during storage or transport, and made confusion in a few labs. It was advisable to divide the validated brands between different labs so that they could rely on the same kit for a longer time. The main problem in such a case would be assuring similar results from different labs.

IT and data management

Collecting data from so many labs and so many tested people needs a very strong IT infrastructure. We found the overwhelming need for a universal hospital information system (HIS) and laboratory information system (LIS) in the country. Lack of such a data transfer and sharing system could lead to confusion in making decisions for a system that needs real-time data on the number of tests, number of positive cases, number of negative cases, and the linkage between clinical and laboratory results. Any change in those figures could help us evaluate the effectiveness of our interventions or taking corrective measures in the labs beforehand. As a temporary solution to this problem, we employed an email based network to collect the testing data from all labs on a daily basis.

Sampling problems

This disease is so new that nobody is sure of many things about it yet, including the proper sampling method. There are many complaints about the low sensitivity of PCR compared to CT-Scan. This is partly due to improper sample collection. One nasopharyngeal swab and one oropharyngeal swab in the same transport tube is still the most recommended sample collection method for many cases in Iran. Having a human gene target in the multiplex PCR test gives some level of assurance that the sample has enough human material but does not rule out if the sample has been collected from a proper site or not. Enough proper swabs and viral transport media (VTM) were not available to us on several occasions. Wooden/cotton swabs or thick ones are not appropriate especially for nasopharyngeal sampling. We are currently studying other sampling methods, like throat gargle or saliva, but the results are not available yet.

Logistics and sanctions

The laboratory campaign to this pandemic has been probably the biggest one in the human history. Though some tests, like for HIV, have been done much more frequently performing so many tests in such a short time for COVID-19 has been unprecedented. This needed a very strong logistic support in every aspect, from PPE to preparing testing material, the equipment, even the plasticware, and finally the human resource. Except for human resources, which was fortunately of high quality and highly motivated, we had several problems with the rest. The unilateral sanctions against Iran, though claimed sparing humanitarian and medical needs, had negatively impacted supplying necessary instrument, for example, real-time PCR machines, or purchasing consumables due to bank sanctions and money transfer limitation. It also had affected post services, which prevented us from receiving control materials from abroad. It is highly recommended to make an international agreement on lifting such sanctions in pandemics like COVID-19.

Future actions required to prepare for the coming winter

Although information from influenza monitoring in the Southern Hemisphere indicated a significant reduction in influenza cases, as a far-sighted and precautionary measure, a multiplex COVID-19/Influenza A and B Real Time PCR kit was designed and distributed among selected laboratories of the network in different parts of the country.

Moreover, a commercial multiplex assay (Anyplex II RV 16 Detection kit (Seegene, Seoul, Korea)) was purchased in order to monitor outbreaks of other common respiratory diseases including Adenovirus, Influenza A virus, Influenza B virus, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Parainfluenza virus

4, Human rhinovirus, Bocavirus 1/2/3/4, Coronavirus 229E, Coronavirus NL63, Coronavirus OC43, Enterovirus, Metapneumovirus, Respiratory syncytial virus A, Respiratory syncytial virus B.

Additionally, for monitoring the quality of network laboratories, regular proficiency testing program was designed and laboratories were required to participate in this program. Continuation of each laboratory activity is conditional on success in the proficiency test.

Final words

The experience of the new coronavirus pandemic further revealed the irreplaceable role of the laboratory in controlling infectious diseases. Many efforts have been made in Iran to set up the COVID-19 laboratory network. However, there are serious challenges such as upgrading laboratory data management systems and maintaining resilience in terms of laboratory equipment and supplies, which should be considered not only for controlling the current epidemic but also for future outbreaks.

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Not required.

Declaration of Competing Interest

None.

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