Review Article

COVID-19: Lab Testing: Review Article

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Abstract

The rapid tests had no role. The standards were laid down and an attempt was made to streamline the diagnostic work. Why enough tests are not being done for Corona-virus? Why so late and so little? Why there is so much discrepancy in the results between the lab? We have developed the test overnight in Pakistan, why not to be allowed to start testing on a mass scale? Why few labs are over-performing than the expectations? Why cannot other labs do when kit and equipment is there? There were many such like questions which made the news. The media need something to talk and that something should pertain to the current situation.

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Introduction

The recent advent of Covid-19 pandemic has raised many questions globally. The diagnosis, data, tracking of disease and its control depended upon virology lab services which were not well-established in Pakistan. Therefore, the laboratories were designed and created in hurry. There were many questions raised about the gold standard test, which was PCR for the causative virus; SARS CoV2. There was a need for its validation, standardization, interpretation and expansion of the service all over the country. The standards were set and guidelines were prepared. There was much confusion about the rapid test. The viral serology was established with caution and reservation. Its main role was in sero-epidemiology and identification of those who have been exposed to the virus. The rapid tests had no role. The standards were laid down and an attempt was made to streamline the diagnostic work.

Why enough tests are not being done for Corona-virus? Why so late and so little? Why there is so much discrepancy in the results between the lab? We have developed the test overnight in Pakistan, why not to be allowed to start testing on a mass scale? Why few labs are over-performing than the expectations? Why cannot other labs do when kit and equipment is there? There were many such like questions which made the news. The media need something to talk and that something should pertain to the current situation. We are in the phase of pandemic which the world is facing now, and we are in it. The response of the nation depends upon the knowledge and resources of a particular nation.[1,2]

Recent crisis of the Covid-19 is caused by Novel Coronavirus (now named as SARS-Cov 2). The tests were not available for all diseases. Moreover, their reagents cannot be purchased over the shelf, which may be run on machines, which are already present there. The virus needs to be characterized, its genome mapped and the host's immune reaction against it studied before the diagnostic test systems are developed. Ordinarily, it may take years. This time, the process was faster than usual but still the diagnostic mechanism for COVID-19 is in a developmental phase. It will surely be sophisticated, over time. Once a kit is developed, its suitability, standards, quality and performance are established. Its performance, linearity, standard deviations and precision is studied and then the kit is validated. Then it is marketed for use in the labs across the word, when the demand is more the production may be in accordance with the need. Then the kits are dispatched under optimum temperature and go through it.[3]

Then the end user is under strict international national, provincial and local regulations. There are many regulatory authorities with their own mandates. They have different standard procedures and requirements. The kits have to be approved by international bodies like the FDA, British standards, and European regulators (CE Marked). Every kit may not be run on any equipment. The available gadgets should be suitable for the kits, and they have to procured, calibrated and regularly maintained. Then there is a consideration for the biosafety, so that workers and the environments remain safe during the specimen collection, transportation and test performance. A right specimen, taken by a right person, in a right container and timely transported under optimum conditions makes a lot of differences. The specimen has to be properly labelled and accompanied by the rightly filled proforma. It must contain, name address, disease symptoms and even the NIC number for matching a reference.[4]

Then we need a trained and dedicated team with Standard Operational Procedures (SOPs), safety equipment (PPE- Personal Protective Equipment) and regular checks. There is a team which collects and transports the specimens and another team records their receipt and checks the labels and integrity of the specimen. Then there is team which performs the test and finally there is a team of laboratory physicians which authorizes the report and the report is released, with popper interpretation. If needed, then the medical staff speaks to the ordering physicians on telephone. Sometime, he has to talk to the patient or his relative, which may not be considered appropriate in the west but in Pakistan, they too have to be explained about the report. Then there should be suitable Laboratory Information Management system (LIMS) in place, to trace the sample, generate reports and maintain the record. The equipment and staff need a well-designed place, which may be different in case of virology related work, as there is a need for stringent biosafety, decontamination and error alarms.[5]

All components of a viral lab must be there. It should in turn be ideally managed by a team of experienced personnel and led by a consultant virologist. A sophisticated work is performed, by a specialized workers and specialized team. The lab needs at least a clinical virologist, which we do not have many in Pakistan. A general pathologist or even a microbiologist may be very sincere and hardworking in profession but his skill cannot match with a person, who was trained as a virologist. According to international standards, he must be medically qualified first and then undergo at least five years training in his specialty. Then his team of technologists and scientists should be specialized in the virology. They must be licensed and accredited. As the medically laboratory deals with human samples and managed by the laboratory physicians, they must be licensed by the respective medical councils.[6]

The general public and even the physicians talk of kit, test and a fully robotic automated machine. The automation needs more careful supervision, better quality management and trouble shooting. That means a supervised laboratory system. Under a stressful situation, which arise in case of a disaster, the lab needs skills, resources and support system.

This time, with the onset of pandemic, the public sector has sincerely tried to develop quite a few regional SARS-CoV2 testing laboratories, and they did it in some way. Others tried to jump in the field without much preparation and were desperate to apply a test which might be quick and simple. Such antibody-based devices came in the market but luckily these were banned by the Govt of Pakistan and quackery was discouraged. The flight operation was at standstill and there was a problem of a regular supply of reagents. Many different kits were brought in the market. These were of different quality and were used by various labs. An already established system of virology may conduct proper evaluation and validation and enhanced the capacity. [7]

Every lab test has its limitation. In case of SARS-Cov2, the relevant virus must be there in the sample, for its detection. The ideal sample is tracheal aspirate, which may pick 93 percent of the cases. A sample taken from the nasopharynx by inserting the swab stick, deep through nose, may yield as low results as 72 percent of infected cases. In our experience, it is definitely more than that but may still miss an infected case. At times, the virus may be present in the lung but not in the nasopharynx. If we take two samples; one from nasopharynx (deep in the nose) and one from the throat, there may be more chances to detect the virus. The ideal specimen may be the tracheal aspirate but that is difficult to obtain and needs to be

taken by a skilled physician. The person who collects the specimen, he and his staff may be exposed. Therefore, we have to compromise in the nature of specimen. The swab should be of made of Teflon and not of cotton or calcium alginate. The staff needs training for specimen collection, containers, biosafety handling and their transport.[8]

The turnaround time (TAT) means the time taken between the collection of samples an issue of report. That is the most important aspect of reporting and ideally it should be within twenty hours. That is needed to release the people from isolation, quarantine and contact tracing. If the reports are made available in a weeks' time, the plight of the person tested may be easily understood.

The laboratories differ in respect of their capacity, capability, manpower and standards. Obviously, the work performed by a lab cannot be compared with that done in another lab. Even a sample taken at different time from the same patients and tested in the same lab may show different results. People at times, do compare different labs by providing the sample to various labs. They take anyone of them as a gold standard, the result of which suits to their own needs. Such cases have been highlighted by the media and the performance of different labs is questioned by the nonprofessional people. In the absence of a system of standardization of labs, there may be a confusion. Initially, the Covid-19 diagnosis was taken a taboo and the people wanted to get the reports to make their own case. That might lead to a question to professionalism and credibility.[9] A good lab tries to use the standardized methodology. Human error is a reality, but one must do his best to avoid such errors and provide an optimum service by adoption of reference intervals. There is a need for better coordination at a national level and standardization of procedures.[10]

Why the viral PCR and why not rapid diagnostic kits? In mid to late 1980s, AIDS have just been introduced to England and was setting itself as a pandemic. Anybody, could get his test done for the HIV (AIDS virus), through his general practitioner, who took his blood sample and sent it to us. When the result was received it made permanent part of his medical record. If it was positive then his GP used to write in his medical proforma sent by a prospective employer, bank (when he applied for a loan or mortgage) and Insurance companies. Surely, he could not get a loan or a mortgage, with a positive status of the virus. The people wanted to know their HIV status, without being recorded. Such rapid devices were available but not used by the National health Service. These were purchased by the free-roaming Gypsies. They set a lab in the back of their minitrucks. They took the blood and tested there and then and told the result verbally. So, we conveniently called them Gypsy Test. Now that test is in the town and everyone wanted to do it, as it is easy to perform and quick to report. The test had a predictive value of 20 percent and the Government of Pakistan banned its use.[11]

What is the most suitable test? It depends upon the stage of infection. The person may fall sick with pneumonia when no test is positive; even the viral RNA by Polymerase Chain Reaction (PCR). The antibodies appear when the infection is established and the immune system reacts to the virus. The response varies from patient to patient. Ordinarily, it takes three weeks for antibodies to mature. Many manufacturers still market the test kits, which pick total antibodies of high avidity. It is too early to say whether these remain detectable lifetime or their level may decline with the passage of time? There are still some unanswered questions about their correlation with protection from the virus, in future.[12] As the pandemic progresses and its waves affect a major portion of the population, their role will gain more importance.

Initially, the test is to detect the virus or its component. The viral isolation is not suggested because of three reasons; need for a highly developed cell culture facility, delay in diagnosis (as it may take up to eight days in the completion of the procedure) and biosafety related issues. Therefore, we bank upon detection of viral antigen or RNA. The antigen test is yet to be established and it might be less sensitive and may be having some problem of its specificity. The viral RNA is picked by the PCR tests, which targets one to four different genes. Different vendors base their test of different targets and the specificity and sensitivity of the test varies. The test remains positive for two weeks of after an asymptomatic or symptomatic infection. In a symptomatic infection and in rare cases, it may remain positive for many weeks or even up to fifty days. Different studies from Singapore and South Korea has shown that the patient remains no

more of a danger to spread infection to others, after two weeks of symptoms, even if the PCR remains still positive. As we pick the RNA and not complete virus in this test, we do not know whether the virus is still capable of infecting others or has been disintegrated and we are picking only the genetic material. The test may be completed in about six hours, after the initiation of the procedure. It has extraction and amplification steps and there may be separate kits for these two steps. Then there is a problem of compatibility of these kits for one test. Many kits have different apparatuses and all equipment cannot be uses across the board. The sensitivity of the test varies according to the day of infection, severity of disease or type of specimen. The super-spreaders may have a high level of RNA even if the person himself is asymptomatic. Most of the children fall in this category. The criteria of interpretation of a test results are stringent.[13]

We as a group of few clinical virologist, which are in Pakistan have reached to a consensus for the interpretation and that document may be shared to any lab in the country, at request. To have a satisfaction that one has cleared the virus, one should have two negative PCR test done, on two consecutive samples taken at least twenty-four hours apart. Why two? Is to be sure because one negative test may turn out to be positive next day. There are still the people who showed two negative tests on such consecutive samples but after a couple of weeks were found to be positive on PCR.

Much emphasis is not laid down on antibody tests. The rapid tests are out due to above mentioned ban. The FDA approved test of Roche and Abbott etc. are available, which are done on chemilumiscence based ELISA. These are in the phase of further improvement and development. Their precision as well consistency is being established. COVID-19 is a Coronavirus and there are four endemic and seasonal coronaviruses which are known to cause a milder human disease. Their encounter leads to the production of antibodies and there may be a cross reactivity, which has to be stringently ruled out. There is a need for further perfection in these tests. They cannot pick an active viral infection and may appear late. Their presence means that the person has been exposed to the virus in the past; whether he is now immune is a moot question. Nobody can say it with confidence but most probably one may not have a second infection. If the infection is ongoing, one may die despite having developed the antibodies. There is a phase when one may have the virus as well as its antibodies and immune complexes may be made. At this stage the test for PCR or antigen may show presence and absence on consecutive samples.[14]

The antibody test may also suit to mass-screening and sero-epidemiological purpose to know how much percentage of a particular population has encountered the virus and those who are positive may be sent back to work. In Singapore, they have done with their expatriate workers. The test is also used to identify the suitable donor for plasma-based therapy. If done with PCR, it may show the dynamicity of the infection. Many people have come up with IgM based kits, which gives very little answer. Yet we do not have IgM based test for the HIV or Hepatitis C virus. All viral infection may, therefore, cannot be detected by an IgM based test. The testing needs algorithm based on a battery of tests, clinical information and radiological findings. Therefore, basing the opinion or cursing a lab on the base of a single test and that too in comparison with the information gathered by other labs is more of a tool for information gathering. Therefore, interpretation is a difficult phenomenon.[15]

The test result is reported to the individual tested as well as public health authorities, who take their action, accordingly. They isolate the patient and trace his contacts for the purpose of quarantine and that is a legal requirement. People curse a lab for informing the health authorities. The policy was made to save the patients and his contacts. Anyhow that seems to be the past as the authorities are exhausted and have dwelled to send home an infected asymptomatic case, if his house has a facility for isolation. It depends upon may factors; mainly the conscience of the patient and his family. The labs are often asked about the secrecy of the result. They have a right but the public health act supersedes their right and they have to be obliged for public health.[16]

The test has many facets as mentioned above and it is more than a kit and machine. That needs a setup of virology and professionalism. It needs virology services and development of skill and expertise. The media should know the limitation of these test and avoid creating the problem for a very few professionals who are at work, under the difficult circumstances.

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