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Dilemma of Laboratory Diagnosis of COVID-19 Patients

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TO THE EDITOR,

If we are talking about a global terror today that is not a nuclear reactor explosion or atom bomb explosion, maybe it is something more threatening. Because currently almost every nation has declared their fights against an invisible enemy who has imbalanced the total homeostasis of the world and this is a virus known as SARS-CoV2 or severe acute respiratory syndrome coronavirus 2 which causes the disease named coronavirus disease 2019 (COVID-19). This has now declared as pandemic by world health organization after influenza pandemic in 1918 to 1919.

The outbreak began in December 2019 in Wuhan and many other provinces in China[1,2]. Although the number of cases was increasing rapidly but information regarding clinical characteristics was not subtle. Only on January 7, a deep sequencing analysis of samples from throat swabs and lower respiratory tract revealed this novel coronavirus. So far it has found that the novel coronavirus is an enveloped non-segmented positive sense RNA virus belongs to the beta coronaviruses. Previously identified other well-known atypical pneumonia viruses are severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle east Respiratory Syndrome Virus (MERS-CoV)[3]. Epidemiological research has found the connection of person to person transmission from corona affected people[4]. The exponential rising curve of affected and dead people is still growing aggressively. If we look at the clinical manifestations of COVID-19, it includes severe version of common flue such as fever, dry cough, myalgia and fatigue. There are also some fewer common symptoms like headache, expectoration and diarrhoea. But about half of the patients have developed severe pneumonia and one third of the patients require intensive care support because of acute respiratory distress syndrome (ARDS) or multiple organ failure[1-5]. However, there are only a few reports about the novel coronavirus pneumonia so far. There is report regarding 75 patients with COVID-19 in the first affiliated hospital of USTC from January 21 to February 16, 2020, Hefei, Anhui province which described the epidemiological, clinical and laboratory characteristics of 75 COVID-19 confirmed patients who admitted in that hospital[6].

Now it is a burning question that who can and should be tested? According to Robert Koch Institute, Germany a test for an infection with the coronavirus only makes sense if there are signs of illness. If someone is healthy, the test says nothing about whether they can get sick. It would also unnecessarily burden the test capacities. If some has respiratory problems with symptoms such as coughing, sneezing, or a sore throat they should be tested. If a person had contact with someone who has been confirmed to have Covid-19 in the past two weeks, or if he/she have been in an area where it has many diseases have already occurred. A test is also advisable if there is a previous illness or if the illness worsens and there is shortness of breath or high fever. In addition, people with symptoms who work in the hospital or in elderly care or who meet risk groups in other ways should be tested[7].

Ideal Specimens

According to WHO the specimens that should be collected for laboratory testing are two types which are upper respiratory specimens like nasopharyngeal and oropharyngeal swab or wash in ambulatory patients and lower respiratory specimens like sputum, endotracheal aspirate, and bronchoalveolar lavage. Additionally, blood and stool may be collected as this virus was identified in these

specimens and coronaviruses are responsible for SARS and MERS. However, the duration and frequency of shedding of COVID-19 virus in stool and potentially in urine is unknown. In case of patients who are deceased, consider autopsy material including lung tissue. In surviving patients, paired serum (acute and convalescent) can be useful to retrospectively define cases as serological assays become available. After collecting the specimens these should be reached into BSL-2 laboratory as soon as possible. Correct handling and proper shipping should be done at 2-8°C temperature and stored in -20°C or ideally at -70°C temperature[8].

Table 1: Specimens to be collected from symptomatic patients and contacts[8]

Source	Test	Type of sample
Patient	NAAT	1) Lower respiratory tract (Sputum, aspirate, lavage) 2) Upper respiratory tract (nasopharyngeal, oropharyngeal swab, wash, aspirate) 3) stool, blood (from autopsy)
Patient	Serology	Blood
Contact	NAAT, Serology	Nasopharyngeal, oropharyngeal swab, serum

Laboratory Tests

Routinely, confirmation of COVID-19 is based on detection of unique sequencing of virus RNA by nucleic acid amplification tests (NAAT) such as real time reverse transcription polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing. So far, targeted viral genes are N, E, S and RdRP. According to the Robert Koch Institute, the pure test time for evaluating the sample is about four to five hours. In practice, it usually takes 24 to 48 hours for the patient to find out the result. New test procedures such as the drive-in centers are to shorten this time[7-8].

As long as the result is not yet available, we should assume that we can infect other people, and therefore stay at home and avoid contacts. The study of USTC showed that they confirmed the patients by respiratory swab collection and followed by real time RT-PCR method. Viral RNA was extracted using QIAamp RNA virus Kit (Qiagen, Heiden, Germany). The diagnostic test was done by using a commercial coronavirus test kit (Shenzhen Huada Yinyuan Pharmaceutical Technology Co., Ltd., Shenzhen). The specific primers and probe targeted to nucleocapsid protein (N) were used and the sequences were as follows: forward primer 5'-GGGGAACTTCTCCTGCTAGAAT-3, reverse primer 5'-CAGACATTTTGCTCTCAAGCTG-3 and the probe 5'-FAM-TTGCTGCTGCTTGACAGATT-TAMRA-3'.

Conditions for the amplifications were 50°C for 20 min, 95^0 C for 10 min, followed by 40 cycles of denaturation at 95^0 C for 15 s and extending and collecting fluorescence signal at 60^0 C for 30s. A cycle threshold value (Ct-value) less than 37 was defined as a positive test result, and a Ct-value of 40 or more was defined as a negative test[6-8]. Nonetheless, hematological parameters including blood routine, blood biochemistry, coagulation profile, and infection-related biomarkers were recorded. Plasma cytokine interleukin 6 (IL-6) levels were detected by ELISA and the CD4+ and CD8+ T cell subsets were counted using flow cytometry. By using all these parameters, they identified the age groups with associated symptoms and signs[6].

Sero-Diagnosis

Another broad spectrum of laboratory profiling of COVID-19 patients can be done by serological surveys. It can aid investigation of outbreak and the retrospective assessment of the extent of outbreak. So far it has been seen leucopenia (16%) and lymphopenia (53.33%) along with increased neutrophil count.41.33% and 37.33% patients showed decreased counts of CD4+ and CD8+ T cell level respectively.

Table 2: In a nutshell of laboratory results of COVID-19 patients[6]

Blood Routine	**Remark**
Leucocyte	↓
Lymphocyte	↓
Neutrophil	↑
Platelet	↓
Hemoglobin	↓
CD4+, CD8+	↓
Coagulation function(APTT,PT)	Impaired
LDH	↑
CRP	↑
ESR	↑
IL-6	↑

Along with lower level of hemoglobin patients also showed impaired coagulation function. Moreover, the USTC study showed impaired liver and renal function in COVID-19 patients which is an

indication of potential internal organ damage like elevated LDH, CRP, ESR. But blood analysis can bring light to another spectrum of laboratory profile. Analysis of blood sample can give idea about the development of protective antibody after an infection and deactivation of virus. Also, blood plasma was used as a potential treatment option in China[6].

Rapid Diagnostic tests

However, to avoid unnecessary blood work-up and diagnostic testing in patients with COVID- 19 a rapid test is also in development process. Many companies are now working on rapid tests. Rapid test results are considered a key to curbing the coronavirus. The US pharmaceutical company Abbott Laboratories has received marketing authorization for one in the United States. The test delivers results in a maximum of 13 minutes and can be used in medical practices, test centers or hospitals. The Food and Drug Administration (FDA) has already approved several tests using the rapid procedure, including one from the Swiss company Roche and one from the US Company Cepheid. The technology group Bosch has also developed a rapid corona virus test. The fully automatic procedure for the detection of viral genetic material should take less than two and a half hours from taking the sample to the result. Crisper-based diagnostics for detecting the coronavirus are also in the works at startups like Sherlock Biosciences and Mammoth Biosciences. These tests use Crisper's programmable gene-seeking capabilities to deliver a diagnosis in under an hour without the need for fussy lab instruments[7].

Conclusion

Finally, we should keep in mind we are obliged to practice our own social distancing, maintain personal hygiene as much as possible and check any of the neighbor or elderly are sick or need supplies of food or not. We should keep in mind also that this is a real fight of survival. Therefore, proper laboratory preparation, being updated with the newest testing procedures will ease the path of war against this invisible enemy SARS-CoV2.

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