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Combat COVID19 Diagnosis with Combined Test, Outcome: Make Up RT-PCR Shortage and RDB False Negative Coverage

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ABSTRACT

COVID-19 diagnosis is now a burning issue in the world. WHO certified only reverse transcriptase polymerase chain reaction (RT-PCR) technique which is used for diagnosis of COVID-19 but another side rapid dot blot (RDB) test is not recognized by WHO due to false positive and false negative issue. RDB test is so much easier and less time consuming than RT-PCR. Around the world huge people affected with COVID-19 daily but only use of RT-PCR in diagnosis is not cover all the existing cases due to some limitation of this test. Real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assays were used for detection of SARS-CoV-2 from respiratory secretions collected by nasal and oropharyngeal swabs. Due to lack of technology, technical support for a large portion of people are to be undiagnosed, especially in developing country. If we programed a combined test RT-PCR with RDB, we can enhance test efficiency and efficacy. RDB test will make up RT-PCR shortage, by initial screening test of large population, and remain population confirmed by RT-PCR that make up the false negative of initial RDB test. The real result is come out if we work with.

KEYWORD: – COVID-19 diagnosis, RT-PCR, RDB test.

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INTRODUCTION

Coronavirus disease 2019 (COVID-19) was discovered in Hubei Province, China in December 2019 (Zhou P *et al.* 2020). By January 10, 2020, samples from patients' Broncho alveolar lavage (BAL) fluid were analysed to reveal a pathogen with a similar genetic sequence to the beta coronavirus B lineage. It was discovered that this new pathogen had ~80%, ~50%, and ~96% similarity to the genome of the severe acute respiratory syndrome virus (SARS-CoV), Middle East respiratory syndrome virus (MERS-CoV), and bat coronavirus RaTG13, respectively. The novel coronavirus was named SARS-CoV-2, the pathogen causing COVID-19. The virus has a diameter ranging from 60 to 140 nm, has an envelope with protein spikes, and has genetic material. The overall structure looks similar to other viruses from the Coronaviridae family.

A novel coronavirus (SARS-CoV-2) was suspected to be the aetiology with Phinolophus bat as the alleged origin (WHO guidance 2020). In just two months, the virus has spread from Wuhan to the whole China, and another 200 countries. By 24:00 on May 03, accumulative 3,34,9786 confirmed cases with 2,38,682 deaths were reported around the world.

Viral protein antigens and antibodies that are created in response to a SARS-CoV-2 infection can be used for diagnosing COVID-19. Changes in viral load over the course of the infection may make viral proteins difficult to detect. In contrast, antibodies generated in response to viral proteins may provide a larger window of time for indirectly detecting SARS-CoV-2 (K. K.-W. *et al*. 2020). Antibody tests can be particularly useful for surveillance of COVID-19. One potential challenge with developing accurate serological tests includes potential cross-reactivity of SARS-CoV-2 antibodies with antibodies generated against other coronaviruses. Previously tested plasma samples from 15 COVID-19 patients against the S protein of SARS-CoV-2 and SARS-CoV and saw a high frequency of cross-reactivity (Lv et al. 2020). Currently, serological tests (i.e.

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blood tests for specific antibodies) are in development. Recently immunoglobulin G and M (IgG and IgM) are detected from human serum of COVID-19 patients using an enzyme-linked immunosorbent assay (ELISA) (Zhang et al. 2020).

A dot blot technique in molecular biology used to detect proteins. It represents a simplification of the western blot method with the exception that the proteins to be detected are not first separated by electrophoresis. Instead the sample is applied directly on a membrane in a single spot and blotting procedure is performed.

A Bangladeshi Scientist Dr. Bijon Kumar Shil invented rapid dot blot (RDB) test for the diagnosis of COVID-19. The kit developed by Bangladesh's Gonoshasthaya - RNA Biotech Limited is similar to one developed in January by scientists in China as the coronavirus outbreak intensified in the Chinese province of Hubei. Within a few minutes this test is diagnosed COVID-19 by detecting blood antibody.

Reverse transcriptase polymerase chain reaction (RT-PCR) another world wide recognized technique for RNA detection. COVID-19 is caused by novel coronavirus (SARS-CoV-2) which is a RNA virus. So, RT-PCR is the best way to diagnose COVID-19 by the band of RNA of particular virus.

MATERIALS AND METHODOLOGY

For RT-PCR the most predominantly used method for diagnosing COVID-19 using respiratory samples (WHO guidelines 2020). Upper respiratory samples are broadly recommended, although lower respiratory samples are recommended for patients exhibiting productive cough (CDC guideline 2019). Upper respiratory tract samples include nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal washes, and nasal aspirates. Lower respiratory tract samples include sputum, BAL fluid, and tracheal aspirates. Both BAL and tracheal aspirates can be high risk for aerosol generation. The detectable viral load depends on the days after illness onset. In the first 14 days after onset, SARS-CoV-2 could most reliably be detected in sputum followed by nasal swabs, whereas throat swabs were unreliable 8 days after symptom onset (Pan Y *et al.* 2020, Yang Y *et al.* 2020). These negatives could result from improper sampling techniques, low viral load in the area sampled, or mutations in the viral genome multiple lines of evidence for patients linked epidemiologically even if the results are negative from nasopharyngeal and/or oropharyngeal swab (Winichakoon *et al.* 2020). RT-PCR should be done according to the established and routine protocol.

Before performing RT-PCR RDB test should be done for the conformation of the positive sample. In RDB test blood serum, saliva and sputum samples are needed. The rapid dot blot (RDB) test, looks for antibodies in the blood that are created in response to a given virus.

To diagnosed large population within short period completely and correctly. We perform combined those two test by random hypothesis on the basis of history and epidemiology.

Case type-1 (COVID-19 sign & syndrome present): SARS-CoV-2 has long incubation period its vary to 4-7 day. Initially maximum cases are considered as common cold, after few days he/she suspected this sign is related with COVID-19. Then he/she decide to test for COVID-19. Already 7 days gone. And antibody produce in patient body. So rapid Ag-Ab binding are effective in this case. Now we can decide and consider, case with COVID-19 sign syndrome present and rapid dot blot test (RDB) positive are COVID-19 positive and isolated those patient. There are some chances to get false negative. So, we perform RT-PCR remain case.

Case type-2 (Asymptomatic: Get exposure less than one week): This type of case, such as accidentally doctor/health worker handle

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COVID-19 patient without protection, patient family member, ambulance driver, police, dead body disposal related person are confirmed by get exposure of corona virus within short period (1-3days). So no antigen-antibody phenomena active within the short period. In this type of case rapid dot blot(RDB) or other serological test is not effect. So only genome of this virus detect via RT-PCR is effective in this case.

Case type 3 (Asymptomatic: Get exposure more than one week): Based on epidemiologic study, some people may get exposure more than 7 day and unexamined people undiagnosed infection people fly infected area to non-infected area. He/she randomly move tea stall, prayer hall etc. after some day he diagnosed as COVID-19 positive. If the remain people already get exposure more than one week, antigen present in those case. So people with rapid dot blot (RDB) test positive are true positive, and people with RDB negative is considerable negative. But they must be in quarantine for 14 days long.

CURRENT TEST CONDITION

The symptoms expressed by COVID-19 patients are nonspecific and cannot be used for an accurate diagnosis. There was a report that 44% of 1099 COVID-19 patients from China had a fever when they entered the hospital and that 89% developed a fever while in hospital reported (Guan *et al.* 2020). They further found that patients had a cough (68%), fatigue (38%), sputum production (34%), and shortness of breath (19%). Many of these symptoms could be associated with other respiratory infections. Nucleic acid testing and CT scans have been used for diagnosing and screening COVID-19.

Molecular techniques are more suitable than syndromic testing and CT scans for accurate diagnoses because they can target and identify specific pathogens. RT-PCR is one of most modern technique. Its need high technology, highly qualified technical person, high level bio-safety (BSL-3) lab. It is also expensive and time consuming. Developing country failed to diagnosis COVID-19 by RT-PCR as rational to their population.

Table-1. Test per million developed country vs. developing country:

Data of RT-PCR test per millions of populations of developed country	Data of RT-PCR test per millions of populations of developing country
USA - 20,424 Spain - 32,699 Italy - 34,879 UK - 16,644 Russia - 27,036 Germany - 30,400	India – 708 Bangladesh - 462 Pakistan - 878 Indonesia- 395 Afghanistan- 284

From the following data we can found that the developing country could not test enough as they need. So a large number of people are undiagnosed.

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COMBIND TEST PLANNING

To makeup this RT-PCR shortage, we can make a test planning on the basis of history and by both RT-PCR & rapid dot blot (RDB) test. Firstly, we perform rapid dot blot (RDB) all of sample patients. Now the program planning is given:

1. History of COVID-19 sign & syndrome presents plus rapid dot blot (RDB) positive = It may be indicated COVID-19 positive.

2. History of COVID-19 sign & syndrome present plus rapid dot blot (RDB) negative = It may be false negative and confirmed by RT-PCR.

3. History: Get exposure to COVID-19 patients/dead-body very recent (7d>) that must confirm by RT-PCR.

4. History: Get exposure (Patients/Dead body) more than one week ago (No sign & syndrome) with rapid dot blot (RDB) test positive = It may be asymptomatic COVID-19 case.

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5. History: Get exposure (Patients/Dead body) more than one week ago (no sign & syndrome) with rapid dot blot (RDB) test negative = It may indication of COVID-19 negative but he/she under quarantine two weeks or more.

Advantage over this combination: 1. We can perform large test within short period. 2.Firstly we found large numbers of positive case. 3. Suspected false negative confirmed by RT-PCR. 4. Makeup RT-PCR shortage by use of rapid dot blot (RDB) test.

RESULTS

As example we need to done 1000 RT-PCR, but our capacity only 200 PCR. And 250 people are affected with COVID-19 among 1000. Selection of 200 (20%) for RT-PCR from 1000 is difficult, and 800 (80%) case remain undiagnosed. And there is great chance to a large number of active case is out of diagnosis. Even if luckily selection is 100% correct, then 200 RT-PCR test results 200 positive, then 50 COVID-19 cases remain undiagnosed. But uncertain selection or disease history based selection may be highest 40 to 50% correct. If the selection is 20% correct then we found 40 corona case, and 210 case remain undiagnosed, if selection is 50% correct then diagnosis case 100 and 150 remain undiagnosed. If we done screening test by RDB test 1000 sample. Then we found 225 (Consideration 10% false negative). Without RT-PCR we diagnosed 225 COVID-19 case, and only 25 case remain undiagnosed. Now we have to done 200 test by RT-PCR to diagnosis 25 cases from 775 sample. From statistical random selection we can diagnosis 6 case (25/775*200). Selection based on clinical history and epidemiological selection it may be enhanced 10 corona case from 775sample by 200 RT-PCR test.

LIMITATIONS

The rapid dot blot (RDB) test kit looks for antibodies produced by the white blood cells in response to the virus rather than the virus itself, there is a margin of error where it could return a false negative if used at the wrong time. The dot blot test detects the specific antibody in the blood created by the white blood cell in response to coronavirus. The test results may be found within few minutes.

The best part of this rapid kit is it's cheap (approximately $3) to produce unlike the RT-PCR testing kit which one is expensive. An RT-PCR kit costs about $120 to $130. A specialised biosafety lab is also needed to house a PCR machine, each of which may cost $15,000 to $90,000.

In underdeveloped country has not enough desired biosafety level to conduct RT-PCR tests. Whereas rapid dot blot (RDB) test can be conducted by most of the laboratories.

Limitations of the rapid dot blot kit looks for antibodies in the blood produced in response to infection by coronavirus, whereas the RT-PCR looks for the virus itself (through RNA extraction) in respiratory specimens. Since the rapid test relies on the presence of a sufficient amount of antibodies in the blood, factors like timing of the test, previous infections, immune status of a person, cross-reaction with other antigens, can produce false results.

CONCLUSION

The availability of established diagnostic technologies has enabled researchers to plug-and-play in the design of COVID-19 diagnostics. Such technologies took decades to optimize, but they are now playing an important role in identifying and managing the spread of COVID-19. Lessons learned from the 2002 SARS outbreak have guided the development of COVID-19 identification and detection. In conclusion, diagnostics are an important part of the toolbox for dealing with outbreaks because they enable healthcare workers to direct resources and efforts to patients with COVID-19.

Multiple diagnosis enhances disease diagnosis process. To combat COVID-19,

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we have to prepare multiple way. The rapid dot blot (RDB) and other serological test support RT-PCR shortage. Diagnosis and isolation is only and only solution to decrease the spread of infectious pathogens and reduce mortality and eradication of COVID-19.

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