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Role of GeneXpert in Diagnosis of Covid-19 Among Armed Forces Personnel of Bangladesh

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Abstract:

Bangladesh is one of the several countries which have successfully faced the challenge of recent Covid-19 pandemic. After the first emergence of the outbreak of SARS-CoV-2 virus in China, it rapidly spread over the whole world. From the very beginning of the pandemic, Bangladesh government has taken all sorts of effective measures to save millions of lives. In the perspective of the Covid-19 situations in Bangladesh Armed Forces, AFIP Dhaka Cantonment has still been contributing very successfully to combat this situation. By December 2020, Covid-19 laboratories of AFIP performed 90,495 RT-PCR tests and 15,595 were found positive. Besides RT-PCR tests another very sensitive and rapid method named GeneXpert is being used to provide the Covid-19 test reports within 1 hour. By using this GeneXpert test system AFIP immensely helped the patients who require emergency treatment intervention, for dead bodies requires immediate religious rituals, personnel who needs urgent reports to go overseas and also for VIPs and VVIPs in situations of urgency. For the last 1 year during this pandemic, AFIP has been providing the Covid-19 diagnostic facilities with utmost accuracy and quality of the tests. It is the honour and pride of this renowned and prestigious institution to render its continuous service in the critical period of our nation.

Key words: Covid-19, Gene Xpert, RT-PCR, AFIP.

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Introduction

The Covid-19 pandemic is a critical situation in the history of present world. The whole mankind has still been suffering from the outbreak of SARS-CoV-2 across the globe. Healthcare systems of all countries have faced a great challenge to contain the outbreak of this new virus for saving the valuable million lives.

This virus was first identified in Wuhan city of China in December 2019. After this SARS-CoV-2 has spread worldwide, on 30 January 2020, the Director-General of the World Health Organization (WHO) declared the outbreak of COVID-19 as a Public Health Emergency of International Concern and issued a set of Temporary Recommendations.In Bangladesh by mid February of 2021 a total 541,038 people have been affected by Covid-19 with 8,285 deaths.[1]

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Rapid detection of the SARS-CoV-2 virus and isolation of the positive cases are the mainstay of preventing the further spread. For diagnosing the Covid-19 in Armed Forces personnel, first RT-PCR laboratory has been established at AFIP, Dhaka Cantonment in March 2020. Gradually RT-PCR laboratories has been established in all the peripheral CMHs. From the very beginning all these laboratories are playing great role for providing prompt and reliable diagnostic facilities for both Armed Forces members and also for civilians. All the CMHs have still been providing highly effective and successful patient cares for Covid-19 patients in a highly disciplined and organized way. Bangladesh Armed Forces has gained immense praises and congratulations for helping the nation in its emergency.

By the end of January 2021, the Covid-19 pandemic has come to the almost control point. The infection rates have been diminished significantly and also the daily death rates. It is the great success of the peoples and the government of Bangladesh.

Coronavirus disease 2019 (COVID-19)

Coronavirus disease 2019 (COVID-19) is a contagious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).The virus that causes COVID-19 spreads mainly when an infected person is in close contactwith another person.Small droplets and aerosols containing the virus can spread from an infected person's nose and mouth as they breathe, cough, sneeze, sing, or speak. Other people are infected

if the virus gets into their mouth, nose or eyes. The virus may also spread via contaminated surfaces, although this is not thought to be the main route of transmission.[2]

COVID-19 can affect the upper respiratory tract (sinuses, nose, and throat) and the lower respiratory tract (windpipe and lungs).The lungs are the organs most affected by COVID-19 because the virus accesses host cells via the enzyme angiotensin-converting enzyme 2 (ACE2), which is most abundant in type II alveolar cells of the lungs.The virus uses a special surface glycoprotein called a "spike" (peplomer) to connect to ACE2 and enter the host cell.[3]

COVID-19 can provisionally be diagnosed on the basis of symptoms and confirmed using reverse transcription polymerase chain reaction (RT-PCR) testing of infected secretions.Along with laboratory testing, chest CT scans may be helpful to diagnose COVID-19 in individuals with a high clinical suspicion of infection.Detection of prior infection is possible with serological tests, which detect antibodies produced by the body in response to infection.[4]

The standard method of testing for presence of SARS-CoV-2 is real-time reverse transcription polymerase chain reaction (rRT-PCR),which detects the presence of viral RNA fragments. As this test detects RNA but not infectious virus, its "ability to determine duration of infectivity of patients is limited." The test is typically done on respiratory samples obtained by a nasopharyngeal swab; however, a nasal swab or sputum sample may also be used.Results are generally available within a few hours to two days.[4]

A number of laboratories and companies have developed serological tests, which detect antigen and also antibodies produced by the body in response to infection.Chest CT scans may be helpful to diagnose COVID-19 in individuals with a high clinical suspicion of infection but are not recommended for routine screening.[5]

Preventive measures to reduce the chances of infection include staying at home, wearing a mask in public, avoiding crowded places, keeping distance from others, ventilating indoor spaces, washing hands with soap and water often and for at least 20 seconds, practicing good respiratory hygiene, and avoiding touching the eyes, nose, or mouth with unwashed hands.Those diagnosed with COVID-19 or who believe they may be infected are advised by the CDC to stay home except to get medical care, call ahead before visiting a healthcare provider, wear a face mask before entering the healthcare provider's office and when in any room or vehicle with another person, cover coughs and sneezes with a tissue, regularly wash hands with soap and water and avoid sharing personal household items.[6]

A COVID 19 vaccine is a vaccine intended to provide acquired immunity against SARS CoV 2. As of February 2021, 66 vaccine candidates are in clinical research, including 17 in Phase I trials, 23 in Phase I–II trials, 6 in Phase II trials, and 20 in Phase III trials.Trials for four other candidates were terminated.In Phase III trials, several COVID 19 vaccines demonstrate efficacy as high as 95% in preventing symptomatic COVID 19 infections. As of February 2021, ten vaccines are authorized by at least one national regulatory authority for public use: two RNA vaccines, four conventional inactivated vaccines, three viral vector vaccines, one peptide vaccine.[7]

Many countries have implemented phased distribution plans that prioritize those at highest risk of complications, such as the elderly, and those at high risk of exposure and transmission, such as healthcare workers.As of 5 February 2021, 123.54 million doses of COVID 19 vaccine have been administered worldwide based on official reports from national health agencies. Pfizer, Moderna, and AstraZeneca predicted a manufacturing capacity of 5.3 billion doses in 2021, which could be used to vaccinate about 3 billion people (as the vaccines require two doses for a protective effect against COVID 19). By December, more than 10 billion vaccine doses had been preordered by countries.[8]

Covid-19 situation in Bangladesh Armed Forces

COVID-19 in Bangladesh is a part of the worldwide pandemic of coronavirus disease 2019 (COVID-19) caused bySARS-CoV-2. The virus was confirmed to have spread to Bangladesh in March 2020. The first three known cases were reported on 8 March 2020 by the country's epidemiology institute, IEDCR.

Since then, the pandemic has spread day by day over the whole nation and the number of affected people has been increasing. First case of SARS-CoV-2 infected person amongst Bangladesh Armed Forces was detected on 8 April 2020. Since then up to 5 December 2020 a total of 142,606 personnel were tested for corona virus and 25,206 were found positive (17.67%). Exclusively at AFIP a number of 90,495 were tested and 15,595 were found positive. Out of this total 142,606 number of patients

90,495 are from Dhaka (63.45%). Among 25,206 positive cases, 15,595 (61.87%) positive cases were from Dhaka.[9]

During this time a total of 5,588 personnel from Bangladesh Armed Forces were deployed in different UN missions and as part of pre-deployment international flight regulation they were tested for SARS-CoV-2 and 260 were found positive (4.65%). Amongst this deployment 4,805 (85.95%) were from Bangladesh Army, 631 (11.29%) form Bangladesh Air Force and 152 (2.72%) deployment were from Bangladesh Navy.[9]

Infection rate was higher amongst UNPKO pre-deployment in Navy (6.57%), then Air Force (6.18%) and Bangladesh Army providing a large numbers of contingent members having relatively lower infection rate (4.39%). Among all this personnel 66.27% were male and 33.17% were female. Up to 5 December 2020, a total 7,834 (8.65%) SARS-CoV-2 positive patients were admitted into the hospital. Out of 7,834 cases 412 lost their life (5.25%).[9]

Xpert Xpress SARS-CoV-2 technique for Covid-19 diagnosis

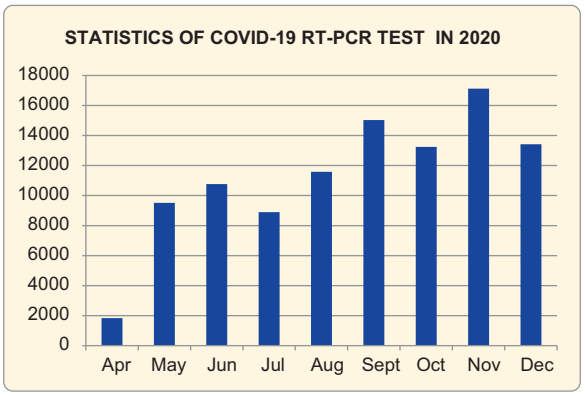

Fig.-1: *Covid-19 RT-PCR test summary at AFIP in 2020 (from April to December).*

The Xpert Xpress SARS-CoV-2 test is a rapid, real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 RNA in upper respiratory tract specimens, such asnasal swab or oropharyngeal swabs collected from individuals suspected of COVID-19.

The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of active infection with SARS-CoV-2; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. The agent detected may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.[10]

Negative results must be combined with clinical observations, patient history, and epidemiological information. Testing with the Xpert Xpress SARS-CoV-2 test is intended for use by trained operators who are proficient in performing tests using either GeneXpertDx, GeneXpert Infinity and/or GeneXpert Xpress systems. The XpertXpress SARS-CoV-2 test is only for use under the Food and Drug Administration's Emergency Use Authorization.[10]

The Xpert Xpress SARS-CoV-2 test is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-CoV-2 and is based on widely used nucleic acid amplification technology.[10]

The Xpert Xpress SARS-CoV-2 test contains primers and probes and internal controls used in RT-PCR for the in vitro qualitative detection of SARS-CoV-2 RNA in upper respiratory specimens.Principle of the Procedure The Xpert Xpress SARS-CoV-2 test is an automated in vitro diagnostic test for qualitative detection of nucleic acid from SARS-CoV-2. The Xpert Xpress SARS-CoV-2 test is performed on GeneXpert Instrument Systems. The GeneXpert Instrument Systems automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in simple or complex samples using real-time PCR assays.[11]

The systems consist of an instrument, computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable cartridges that hold the RT-PCR reagents and host the RT-PCR process. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, see the GeneXpertDx System Operator Manual or the GeneXpert Infinity System Operator Manual. The Xpert Xpress SARS-CoV-2 test includes reagents for the detection of RNA from SARS-CoV-2 in nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab and/or nasal wash/aspirate specimens.[11]

A Sample Processing Control (SPC) and a Probe Check

Control (PCC) are also included in the cartridge utilized by the GeneXpert instrument. The SPC is present to control for adequate processing of the sample and to monitor for the presence of potential inhibitor(s) in the RT-PCR reaction. The SPC also ensures that the RT-PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the RT-PCR reagents are functional. The PCC verifies reagent rehydration, PCR tube filling, and confirms that all reaction components are present in the cartridge including monitoring for probe integrity and dye stability.

The nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swab specimen and/or nasal wash/aspirate specimen is collected and placed into a viral transport tube containing 3 mL transport medium or 3 mL of saline. The specimen is briefly mixed by rapidly inverting the collection tube 5 times. Using the supplied transfer pipette, the sample is transferred to the sample chamber of the Xpert Xpress SARS-CoV-2 cartridge. The GeneXpert cartridge is loaded onto the GeneXpert Instrument System platform, which performs hands-off,automated sample processing, and real-time RT-PCR for detection of viral RNA.[11]

Role of GeneXpert for Covid-19 diagnosis in Bangladesh Armed Forces

AFIP has played a significant role to combat the Covid-19 pandemic in Bangladesh Armed Forces. To save millions of valuable life from this new disease it is most important to diagnose the infection as soon as possible and then isolation of the positive case to prevent further spread of the virus.

For diagnosis of Covid-19 two RT-PCR labs was established in AFIP within shortest possible time after the outbreaks of the virus. The first RT-PCR laboratory was established at Microbiology Department of AFIP on 22 March 2020. As the requirements of Covid-19 test was increasing tremendously, it was urgently needed to establish another laboratory at AFIP. For this necessity second Covid-19 laboratory was established at the Genetic Lab department of AFIP on 2April 2020.

Besides two RT-PCR laboratories, GeneXpert test facilities have been incorporated at AFIP from 7 July 2020. After installment of two GeneXpert Machines a new dimensions had been added for Covid-19 diagnostics. By using this method test results are being delivered within one hour time which has still been playing a magnificent role to overcome Covid-19 pandemic.

From the very beginning of the pandemics AFIP has been providing the regular diagnostic supports for Covid-19 among Armed Forces with its utmost capabilities. Pathologists of all departments of AFIP were involved in the Covid-19 laboratories to face the huge load of samples collected from suspected Covid-19 patients. All personnel of Covid-19 laboratories have still been giving their best possible efforts to combat Covid-19.

Everyday around 500 samples are tested in the Covid-19 laboratories of AFIP. It is worth mentioning that AFIP is capable of providing the Covid-19 test results within the day of receiving samples. This prompt delivery of test results contributed significantly to the management of Covid-19 patients and also for controlling the spread of SARS-CoV-2.

Sometimes it becomes very crucial to know the Covid-19 test results as early as possible for the management of various emergencies. Few practically experienced situations are as follows:

Scenario-1: A 72-year-old man died of the complications of Covid-19 after 4 weeks of all possible treatments at the ICU of CMH Dhaka. For accomplishment of all religious ritual activities before immediate burial of this patient, it was very urgent to know the present Covid-19 status of this patient.

Scenario-2: A 55-year-old retired army soldier presented to the emergency department of CMH Dhaka with the complaints of chest pain and sweating. After doing necessary investigations he was evaluated as a case of myocardial infarction and immediate resuscitative treatment was given accordingly. For further necessary management of this patient it was required to know the Covid-19 status of the patient on immediate basis.

Scenario-3: A 31-year-old female was evaluated as a case of ruptured ectopic pregnancy at the obstetric department of CMH Dhaka. Surgery was planned on urgent basis to manage this case. But before all arrangements it was necessary to know the Covid-19 status of this patient.

Scenario-4: A senior officer of Bangladesh Army was detailed by Bangladesh Government for attending an international conference abroad. On the day of his

departure for abroad, suddenly due to few unavoidable circumstances his flight had been cancelled. He was tested negative for Covid-19, but the 72-hours time limitation of airways authority ended by that time. To avail the next flight he required another Covid-19 test result on urgent basis.

The four urgent situations aforementioned are very usual phenomena in Covid-19 laboratories of AFIP. In all these situations we received samples with the request of urgent delivery of Covid-19 test results.

It is understandable that any delay of report delivery in such situations would result in tremendous sufferings of the persons. In RT-PCR method at least 5 hours required to complete a run. In RT-PCR methods Covid-19 tests are usually done batch wise. Each batch contains 36, 72 or 96 samples in accordance with the test slot used. So single sample is usually not tested in a specific PCR run, because it will not be economic in regard to time, expense and manpower. In case of urgent and immediate necessity it finds very difficult to provide Covid-19 test results by RT-PCR methods.

To perform the Covid-19 test results on immediate basis GeneXpert technique is used in the Covid-19 laboratories of AFIP as a very useful and effective method. This test is performed using the principle of Nucleic Acid Amplification Technique (NAAT). It takes only 1 hour to provide the result of the test after running 45 PCR cycles. It has some other advantages, like comparatively less requirements of skilled manpower and sophisticated laboratory maneuvers.

Table-I

Statistics of Covid-19 tests done by GeneXpert technique at AFIP

Tests	No of samples	Positive	Negative
Dead body	345	192	153
VIPs and VVIPs	134	38	96
Emergency cases (OT, Critical patients, Foreign mission etc.)	811	194	617
Total	1,290	424 (32.87%)	866 (67.13%)

Up to December 2020 a total 1290 GeneXpert tests have been performed which includes 811 various emergency cases, such as preoperative patients, critically ill patients and foreign mission candidates, 345 dead body samples and 134 VIP/VVIP samples. All of these samples were tested for COVID-19 on emergency basis to provide the test results as immediately as possible. Among 1290 samples 424 were positive (32.87%).

Conclusion:

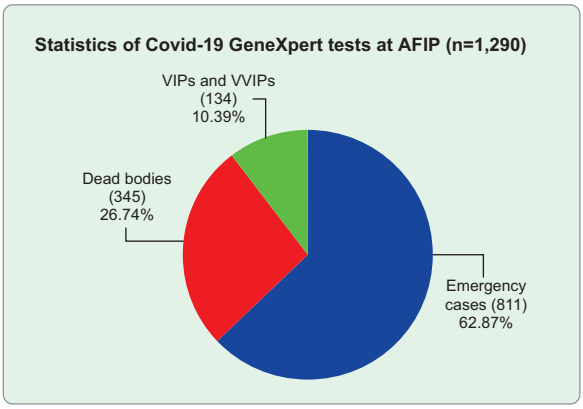

Fig.-2: *Distribution of all samples of Covid-19 GeneXpert tests at AFIP.*

Covid-19 pandemic is a great challenge for the world health care system. Considering the natural characteristics of this newly emerged SARS-CoV-2 virus it has been well understood that the early diagnosis, quarantine and immediate isolation of Covid-19 positive patients are the most important measures to prevent the spread of this virus. GeneXpert technique is undoubtedly a significant innovation for the earliest and one of the most effective diagnostic methods for Covid-19. In perspective of Bangladesh Armed Forces AFIP has still been playing an outstanding role for containing Covid-19 pandemic.

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