

# **Report on**

## **Sample collection and sample testing for SARS-Co-2 in Bangladesh: A descriptive qualitative study**

**Sponsored by:**

**Bangladesh Health watch**

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## ABBREVIATIONS

<b>Acronym</b>	<b>Full terminology</b>
ADG	Additional Director General
BCSIR	Bangladesh Center for Scientific & Industrial Research
BSL	Biosafety Level
BSMMU	Bangabandhu Sheikh Mujib Medical University
BRAC	Bangladesh Rural Advancement Committee
CDC	Center for Disease Control & Prevention
cDNA	Complementary Deoxyribonucleic Acid
CMSD	Central Medical Stores & Depots
Covid	Coronaviral Disease
CS	Civil Surgeon
DGHS	Director General of Health Services
DMCH	Dhaka Medical College Hospital
DNA	Deoxyribonucleic Acid
EPI	Expanded Program of Immunology
HEPA	High Efficiency Particulate Air
HMIS	Health Management Information System
ID	Identification
IEDCR	Institute of Epidemiology Disease Control & Research
IPH	Institute of Public Health
KII	Key Informant interview
LD	Line Director
MMC	Mymensingh Medical College
MNCAH	Maternal Neonatal Child & Adult Health

MT	Medical Technologist
NIPSOM	National Institute of Preventive & Social Medicine
PCR	Polymerase Chain Reaction
PPAE	Personal Protection Equipment
RNA	Ribonucleic Acid
rT	Real Time
RT	Reverse Transcriptase
SARS Cov	Severe Acute Respiratory Syndrome Corona Virus
SOP	Standard Operating Procedure
UHC	Upazila Health Complex
USA	United States of America
VTM	Viral Transport Medium
WHO	World Health Organization

## INTRODUCTION

Bangladesh Health Watch and James P Grant School of Public Health are sponsoring quick surveys on the different aspects of Covid19. It is imperative that a quick and small survey is also done on the procedures of collection of clinical samples from the suspected patients, and its problems. At the same time, it should be useful for future reference as to what are the problems faced by the personnel involved with sample collection and sample testing.

The samples in Bangladesh are collected from the nasopharynx and the oropharynx of a suspected patient or interested person by a medical technologist, following specific procedures, i.e. sample collection with a swab stick in a procedurally correct manner, store in a collection tube, preserve in an EPI cold box and transport to the PCR laboratory for testing within 72 hours.

The sample in the PCR laboratory is treated with several chemical solutions that remove substances, such as proteins and fats from the sample, extract only the RNA present in the sample and person's own DNA, and, if present, the coronavirus RNA.

Samples are tested through an r-T-PCR (real time polymerase chain reaction). Reverse transcription polymerase chain reaction (RT-PCR) is a laboratory technique which uses a reverse transcriptase to convert the viral mRNA into a single stranded DNA (called complementary DNA or cDNA). Next, DNA polymerase is used to convert the single-stranded cDNA into double-stranded DNA. These DNA molecules can now be used as templates for a PCR reaction. Additional short DNA fragments, as primers, are then added that are complementary to specific parts of the transcribed viral DNA. These fragments attach themselves to target sections of the viral DNA if the virus is present in a sample. Some of the added genetic fragments are for building and amplifying specific DNA strands using polymerase chain reaction, while the others are for adding marker labels to the strands, which produce a fluorescence, that signals the presence of the viral RNA.

The mixture is placed in a rT-PCR machine. The machine cycles through temperatures that heat and cool the mixture to trigger specific chemical reactions that create new, identical copies of the target sections of viral DNA. The cycle repeats over and over to continue copying the target sections of viral DNA. Each cycle doubles the previous amount: two copies become four, four copies become eight, and so on. A standard real time rT-PCR setup usually goes through 35 cycles, which means that by the end of the process, around 35 billion new copies of the sections of viral DNA are created from each strand of the virus present in the sample.

As new copies of the viral DNA sections are built, the marker labels attach to the DNA strands and release a fluorescent dye, which is measured by the machine's computer and presented in

real time on the screen. The computer tracks the amount of fluorescence in the sample after each cycle. When the amount goes over a certain level of fluorescence, this confirms that the virus is present. The number of cycles taken to reach this level estimates the severity of the infection: the fewer the cycles, the more severe the viral infection is.<sup>1</sup>

The real time rT-PCR technique is highly sensitive and specific and can deliver a reliable diagnosis as fast as in three hours. It continues to be the most accurate method available for detection of the coronavirus. Although the samples taken from the nasopharynx or the oropharynx, is 67% sensitive that taken from the gastric lavage is almost 99% sensitive. The Gene Xpert machines, used for detecting tuberculosis bacillus may also be used for detecting SARS-CoV-2 virus, which however, would require a different software and kit. It is said to be 100% sensitive and is available in at upazila levels. The usage of the machine is being scaled up now in the country.

Despite the fact that PCR is a very sensitive test, false negative in COVID-19 may be caused by:

- Presence of inhibitors in sample.
- Degraded samples\*
- Strain diversity reduction-
- Primer target homology
- Probe target homology
- Degraded reagent\*
- Malfunctioning equipment
- Aliquoting errors; sample or reagent
- Transcription errors during resulting and record keeping.

False positive results, on the other hand may occur due to:

- Contamination from prior sample testing by auto analyzers.
- Amplicons from previous amplifications of the same target.
- Aliquoting errors; sample or reagents.
- Transcription errors during resulting and record keeping.

## **OBJECTIVES OF THE SURVEY**

To find out the quality of sample collection and safety of rT-PCR testing for SARS-CoV-2

## **METHODOLOGY**

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<sup>1</sup> <https://www.iaea.org/newscenter/news/how-is-the-covid-19-virus-detected-using-real-time-rt-pcr>

### Sampling and sample size

The sampling was purposive. Sample size was additive and sequential, as per need. The sample size has been enumerated below.

### Survey instruments

- a. An observational checklist was given to the research associates on how the samples are taken from the test seekers, how these are recorded, stored and transported. The guideline was developed based on the standard protocols of CDC, USA and WHO;
- b. A site visit was made to the institutes which conduct PCR testing, to see the safety measures, as per the standard prescribed by WHO;
- c. A guideline was developed for key informant interviews, who were of varied types and background. These KII respondents were directly or indirectly involved with the sample collection and testing, including supervision, monitoring etc. They were interviewed by the Coordinator of the Survey through telephone;
- d. Validation was sought through cross interviews over phone with some other officers at district level who were related with sampling and testing services;
- e. Newspaper columns and television channels were studied for triangulation.

### Respondents of the KII survey

Since there was considerable time constraint, the following 28 representative numbers of officials from five divisions of the country were interviewed, on a representative basis (Dhaka, Mymensingh, Chattagram, Rajshahi, and Barishal), besides high officials from Dhaka.

1. Officials of DGHS, Line Directors and a Medical Officer: 5 (ADG, Administration, DGHS; LD, TB-Leprosy Control and AIDS & STD Program; LD, MNCAH; and LD, HMIS; and Medical Officer of TB-Leprosy Control Program);
2. Professional staff from IEDCR: 3 (Director, a Chief Scientific Officer, and an Asst. Professor);
3. Former Director of HMIS, DGHS: 1 (Coordinator of the sample collection process)
4. Civil Surgeons: 3 (Manikganj, Narayanganj and Mymensingh);
5. Upazila Health & Family Planning Officers: 3 (Chouddogram of Cumilla, Chatmohor of Pabna, and Bakerganj of Barishal);

6. Medical College and District Health Officials related with sampling and testing services: 3 (Junior Health Education Officer, Narayanganj, A physician of Mymensingh Medical College, Principal of Cumilla Medical College);
7. Laboratory technicians who collect samples: 2 (Narayanganj and Chatmohor, Pabna);
8. Supervisor of PCR testing process: 5 (IEDCR, NIPSOM, IPH); and
9. Others (retired health department officials): 3 (Former Senior Health Education Officer, Chattagram).

As stated above, 4 television channels (The Independent, DBC Television, Shomoy, Channel 24) and 4 newspapers were also studied (Prothom Alo, Bangladesh Protidin, The New Age, and occasionally the daily Star)

*Total observations made were 24 (8 from television channels and 16 from the field)*

#### Work plan

In five divisions, five research associates were deployed after thorough orientation on video and over phone. Each one observed at least two sample collection spots. Survey coordinator visited three PCR laboratories. Observation checklist was filled up by the research associates. They also took photographs and videos as a part of their observations, after seeking permission for conduction of the observation and for photography and videography. The findings were sent to the PI and survey coordinator through internet- e-mailing.

## FINDINGS

The findings reported below were based on qualitative data. One stream of the qualitative data, which was primary in nature, was collected through key informant interviews, following a guideline. The second stream of the qualitative data came from unstructured observations, i.e. without any predefined schedules or checklists. It was based on studying/ observation of some pre-enlisted newspapers and television news portals. The third stream was based on two pre-developed observational checklists for the sample collection and sample testing procedures.

The first and the second stream of qualitative data chronicled under a single sub-head, some events and experiences. This provided the readers interesting features of how the clinical samples were collected, what happened before and during sample collection, how samples were transported to the testing laboratories, what were the experiences at the testing laboratories. The third stream, which has been based on scheduled observational checklists, reported on the quality of the sample collection procedures and the safety measures adopted in the testing laboratories.

### 1. Key Informant Interview and unstructured observation based findings

In the beginning, i.e. in the month of March, the Institute of Epidemiology Disease Control & Research (IEDCR) collected samples first at IEDCR and then from the homes of the suspected patients, to avoid transmission of the virus among the people who were lining up for getting tests at IEDCR. Samples however, were being collected now only from homes of pregnant women and debilitated and elderly people. Others can also provide samples from homes. Since mid-July Taka 500 was levied for home collection, which has been reduced in August to Taka 300.<sup>2</sup> In upazilas from the beginning and even now samples are collected from homes, free of cost in the beginning and at Taka 300 now. For collecting samples from homes, first the people who wanted to get their samples tested have to call an assigned medical officer of the relevant health complex and inform about the clinical features. On ascertainment the medical officer sends two to four medical technologists to homes of the eligible.<sup>3</sup> In the beginning samples were taken from anyone who would like to get tested, then only from people suggestive of infection with Covid19 and their contacts and lastly, from patients who have four pathognomonic symptoms- fever, throat pain, cough and pain in the throat.<sup>4</sup> After a price was applied anyone can now get tested.

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<sup>2</sup> Source: respondents from IEDRC

<sup>3</sup> Enlisted Civil Surgeons and Health & Family Planning Officers

<sup>4</sup> Enlisted newspapers

Although the country reported the first cases of covid-19 on 8 March 2020, the tests for the same started in the last week of January. Up until the end of March IEDCR alone was testing for SARS-Cov-2.<sup>5</sup> IEDCR's decision to collect samples from homes throughout the country, as the sole testing organization, was not based on its capacity, while it put the staff unnecessarily to harm's way. This resulted in infection of its laboratory personnel and in fact the management officials also.<sup>2</sup> Its contemplation that if the suspected patients thronged IEDCR than that could spread the disease among the service seekers was not based on experience, since people were already crowding other places in the Dhaka City, without any physical distancing. It could advise the service seekers to wear surgical masks and gloves before coming to get tested. That could be a more practical approach to prevent infection.

Directorate General of Health Services had a plan to establish rT-PCR laboratories even at upazila level but by early, 37 out of 64 of the districts remained without any PCR laboratories; although by end-August the country got 93 PCR laboratories, about 54 of these were in Dhaka including those in the private sector. In July a decision was taken to use the Gene Xpert machines in the country, procured and used for the diagnosis of Tuberculosis for SARS-CoV-2, as both are nucleic acid based diagnostic apparatus. This would require a software and a kit for the latter, which USA, the only producer was not selling to any party. WHO supported a French manufacturer to produce the kit and ensured adequate supply now. The only disadvantage is, the Gene Xpert machines can test either 4 or 16 samples per batch. Only a dozen of the later type however, is available in Bangladesh. But the advantage of these machines is, each batch of samples takes about one hour to give result. It is claimed to be 100% sensitive and testing of each sample costs about Taka 2,000, instead of Taka 2,888 taken by the RT-PCR per test. Even if 4 samples are tested per batch, availability of adequate MTs may ensure testing of 64 samples between 7 AM 11 PM- 16 hours (in two sessions), i.e. all the samples collected on average per upazila per day may be tested in each upazila health complex the same day.

The public PCR laboratories in Dhaka, however, were not collecting samples for themselves<sup>6</sup>, as BRAC was collecting samples for the public PCR laboratories. Private hospitals were collecting samples in their own premises. The private sector PCR laboratories charge officially Taka 3,500 but to get tested the aspirants give Tk. 1,000 in addition, as fee for physical examination first by a physician. For home collection also an additional fee of Taka 1,000 is charged by the private sector. The recent drama on sample collection and fake test certificates provided from some private sector hospitals and health facilities was noted with alarm, e.g. JKG and the Regent

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<sup>5</sup> WHO Coronavirus Disease (COVID-19) Dashboard

<sup>6</sup> Coordinator of sample collection

Hospital. Staff of some of the sample collection booths also minted money by offering favor to collect samples and providing test results faster.<sup>7</sup> Some public sector sample collectors have been alleged to collect additional money from intending sample providers during home visits.

In each PCR machine, at a time, in 3 to 4 hours, i.e. in one batch, 94 samples may be tested besides one negative and one positive control. Usually two batches or two plates are run, in most laboratories per day, so in any one laboratory at least 188 samples get tested. Some laboratories even run three batches, round the clock, while some because of the dearth of medical technologists run only one session. In some laboratories, samples are wasted during handling and processing, as these laboratories report results of less than 94 samples or less than their multipliers. This perhaps results from the inefficiency of the MTs, who run the PCR machines.

The Prime Minister of Bangladesh advised the health department to collect at least 10 samples from each upazila, but in many of the upazilas the number of samples collected was even up to 60 and 70, collection based on cases suggestive of Covid-19 infection. To facilitate sample collection comfortably for the sample donors, in Kalai, Jaipurhat, samples were seen to be collected from fixed union level booths and through mobile vans. This innovative approach was adopted later in almost all upazilas.

Samples from upazilas were sent to the Civil Surgeon's (CS) office by an MT. The UHC ambulance was used to transport the samples. An administrative staff at CS office determined, based on the daily allocation, how many samples would be collected per day and where would these be sent for testing. But samples from upazilas were collected without any control on the number. These additional samples overwhelmed the system and these samples remained untested for quite sometime. These however, were preserved in -20°C until tested, while in some laboratories these were preserved at -70°C.<sup>8</sup>

Collection of sample since mid-April was more than the capacity of the laboratories to test, as a result in almost every laboratory there was a backlog. Test seekers came in flocks to get their samples tested, some waiting from before sunrise in BSMMU for example during Ramadan. But many of them were refused when the daily quota for testing were full, so many returned home dejected without getting test after waiting for long.<sup>9</sup>

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<sup>7</sup> Enlisted Medical Technologists, TV channels and newspapers

<sup>8</sup> Enlisted Civil Surgeons, Upazila Health & Family Planning Officers, relevant officers of Civil Surgeon Office and officials of the testing institutes

<sup>9</sup> The enlisted newspaper

Poor control on the number of samples to be collected were the main reason of the sample backlog. The original plan to test only suggestive cases gave in and samples were collected from any and every medical care providers, their relatives and friends and family members of influential people including their drivers and maids. This in fact caused not only backlog but unnecessary costs and inefficiency in the testing activities.<sup>10</sup>

In the month of May also more samples were collected than the testing capacity. As a result a bigger backlog started to develop, which became more acute with time. Since samples were getting piled up, in mid-May, a new operational definition of ‘cured’, was developed. This was based on: recovery from fever; no medication was needed; and decrease of respiratory symptoms remarkably. A test for the virus before release from hospital was prescribed, but this was not mandatory for those who showed mild symptoms for three days. The released patients were advised to be in isolation at home or elsewhere for 14 days and then undergo for test. This reduced the mandatory testing of these ‘cured’ patients<sup>11</sup> and reduced the number of testing, relieving the backlog to some degree.

Since 3 May 2020, the responsibility of coordination of testing was given to the Additional Director General (ADG) of Health Service, who is in-charge of administration. The contribution of another ADG, a microbiologist, in the Covid-19 prevention and control did not come into the limelight. To help ADG, and to better coordinate and ensure efficiency in sample collection, DGHS recruited a retired Director of MIS, who was looking after sample collection in the field. On 5 May 2020, after two months of practice of collecting samples from people’s home, decision was taken to open 600 sample collection booths throughout the country in phases in health facilities. These booths were being mostly managed by BRAC, and funded by DFID; staff was recruited by BRAC, and trained by the IEDCR officials.<sup>12</sup> The number of these booths however, stood at about 100 even in September.

In the month of June, the huge backlog of the samples and testing, resulted in a delay by about one to two weeks. To control backlog of testing and facilitate better efficiency, government again decided that samples will be taken from those who have all of the following clinical features only: cough, fever, pain in the throat and difficulty in breathing, since the second week of July, as mentioned earlier.

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<sup>10</sup> Enlisted respondents from testing institutes

<sup>11</sup> Enlisted respondent from DGHS and newspapers

<sup>12</sup> Coordinator of sample collection and the enlisted newspapers

It has been opined that, as a result of charging fees and restricting testability, the number of sample collection fell to 15 per booth, instead of 30 or 40. Of the 15, 10 were to be registered online and 5 through onsite visit. Online registration however, proved to be too cumbersome for many. Restriction in onsite registration to 5, compelled many to return heart broken. This approach reduced the total number of tests done on a daily basis since the second week of July, while the test positivity shot to more than 24%, since more serious patients now provided samples and those who were getting tests, just because it is free, had fallen.<sup>13</sup>

Large number of infection with SARS-CoV-2 among medical technologists (MTs), affected the pace and amount of sample collection in many locations. At one stage sample collection had to be halted almost completely in Barishal and Mymensingh for example and samples from Narayanganj and Cumilla had to be sent to Dhaka for testing. More than 200 MTs were infected in the country by mid-June. This strained the availability of MTs, posts of many of who were already vacant, as recruitment of MTs was on hold in the last 10/12 years due to some legal entangle. In many places, e.g. Rajshahi, samples were collected by untrained Health Assistants. This might have compromised sample quality ending in inaccurate result in the month of May and June. This was one cause of collection of fewer samples. Also, because of dearth of MTs in testing laboratories test results and reporting were delayed.<sup>14</sup> Some 2,000 of the MTs were recruited however, in early May 2020 and declaration was made that another 3,000 will be recruited to cope with the Covid19 situation, but which did not materialize.

Dhaka University PCR laboratory was closed and for sometime the laboratories at the Live Stock Institute in Savar and laboratories in Chattagram, Noakhali. Sirajganj had to halt their testing because of contamination of the laboratories, shortage of kits and infection of testing personnel.<sup>13</sup>

IEDCR, as the central reference laboratory for surveillance provided virtual training to the medical technologist through internet videos. IEDCR however, as the only national reference laboratory for communicable diseases in the country, was not conducting any sort of monitoring of the quality of sample collection and sample testing. Its training quality has been questioned by the trainees themselves because of short period of training and also because of too much reliance on remotely conducted video based training.<sup>15</sup>

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<sup>13</sup> Enlisted newspapers.

<sup>14</sup> Research Officers of SSMF

<sup>15</sup> Enlisted Medical Technologists and IEDCR respondents

Notwithstanding the fact that more samples were collected than the capacity to test those, the number of tests done was far below the standard prescribed by WHO- testing of 10 to 30 samples against one infection. In Bangladesh it is about 5 to 6. The main reasons were shortage of rT-PCR machines, transportation media, sample collecting kits and reagents and trained personnel to collect and test samples.

The recording of the tested people was not smooth from the beginning. The details of the address of the tested people were not recorded meticulously. Many people did not give their address correctly to avoid harassment and stigma. Some were tested twice or thrice but each time separately. This gave a higher number of negative results in the beginning. Some mix-up of addresses of the tested individuals even transcended district boundaries. In some cases people were asked to provide their sample again, whereas they in fact never gave any sample earlier.<sup>16</sup>

The sample collecting swab has to be made of plastic handle and synthetic wools/ cotton tip, other-wise the sample will interfere with the testing of the sample in the PCR machine. For sometimes after the initial period, samples were collected even with a swab that had cotton tip and wooden or broomstick handle. Some sample collection tubes were supplied with swab stick inside and with normal saline as transportation media; some tubes were without any swab sticks and without any media, which the laboratory technicians had to prepare from cotton buds and whatever stick were available, while using normal saline from the laboratory as the transportation media. Some swab sticks were covered in some kind of foil, supplied separately with empty tubes; and some swabs supplied without any cover.<sup>16</sup>

The test tube should hold the sample in universal transportation media, but after sometime these test tubes ran out of stock and test tubes of different shapes and sizes were procured from local markets and normal saline from the local health department laboratories were used for sample collection.<sup>17</sup> In Chattagram samples were collected even in polythine bag with normal saline.<sup>18</sup> How much it affected the sensitivity or specificity of the test result is not known. A lower test positivity in April, May and early June could be because of these poor practices. At present screw capped tubes are used with swab sticks supplied separately, which are longer than the tube, so only the synthetic cotton tip is left into the sample collection tube with transport media or normal

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<sup>16</sup> Research Officers of SSMF

<sup>17</sup> Enlisted staff involved with sample distribution

<sup>18</sup> Research Officer of SSMF

saline and the swab stick is disposed off.<sup>19</sup> In the beginning when swab sticks were made from wood and broomstick, these were longer than the test tube, so insertion of these swab sticks without fitting the length of the test tube used to prevent closing of the lids snugly, spilling the media with sample, which created opportunities of infection to those who handled the samples subsequently for testing in PCR machine.<sup>20</sup>

In the beginning, IEDCR made the distribution list of the test kits, collected these from CMSD and sent these to the nearby districts. The others collect the kits from the Central Medical Stores & Depots (CMSD) directly. CMSD also procured sample collection tube, swab and transport media. Three to four days kits were kept in reserve at district level. Sample collection tubes with transportation media were procured by CMSD from a local national manufacturer (JMI). Line Director, MNCAH was given the responsibility of distributing sample collecting vacutainer, with swab stick in early April. In the beginning the sample collection tubes used to be procured at Taka 500 a piece from China. Now BCSIR produces WHO standard viral transport media (VTM) supplied in screw capped tube with swab stick and tip (the whole set) in zip lock bag at Taka 160 a set. CMSD is still the procuring agency.<sup>19</sup> Sample collection sets and the testing kits are sent to the divisional levels from CMSD. Civil Surgeon's office collects those from the divisional offices and upazila health authorities and the testing laboratories collect the same from the district office.

About 5-10 percent samples were found to be useless for testing, which was because of the inappropriate way of collecting the samples due to poor training of the technicians, poor quality of the sample collection tools, e.g. transport media, swab stick, spillage of samples from the collection tubes etc. Sometimes suspected patients were given the swab to introduce it in their own nostrils themselves and then put it in the collection tube.<sup>21</sup>

Some of the samples, saved in the cold box of EPI, could not be transported from upazilas to Dhaka within 72 hours in the beginning, and for sometimes thereafter when the peripheral PCR laboratories, because of the burden of too many samples or shortage of laboratory technicians, were sent to Dhaka. Transportation to Dhaka based laboratories also was necessary at times as the local testing laboratories were giving wrong results, because of the poor quality of the testing kits. Sometimes stocking of the samples and waiting to send to Dhaka en masse took more than

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<sup>19</sup> Enlisted officer assigned with the responsibility of distribution of sample collecting tubes (LD, MNCAH)

<sup>20</sup> Enlisted Civil Surgeon

<sup>21</sup> Enlisted respondents from IEDCR and Civil Surgeons

72 hours. Because of these problems, some sample donors were requested to give their samples again. This delay reduced test sensitivity, giving false negative results.<sup>22</sup>

Samples taken from nasopharynx or oropharynx are sensitive in less than 70% cases, i.e. about one third giving false negative results. This is the reason that some people tested positive and negative on the same day, while some got positive result after several negative tests, which however, should also be interpreted as test positivity due to dead virus.<sup>23</sup>

MTs (laboratory) were trained periodically by IEDCR on the different steps and processes of collection of samples and for testing pathological samples, but not for SARS Cov-2 per se. But in the emergency situation, created by the Covid19, MT (EPI) have also been pressed into sample collection. EPI technologists were not trained on sample collection processes earlier. In the prevailing situation they were trained online by IEDCR for approximately two hours. As stated above, this training was not adequate. At times samples collected were actually sputum, not taken from the nasopharynx or oropharynx.<sup>24</sup> Sputum samples may have reduced, in the beginning, the test positivity rate.

The former director of HMIS, DGHS who was given the responsibility of coordinating the sampling activities, informed that he requested the outdoor medical officers in medical facilities to see the suspected patients and issue certain number of sampling tickets, according to the capacity of the relevant laboratory. This did not materialize. This incidentally also showed the weakness in management of the sample collection by number and transportation.

The Chairman of the 19 member Technical Committee, formed by the Ministry of Health & Family Welfare, advised in the beginning that testing capacity should be increased to 10,000 per day. In fact, unknown to him, the country had capacity of conducting 10,000 samples at that time. The Chairman, later urged that the testing capacity should be increased to about 20,000 per day.<sup>25</sup> But these numbers of testing of ten or twenty thousand was a suggestion, as these numbers only would provide a trend but not the expanse of infection and the numbers were arbitrary.

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<sup>22</sup> Enlisted Civil Surgeons and Research Officers of SSMF

<sup>23</sup> Moonmoon Shormin and, Md. Abdullah Yusuf. Laboratory Detection of Covid19 Cases: A Systematic Review. Bangladesh Journal of Infectious Diseases April 2020, Volume 7, Number Suppl\_1, Page S11-S17

<sup>24</sup> Research Officers of SSMF

<sup>25</sup> The enlisted newspapers

The Directorate General of Health Services probably could not fathom the volume of work that they will have to manage in terms of sample collection and testing, the paraphernalia to be required and the human resources readiness. It took authority and the higher level decision makers about one month to understand that the testing facilities should be decentralized. As a result, the second most important hotspot, Narayanganj had to wait for a PCR machine until end-May, but which had to bring in medical technologists to conduct the tests from Sonargaon and Rupganj upazila health complexes, as the hospital itself had no such technologists.<sup>26</sup>

IEDCR's mandate is surveillance, outbreak investigation and capacity building for surveillance at peripheral units, including training. It should not have been entrusted even with training of physicians on how to treat patients. Stretching of IEDCR's capacity compromised with its own safety measures- the head of the institution and the focal point of the Covid19 testing along with others, related to testing were infected and had to spend 14 days in quarantine, as stated above. The laboratory itself remained non-functional a few days to mop up the infection.

Non-availability of data for future reference and epidemic management is a loss. This sprang from the non-conduction of random samples to find out what percentage of people suffering from asymptomatic, mild and moderate degree of clinical features are in fact already infected and what percentage of these hitherto non-tested people have already spread the disease and to how many. According to a Daily Star news item, referencing the Deputy Civil Surgeon, in Hobiganj, of the 52 people tested for Covid19 in Habiganj, 51 were without any symptoms.

Some people die daily with clinical features, but are not tested, while many are certified as dead with clinical features suggestive of Covid19, as they could not be tested. In Chattagram for example, the actual number of covid-19 could be many times more than were identified through tests and it was believed that five times more people died from covid-19 than the death among the people identified through the test.

A Chinese delegation which came to Bangladesh and looked into what Bangladesh had been doing with regard to Covid-19 gave some sample testing related suggestions, e.g. increasing of sample testing capacity and improvement of testing quality. They also suggested against sample collection from homes, increase training on biosafety, ensure awareness and safety gears for supportive staff, standardization of the testing laboratories, ensuring availability of testing

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<sup>26</sup> Enlisted respondents from Narayanganj

facility in institutes where medical education is given, improving test quality.<sup>27</sup> Many of these recommendations were not implemented even long after the team left Bangladesh.

### Experience in districts

By 30 April, two physicians and three support staff from the Mymensingh Medical College (MMC) PCR laboratory got infected. The reason could be non-use or improper use of PPEs or non-supply of effective masks. Hiding of Covid19 status by some patients made health care providers casual in approaching them.<sup>28</sup>

In contrast to the number of samples instructed to be collected from upazilas, the Civil Surgeon of Mymensingh had to amass, on average, about 70 to 80 samples from each of the 13 upazilas. In Mymensingh only the MMC laboratory tested samples at the time of this reporting. When samples also included suspected contacts to Covid19 patients, collection went up to even 100 to 110 per day from each upazila. So sample testing and reporting could not cope up with the collection. In 13 days of May, 1,054 samples were collected from Mymensingh upazilas, report came for 731, including some, which had to be sent to ICDDR,B. MMC had capacity in the beginning to test 94 samples in each shift and the laboratory was running two shifts. Effort was being taken to initiate three shifts, i.e. test 282 samples per 24 hours. MMC received two more PCR machine later and it started testing 396 tests per 24 hours. MMC was supposed to test samples from four districts in the division, but sometimes samples were also submitted from Kishorganj and Tangail districts, which overwhelm the test capacity of MMC.<sup>29</sup>

In the beginning Dhaka Medical College Hospital (DMCH) had a backlog of testing, as it was testing 188 samples in two shifts with suggestive clinical features from the emergency and outdoors with only one PCR machine. Another machine was supplied to DMCH but it could not be used because of its poor quality. From 5 May, the authority decided to collect samples only from the Covid19 section of the hospital. Also, there was shortage of medical technologists in DMCH, while the hospital required 20 technologists, it had only six, who were conducting other types of tests as well.<sup>30</sup>

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<sup>27</sup> Enlisted newspapers.

<sup>28</sup> Enlisted Asst. Registrar of Medicine, MMC and Civil Surgeon of Mymensingh

<sup>29</sup> Civil Surgeon Mymensingh

<sup>30</sup> Assistant Professor of Virology, Dhaka Medical College

DMCH by 12 May, experienced 91 deaths in its Covid-19 unit, many of who were not tested, because of the limitation of the capacity in the virology department of the medical college. So these deaths were not accounted for among the dead.<sup>31</sup>

In Dhaka city, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College (DMC) and Mugda General Hospital had a combined capacity to test 700 samples by mid-May (with one PCR machine each in Dhaka and Mugda Medical Colleges and two in BSMMU). But the test seekers everyday ran to several thousands. So the management in these testing facilities started issuing tickets to the queuing through guards, according to capacity. It has been alleged that the guards issued fewer tickets. Also, nobody were informing the queuing test seekers how many should wait for getting testing and who should not wait. This made lots of people to wait even for eight hours and leave without getting the testing service. In many places refused test seekers were seen agitating. As mentioned earlier, to ease this situation, Government allowed BRAC to open 40 sample collection booths in the Dhaka city and collect 40 samples in each booth and JKG as well in Dhaka, Naryanganj, Narsingdi and Gazipur (which became awry-that is another story, which now is well known).<sup>32</sup>

From end May, 2020 sample collection from NGO booths in Dhaka city, was much more than the testing capacity in the designated laboratories. Sample collection was done in uncoordinated manner. Due to collection of more samples than the number that could be tested, backlog started to pile up and in end-May and June in Dhaka also. This picture got common throughout the country further up to mid-July.<sup>31</sup>

In Narayanganj, the Civil Surgeon (CS) had to buy test tubes of various types from the market, since the supply was not adequate in March. Swab sticks came separately. Of the four medical technologists, who were collecting samples through home visits, two got Covid19 infected. Local private diagnostic centers and hospitals lent five medical technologists to the CS. MT-Lab and MT-EPI used to collect the empty sample collection tubes from the CS office, with identity number and name of the person who the samples will be collected from. The sample collectors travelled by ambulances wearing PPEs. CS office also received samples from the upazila health complexes (UHCs), brought by the MT-Lab of the UHC in EPI cold box, travelling by the UHC ambulance. The Store keeper of CS office collected those samples, entered ID, name and address of the sample donors into register and stored those overnight. The next day these were sent to the diagnostic laboratories- in the beginning to IEDCR and IPH and then to the PCR laboratory in

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<sup>31</sup> Enlisted newspapers

<sup>32</sup> Enlisted newspapers and Research Officers, SSMF

the 300 bed hospital in Naryanganj. Samples were also sent the Rupganj Private Hospital alias US-Bangla Hospital, which commissioned a PCR laboratory. Earlier, the Narayanganj RAB and the police used to send samples regularly to the CS office, but later they started sending their samples to the Rupganj upazila based private US-Bangla Hospital. The overnight storage of samples was necessary since the samples were received in the afternoon. But after the availability of the testing laboratory in the Narayanganj 300 bed hospital itself, the samples could be sent to the laboratory on the same day, but the old practice still prevailed, till the last information collected from Narayanganj in June.<sup>33</sup>

The 300 bed Naryanganj hospital did not have any MT conversant with the PCR machine. The rT-PCR machine operating MTs were brought from Rupganj and Sonargaon upazila health complexes.<sup>34</sup> This however, might have reduced the efficiency of the two upazila health complex laboratories of the district.

Private source MTs were quite interested to collect samples from homes. It was later known that they were making extra bucks through home visits and they wanted to share their proceeds with the public sector MTs. As the CS came to know this he stopped home visits by these MTs.<sup>35</sup>

JKG, booths had exhibited non-conducive environment from the beginning. People used to come and queue up without any physical distancing for giving samples. The samples used to be sent to IEDCR, about which the CS had no information of. The results of testing of these samples were not conveyed in two weeks. This made the testing meaningless, as when a positive result does not come in time, an infected person is not quarantined, so s/he gets lots of time to infect others.<sup>33, 34,36</sup>

After IEDCR was declared the national reference laboratory for Covid-19 and it stopped testing of samples, CS of Narayanganj started sending the same to the Institute of Public Health (two days per week) and to the Rupganj private hospital (two days a week). Although the 300 bed Narayanganj hospital was given a PCR machine, it was not commissioned even in one week time. After commissioning, the 300 bed hospital with one PCR machine, on some days, tested only 50 samples, while sample collection was too many. For example, JKG collected 380

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<sup>33</sup> One of the officers of Narayanganj Civil Surgeon Office, responsible for managing sample collection

<sup>34</sup> MTs of the 300 bed Narayanganj Hospital

<sup>35</sup> Research Officers of SSMF

<sup>36</sup> Enlisted newspapers

samples alone on 11 May. On the same day, the samples collected by the CS office staff could have run to 250 from five of its upazilas. But as stated earlier, a PCR machine could test a maximum of 188 samples in two shifts per day, if everything else went fine.<sup>37</sup>

Among the four rT-PCR testing units in Chattagram, every sample in the Chattagram University tested positive in May. Testing had to be stopped therefore in the laboratory. The capacity of testing SARS-CoV-2 in Chattagram fell and samples had to be sent to the National Institute of Laboratory Medicine at Dhaka. Bangladesh Institute of Tropical and Infectious Diseases (BITID) laboratory chief got infected in mid-May 2020, so the laboratory had to reduce the number of tests for about two weeks. The laboratory had three PCR machines but working only for one shift each, testing therefore 282 samples per day, whereas collection of sample ran to 1,000 per day. The laboratory, if ran in two shifts as in many other laboratories, could test 564 samples.<sup>37, 38</sup>

Cumilla on 25 May 2020, collected 495 samples from the whole district, whereas it had only one PCR machine at the Cumilla Medical College Hospital. Some over the capacity samples collected therefore had to be sent to IEDCR, overwhelming the capacity of the latter as well. As may be surmised, although IEDCR stopped its own sample collection and testing, it had to test samples sent from other sample selection units.<sup>39</sup>

#### Reporting issues

Most testing laboratories did not report their test results to the test seekers directly. Test results were sent from the laboratories to the central coordinating unit of DGHS on the same day of testing, these were available to the test seekers in about three days. Sometimes the IDs, names of sample donors and the sample location got mixed up in the beginning, as stated already. As a result, the location of the tested people could not be ascertained.<sup>40</sup>

#### Effect of definitional changes on the number of sample collection

In the first week of May a change was brought in the definition of ‘cured from covid-19’. Earlier the definition was, hospitalization for 14 days, no clinical features for three days and two negative result on the samples collected 14 days apart and tested. The modified definition was based on no clinical features for three days after seven days of the first appearance of the clinical features, sending the patient on home isolation for 14 days and two negative tests taken 24 hours

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<sup>37</sup> Research Officers of SSMF

<sup>38</sup> Enlisted newspapers

<sup>39</sup> Principal Cumilla Medical College

<sup>40</sup> Survey Coordinator

apart from home and tested. In mid-June the necessity of two negative tests for declaring a patient 'cured' was waived, as a result the number of sample collection and tests fell, as stated above already. In the second week of July, sample collection from contacts and those not suffering from cough, fever, throat pain and breathing difficulty ceased, which caused another fall in the number of sample collection, tests and the number of Covid-19 patients, but increasing the test positivity rate. As of now, anyone paying for tests is tested, besides the eligible.

## 2. Observational checklist based findings

### Quality of sample collection procedures

The following data was collected by the SSMF Research Associates and the survey Coordinator. In total 24 observations were made; 16 were made from 7 sample collection locations and 8 from television news portals, in light of the standard guidelines for sample collection for Covid19.<sup>41</sup>

1. In April and the first week of May, the swab fiber used to be made of cotton in at least 40% of cases, as there was shortage of supply of the appropriate swab sticks. This did not fulfill the required condition for accurate diagnosis of SARS-CoV-2, which therefore might have given a false negative result;
2. In April and the first week of May sometimes the swab stick used to be made of broom stick, wood or even hair clip (in 45% cases). This might have interfered with the accuracy of diagnosis of SARS-CoV-2. These sticks used to be bigger than the sample collection tubes, so the sticks had to be broken to insert into the tube and close the tube cap snugly, which in about 10% cases was not fitted well into the tubes with samples, causing spillage of the sample even before the samples reached the diagnostic laboratories;
3. The swab stick was grabbed by the sample collectors by the distal end of the handle without fail, as per the standard in 100% cases;
4. If samples from both Nasopharyngeal and Oropharyngeal were collected, which occurred in about 15% cases, both the swabs were put in a single tube in 100% cases. But mostly sample was taken only from one nostril (in 80% cases). In about 10% cases saliva was taken as sample, which was also a correct way of sampling;
5. For nasopharyngeal swab the minitip was supposed to be entered through the nostril parallel to the palate, until resistance was encountered or the distance was equivalent to that from the ear to the nostril of the patient (training given mentioned entrance of one third of the swab stick, which was a wrong instruction, as the length of swab sticks varied at different times). But while the swab stick was entered until it met resistance as per the standard, the minitip was not entered parallel to the palate in 80% of the cases. This might have had a higher false negative result;
6. The swab stick was supposed to be gently rubbed and rolled constantly while it was being entered; but in 40% cases it was not rolled along the entire length of the Nasopharyng. It was not rolled constantly when it was being withdrawn from the nostril either in 90% of cases. This might have hampered with the accuracy of diagnosis of SARS-CoV-2;

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<sup>41</sup> CDC. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19). <https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html>

7. Swab was supposed to be left in place for several seconds into the Nasopharynx, to absorb secretions, after pushing it the entire length of the nasal turbinate, but it was not practiced in 90% of the cases. This might have given a higher false negative result;
8. Patient's head was supposed to be tilted back 70 degrees but in 65% cases the head was not tilted to 70 degree angle. This might have given a higher false negative;
9. Training on sample collection was given for two hours only through online videos and 100% of the trained said the timing and the process of training were not adequate;
10. The swab was placed immediately into a sterile transport tube containing 2-3mL of either viral transport medium (VTM) or sterile saline for transportation in 80% of cases; but in the months of April and beginning of May saline was used from laboratory of upazila health complexes in almost 70% cases, so its sterility could be doubted and that could have increased false negative result;
11. Specimen was stored at 2-8°C for up to 72 hours after collection in the EPI cold box, which maintained a temperature of 2-8°C for 72 hours. In about 15% cases however, the limit of 72 hours crossed;
12. Each specimen container was labeled with the patient's name, ID number (e.g., medical record number), address and the date the sample was collected but addresses were missing or mixed up in 35% cases till mid-May;
13. Patient specimens from suspected or confirmed cases should have been transported under the banner of UN3373, "Biological. Substance, Category B", as per the WHO standards, which however, was not in practice at all;
14. PPEs were used meticulously by the sample collecting and transporting staff in 80% cases but wearing of the face shield and the shoe coverings were seen to be in practice only in 20% cases;
15. People waiting to give samples or people who accompanied the sample donors did not maintain physical distance at all and 40% of them also did not use any mask or did not fit their mask snugly or appropriately;
16. In one instance a patient was brought from the indoor to the outdoor where a sample collection booth was functioning. The sample was collected from the patient lying down on the bed, while the sample collector was himself standing in the sample collecting booth at a higher level than the patient. These postures and positions did not help in collecting sample as per the standard;
17. The location, date, identity and the ID number of the sample was entered into a register and also onto the sample tube after collecting the sample, in 100% cases in sample collection booths, which could have compromised the safety of the recorder. After collection of a sample the sample collector's hands covered with gloves were washed with sanitizer, but the same was not done by the other staff, who also held the samples;

18. Each sample collection took about 30 to 40 seconds in 100% cases, which was the standard time required for collecting a sample.

### Quality of safety measures in PCR laboratory

The following description was based on visit by the Coordinator of the survey to three PCR laboratories, in light of the laboratory biosafety guidelines, suggested by WHO.<sup>42</sup>

1. Site-specific risk assessment, a standard process to identify and mitigate risks in PCR laboratories, was not practiced in any of the visited laboratories;
2. The disinfectant(s) used in the laboratory for disinfecting surfaces and others were:
  - 1000 ppm sodium hypochlorite (bleach) for general surface disinfection;
  - 10000 ppm sodium hypochlorite (bleach) for disinfection of blood spills;
  - 62-71% ethanol;
  - 0.5% hydrogen peroxide (used in some laboratory but not in all);
  - Disinfectants were used for 15 to 30 minutes (better if left for two hours);
  - Gloves are changed after adding patient specimens to the instrument correctly;
3. None of the laboratories had controlled ventilation system for inward directional airflow into the laboratory room;
4. In two of the three laboratories exhaust air from the laboratory room was re-circulated to other areas within the building without any processing;
5. Exhaust air from all the three laboratory rooms was thrown out with air conditioning exhaust only;
6. No laboratory had a high efficiency particulate arrestance (HEPA) filter in functioning form;
7. All three laboratories had BSL2 level safety for handling the samples and for conducting PCR test;
8. Other resources available/ not available were:
  - All the laboratories need -70°C refrigerator;
  - Hand hygiene and the use of personal protective equipment (PPE) were practiced meticulously in all the three laboratories;
  - The availability of automated instruments and analyzers:
    - ❖ Automated sample processor and analyzer were not adequate;

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<sup>42</sup> WHO. Laboratory biosafety guidance related to the novel coronavirus (2019-nCoV) Interim guidance 12 February 2020. [https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847\\_2](https://www.who.int/docs/default-source/coronaviruse/laboratory-biosafety-novel-coronavirus-version-1-1.pdf?sfvrsn=912a9847_2)

- ❖ Final packaging of specimens for transportation to diagnostic laboratories for additional testing was done in a sealed, decontaminated primary container in all the three laboratories;
  - ❖ None of the laboratories had any system for using inactivated specimens, such as for nucleic acid extraction;
9. Staff in the laboratory were not trained adequately yet on: (i) proper use of the instrument, (ii) ways to minimize the risk of exposures, and (iii) decontamination/ disinfection
  10. Other laboratory based practices and incidences were as follows:
    - Donning and doffing spaces for PPE were available inside all the three laboratories but in separate places;
    - Contaminated specimens were disposed off in hypochlorite solution in one laboratory, while in other two, the decontaminated materials were put in biohazard bag without treatment with hypochlorite;
    - Sharp materials were disposed off in hypochlorite solution in one laboratory while in other two these were put in red colored bins without treating with hypochlorite;
    - Eye splash during sample processing occurred a few times in one laboratory,, while in another it never happened yet, The third laboratory officials did not respond on it;
    - Samples were received in leaking condition occasionally by all the three laboratories;
    - Samples leaked occasionally during loading and unloading of sealed centrifuge cups in all the three laboratories;
    - Leaking occurred occasionally during grinding, blending, vigorous shaking or mixing, or sonic disruption in one laboratory, two other laboratories did not mention this;
    - Opening of containers with high internal pressure and splashing did not occur in any laboratory;
    - Food or drink storage in all the three laboratories was strictly prohibited
    - Personal items such as coats and bags are kept inside some of the laboratories
  11. Eating, drinking, smoking, applying cosmetics were strictly prohibited inside laboratory;
  12. All three laboratories had warm running water and soap;
  13. Staff washed their hands with soap after entering the laboratory and before leaving the laboratory. In one laboratory frequent hand washing is practiced in between;
  14. In one laboratory, a cut or broken skin was covered within 2 minutes (a standard practice) and in the other two laboratories there was no such experience yet;
  15. Mobile electronic devices (for example, mobile telephones, tablets, laptops, flash drives, memory sticks, cameras and/or other portable devices) were used in all the three laboratories when necessary, which was not in accordance to the safety guideline;

16. An SOP for emergency scenarios was available in one laboratory, while in one this will be developed. The other laboratory staff did not mention this;
17. All the three laboratory has adequate first aid box;
18. A system for written record of accidents and incidents existed in one laboratory. It was not available in the other two laboratories, where it will be developed;
19. An SOP for investigating and reporting accidents in one laboratory. In the other two this will be developed;
20. A written procedure for cleaning and decontaminating spills existed in one laboratory, while other two will develop the same;
21. Covid-19 tests for laboratory staff was done 14 days in all the three laboratories.

## RECOMMENDATIONS

### Recommendations pertaining to sample collection

1. A standing standard of logistics should be made, with alternatives, e.g. if a standard transportation medium is not available then a laboratory grade normal saline could be used;
2. DGHS should have a standing criteria based on the recent experience on from who, when, how and wherefrom samples would be collected. These should not be changed in the middle of an epidemic, since the interpretation to the test results and estimates obtained might then give confusing and even wrong messages;
3. The responsibility of need assessment for logistics and procurement; process of procurement from multiple sources of procurement and modes of distribution and monitoring of the logistics at different tiers need be identified by DGHS as a standing procedure, which should come into play automatically as and when situation arises. Standing standards of sample collection paraphernalia should be ready and adhered to. No delay or failure in procuring those as per the agreed standards and amount should occur;
4. A standing step-by-step sample collection, storing, and transportation procedure should be developed for the various types of samples by IEDCR. Officials should be identified and notified as supervisors and monitors of these procedures and on the spot deciders on the bulk of samples to be collected;
5. IEDCR's role should be to lead, coordinate, supervise, monitor and develop capacity and ensure quality for sample collection and sample testing at all relevant levels in the country;
6. Health education sessions should be conducted among the attendants of the patients and companions of those who come for giving sample, emphasizing on wearing mask and maintaining physical distance when relevant. Correct ways of wearing mask should be demonstrated. Emphasis should also be given that an epidemic is not Allah's wrath but of human error and make and that there is nothing shameful if someone is afflicted. No disease in present era is so easily transmissible and so there is no place for fear-mongering;
7. Health Assistants and NGO field staff should be trained on the criteria of those who should provide samples to let them make people aware at large of these criteria. They should ensure interest of the eligible people to visit CCs/ UHFWCs and give samples;
8. Training to the sample collectors should be efficient through adequate period of the training and through theoretical and hands-on demonstration and practice for efficient sample collection and more accurate test result. Sample collectors also need training on: proper use of the swab stick, insertion into the sample collection tube, proper disposal of the swab stick if necessary; storage conditions at different locations, registration, ways to minimize the risk of exposures, and decontamination/ disinfection;
9. Safety of the sample providers should be ensured through ensuring wearing of mask among the waiting people appropriately and through proper physical distancing of at least six feet;

10. Sample collection booths should be opened in epidemics at union health & family welfare centers (UHFWCs) and at community clinics (CCs). At least one staff in these facilities (preferably medical assistants and health assistants respectively) should be trained on how to collect Naso-pharyngeal, Oro-pharyngeal and other samples. Training should emphasize on -accurate process of sample collection, safety measures to be adopted including proper storage of sample and transportation, and how to do registration completely;
11. Sample collection booths in urban areas should be opened in NGO clinics, Government urban dispensaries, municipality and city corporation clinics, Government and private hospitals. Samples may be tested at the spot of sample collection if feasible other-wise samples should be transported from there to the nearby Government laboratories, for which a priori arrangements should be ready. Additional booths should be opened with the help of NGOs, prior readiness for which should be made if necessary under supervision of IEDCR;
12. On the spot registration could be done first, before sample collection and the samples could be saved directly into the transportation box promptly thereafter, instead of holding the sample test tube in one hand and then fill up the register with another. Registration should be complete and accurate. Those who register the sample providers should check, on the spot, the veracity of phone number entered into the register randomly;
13. The ID, names of sample giver, his/ her phone number, date and site of sample collection should be recorded meticulously and completely;
14. Levying of fee should be staggered from Taka 20 to 100 for on the spot collection of sample or Taka 50 to 300, based on the appearance of the sample givers or their households. Charging a fee is necessary to stave off people who would not require a test and not for cost sharing. But the fee should be collected on the spot and directly;
15. No sample giver should be turned back, or made to wait for long, so that no wrong signal goes to those who would be interested to give samples. The number of sample collection booths should be decided in light of the probable number of sample providers and a standing plan should be ready to increase the number of the booths whenever necessary;
16. Attempts should be taken to send the samples collected in the Civil Surgeon Office to the testing laboratory on the same day. This could improve the chance of better viability of the virus in the sample and less scope to infect the staff who handle the samples;
17. Delay in reporting should be prevented by allowing the individual testing units to report to the test seekers directly with copy to the DGHS/ IEDCR;

### Recommendations pertaining to testing laboratories

1. Type 16 Gene Xpert machines should be installed immediately in the 37 districts which still do not have any PCR machines, instead of the latter. Type 4 Gene Xpert machines can cover most of the upazilas for testing SARS-CoV-2;
2. PCR/ Gene Xpert laboratories should have at least six well trained medical technologists to run these in the BSL2 laboratories and these machines should be run efficiently in as many shifts as necessary per day. Two data entry operators should also be available to enter and notify the test results. A microbiologist should be posted as a supervisor if possible or a medical officer should be trained to supervise the testing procedures;
3. Payment should be made after commissioning of the machines by the supplier. This should be done under the supervision of IEDCR. IEDCR should also complete training of the medical technologists to run the machines at the same time;
4. Recruitment of adequate medical technologists should be ensured without delay, as per standard of WHO;
5. Laboratories which do not test at least 188 samples per day in PCR machines or the relevant numbers in the Gene Xpert machines should be inquired by IEDCR for the reasons of their failures on day to day basis;
6. IEDCR as the national reference laboratory and the apex agency for surveillance should work for enhancing capacity and standardization of testing and testing environment including wearing of PPEs at nodal points throughout the country for sample testing (and also at sample collection points). It should also monitor the quality of the sample testing procedures in the country including those in the private sector;
7. IEDCR's capacity should be increased to enable it to undertake the recommendations given in this paper;
8. Safe disposal system and process should be built up for the discarded samples;
9. Protocol for site-specific risk assessment in the PCR laboratories needs to be developed and practiced;
10. Every laboratory has to have controlled ventilation system for inward directional airflow into the laboratory room;
11. Exhaust air from the laboratory room should not be re-circulated to other areas within the building without HEPA filtering. Like-wise exhaust air from the laboratory should not be expelled without HEPA filtration;

12. Laboratory resources should be adequate in terms of -70°C refrigerator, wash basin with hot running water, automated sample processor and analyzer, system for using inactivated specimens (e.g. specimens in nucleic acid extraction buffer);
13. Contaminated specimen should be disposed off in hypochlorite solution followed by their disposal in a biohazard bag;
14. Sharp materials should be disposed off in red color bin after treating with hypochlorite solution;
15. Enough precaution should be ensured to prevent eye splash during sample processing;
16. Leaking during loading and unloading of sealed centrifuge cups and during grinding, blending, vigorous shaking or mixing, or sonic disruption should be prevented;
17. Personal items such as coats and bags should be kept outside the laboratory;
18. Hand washing should be practiced every half an hour;
19. Mobile electronic devices (for example, mobile telephones, tablets, laptops, flash drives, memory sticks, cameras and/or other portable devices) should not be used inside the laboratory and during the laboratory work;
20. A SOP for managing emergency situations should be available in every laboratory;
21. A system for written record of accidents and incidents should be available in every laboratory;
22. An SOP for investigating and reporting accidents should be available in every laboratory;
23. A written procedure for cleaning and decontaminating spills should be developed for every laboratory;
24. All laboratories should have standardized skills, practices, protocols and resources;
25. Old PCR machines should be replaced;
26. Gene Xpert machines should be used with apropos kit in districts and upazilas where PCR machine could not be provided yet;
27. Laboratories should not have unnecessary tools, furniture, machines, and provisions, e.g. telephone, books, documents papers, or waste bins.

#### [Recommendations on Reporting and Recording](#)

1. Data collection should aim future use of the data and hence should be complete, timely and accurate. From the beginning of an epidemic adequate and appropriate measures should be in order to comply with these requirements;
2. The Covid-19 experience was pathologically incomplete with regard to providing a complete picture of the problem in the country, in absence of any large scale random sample based survey and absence of any antibody based assessment to find out the infection rates among different sorts of people by sex, age, socio-economic and occupational groups and geographical location. These information could provide invaluable guidance on how to proceed with preventing and controlling of similar, if not the same types of epidemics and who to prioritize for intervention;

## Annex 1. Observational checklists

### Laboratory procedures

1. Is any site-specific risk assessment done to identify and mitigate risks: Y / N
  - i. IF yes. what are looked into (please mention just three):
  - ii. ....
  - iii. ....
  - iv. ....
2. Is any of the following disinfectant(s) used in the laboratory for disinfecting surfaces and others?
  - Disinfectant used is 1000 ppm sodium hypochlorite (bleach) for general surface disinfection: Y / N
  - Disinfectant used is 10000 ppm sodium hypochlorite (bleach) for disinfection of blood spills: Y / N
  - Disinfectant used is 62-71% ethanol: Y / N
  - Disinfectant used is 0.5% hydrogen peroxide: Y / N
  - Disinfectant used is quaternary ammonium compounds: Y / N
  - Disinfectant used is phenolic compounds: Y / N
  - All these disinfectants are used for 5 minutes/ 10 minutes/ 15 minutes/ 30 minutes
  - Gloves are changed after adding patient specimens to the instrument: Y / N
3. Is there any plan for controlled ventilation system to inward directional airflow into the laboratory room: Y / N
4. Is the exhaust air from the laboratory room recirculated to other areas within the building: Y / N
5. How is the exhaust air from the laboratory room thrown out: long chimney/ through an exhaust pipe outside the wall/ Any other ( )
6. Will the laboratory install a high efficiency particulate arrestance (HEPA) filter in functioning form: Y/ N (Comment )
7. A BSL-2 laboratory is used for handling the samples and for conducting PCR test: Y / N
8. Training will be arranged for competency level of the personnel who perform the procedures: Y / N (Comment: )
9. Other resources available include:
  - Will the laboratory procure refrigerator for temperature less than at -70°C or below: Y / N (Comment )
  - A dedicated hand-wash sink exists in the laboratory: Y/ N

- Hand hygiene and the use of personal protective equipment (PPE): Y / N
- a. Will the laboratory install automated instruments and analyzers for:
    - ❖ Processing samples: Y / N (Comment )
    - ❖ Final packaging of specimens for transport to diagnostic laboratories for additional testing (specimens should be in a sealed, decontaminated primary container): Y / N
    - ❖ Will install system for using inactivated specimens, such as specimens in nucleic acid extraction buffer: Y / N (Comment )
  10. Staff in the laboratory will be trained on: (i) proper use of the instrument: Y / N, (ii) ways to minimize the risk of exposures: Y / N, and (iii) decontamination/ disinfection: Y / N (Comment )
  11. Other laboratory based practices and incidences are as follows:
    - a. Donning and doffing space for PPE is available inside the laboratory: Y / N
    - b. Contaminated specimen are disposed off in:
    - c. Sharp materials are disposed off in:
    - d. Eye splash during sample processing occurred a few times: Y / N
    - e. Received samples leaked occasionally (in case of sample reception): Y / N
    - f. Samples leaked occasionally during loading and unloading of sealed centrifuge cups: Y / N
    - g. Leaking occurred occasionally during grinding, blending, vigorous shaking or mixing, or sonic disruption: Y / N
    - h. Opening of containers with high internal pressure and splashing: Y / N
    - i. Food or drink is stored in another refrigerator in the laboratory: Y / N
    - j. Personal items such as coats and bags are kept in the laboratory neatly: Y / N
  12. Eating, drinking, smoking, applying cosmetics are sometimes performed in the laboratory with caution: Y / N
  13. Laboratory has warm running water and soap: Y / N
  14. When does the staff wash their hands with soap: After entering the laboratory/ Every now and then/ Before leaving the laboratory
  15. How long it takes to cover a cut or broken skin: within 2 minutes/ 5 minutes/ 10 minutes
  16. Mobile electronic devices (for example, mobile telephones, tablets, laptops, flash drives, memory sticks, cameras and/or other portable devices) are used in lab when necessary: Y / N
  17. An SOP will be developed for emergency scenarios: Y / N (Comment )
  18. Laboratory will buy first aid box: Y / N (Comment )
  19. A system for written record of accidents and incidents will be developed : Y / N (Comment )
  20. An SOP will be developed for investigating and reporting accidents: Y / N (Comment)

21. A written procedure will be developed for cleaning and decontaminating spills: Y / N
22. How frequently Covid-19 tests for laboratory staff is done: every 10/ 14/ 21 days

## Sample collection procedure

1. Swab fiber is synthetic: Y / N
2. Handle of the swab sticks in plastic or made of wire: Y / N
3. Swab is grabbed by the distal end of the handle: Y / N
4. If both Nasopharyngeal and Oropharyngeal swabs are collected, they are combined in a single tube: Y / N
5. For nasopharyngeal swab the minitip is entered through the nostril parallel to the palate (not upwards) until resistance is encountered or the distance is equivalent to that from the ear to the nostril of the patient: Y / N
6. Swab is gently rubbed and rolled: Y / N
7. Swab is left in place for several seconds to absorb secretions: Y / N
8. Swab is removed slowly while rotating it: Y / N
9. Patient's head is tilted back 70 degrees: Y / N
10. Swabs is placed immediately into a sterile transport tube containing 2-3mL of either viral transport medium (VTM), Amies transport medium, or sterile saline for transportation: Y / N
11. If non-bacteriostatic saline is used to collect the specimen it is placed immediately into a sterile transport tube: Y / N
12. Sterile swabs for upper respiratory specimen collection is packaged individually wrapped / in bulk number
13. Specimens is stored at 2-8°C for up to 72 hours after collection: Y / N
14. Each specimen container is labeled with the patient's name, ID number (e.g., medical record number), address and the date the sample was collected: Y / N
15. Patient specimens from suspected or confirmed cases should be transported as UN3373, "Biological. Substance, Category B".
16. The donning and doffing of PPE is done in the same room: Y / N

## **Annex 2. List of the interviewed personnel and sources of information**

1. Prof. Nasima Sultana, ADG (Adm.), DGHS, MoHFW;
2. Prof. Dr. Sabrina Flora, Director, IEDCR, DGHS, MoHFW;
3. Prof. Samiul Islam, Line Director TB-Leprosy and ASP, DGHS MoHFW;
4. Prof. Dr. Mostafa Kamal, Principal Cumilla Medical College;
5. Dr. Shamsul Haque, Line Director, MNCAH, DGHS, MoHFW;
6. Dr. Samir Kumar Sarkar, Coordinator, Sample collection for Covid-19;
7. Dr. A.S.M. Alamgir, Chief Scientist of Epidemiology, IEDCR;
8. Dr. Fahmida Khatoon, Assistant Professor, NIPSOM;
9. Dr. Moshiul Alam, Civil Surgeon, Mymensingh;
10. Dr. Shamshuzzaman, Superintendent, in-charge, Narayanganj 300 bed Hospital;
11. Dr. Imtiaz Hossain, Civil Surgeon, Narayanganj;
12. Manjur Hossain Khan, Assistant Professor, IEDCR & National Influenza Center;
13. Dr. Khondokar Mahbuba Jamil, Virologist, IPH;
14. Dr. Mohammad Jahangir, UHFPO, Birol, Dinajpur, now Asst. Director, Coord., DGHS;
15. Dr. Mohammad Shuaib, UHFPO, Chatmohor, Pabna;
16. Dr. Hasibul Islam, UHFPO, Chouddogram, Cumilla;
17. Dr. Zahidul Islam, UHFPO, Narayanganj Sadar;
18. Dr. Mohammad Afzalur Rahman, Medical Officer, DGHS;
19. Dr. Masud Uddin, Medical Officer, TB-Leprosy Control Program;
20. Dr. Kawsar Uddin, Mymensingh Medical College;
21. Mr. Subhan, Retd. Sr. Health Education Officer, Bureau of Health Education; Chattagram;
22. Mr. Shakir Hussain, Jr. Health Education Officer, Narayangnaj;
23. Mr. Mizanur Rahman, Medical Technologist, Narayanganj 300 Bed Hospital;
24. The Daily Prothom Alo;
25. The Daily New Age;
26. The Daily Bangladesh Protidin;
27. ATN News TV channel;
28. Independent TV channel;
29. Shomoy Television;
30. Channel 24
31. DBC Television.

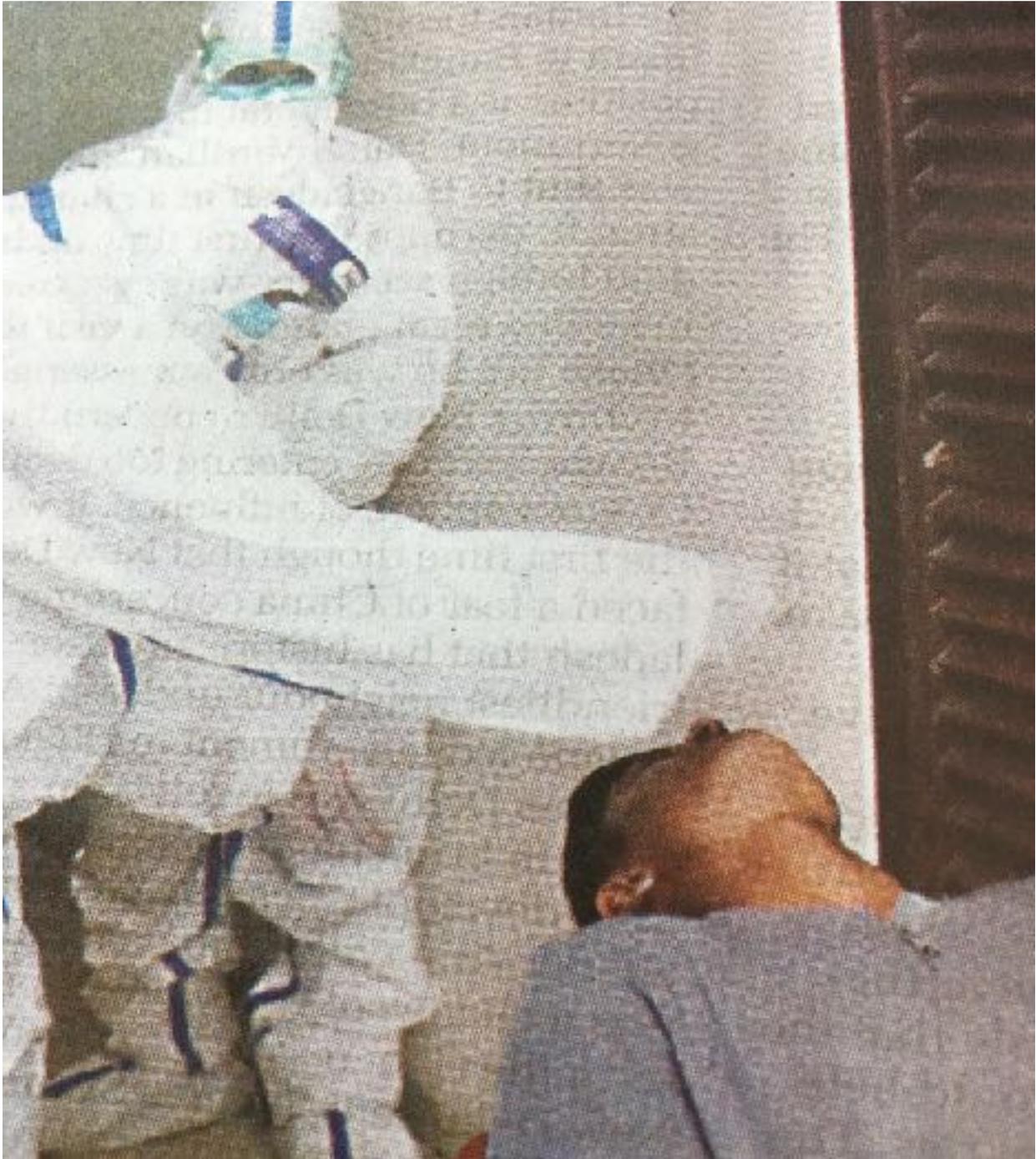


### Annex-3. Photos

#### 1. Proper methods of sample collection

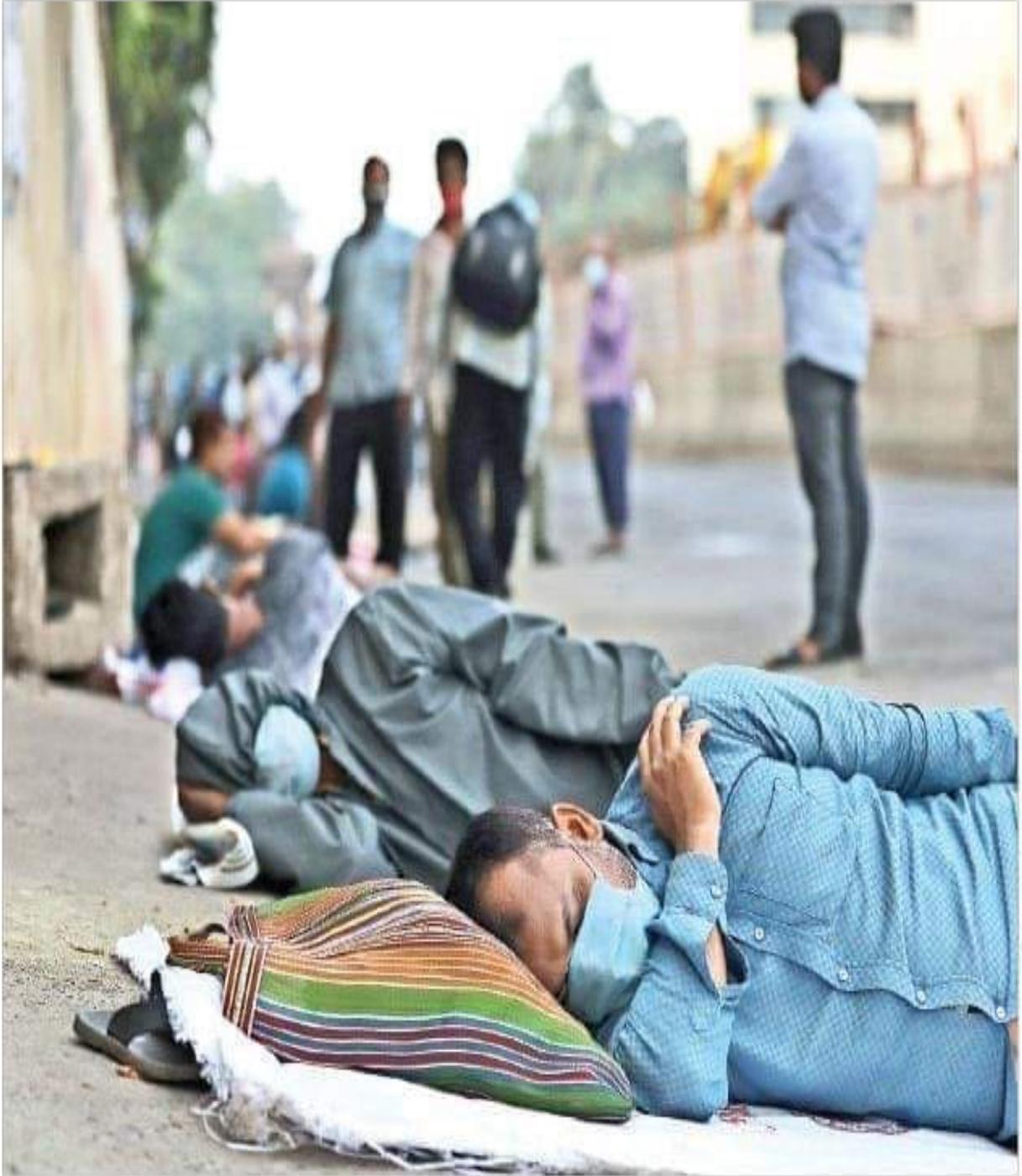


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Source: The Daily New Age

## 2. Waiting for giving samples in BSMMU



করোনাভাইরাসের নমুনা পরীক্ষার সিরিয়াল পাওয়ার জন্য লোকজন রাতের বেলা হাসপাতালের সামনে গিয়ে ভিড় করেন। পেপার, চামড় বিছিয়ে শুয়ে থাকেন ফুটপাথের ওপর। শনিবার ভোর সোয়া ৫টায় বঙ্গবন্ধু শেখ মুজিব মেডিকেল বিশ্ববিদ্যালয়ের বেতার ভবনের সামনে থেকে ছবিটি তোলা

■ কবেল রশীদ

3. Crowded queue for giving samples, without honoring physical distance



4. Sample collection of an indoor patient in the outdoor of a hospital in Dhaka



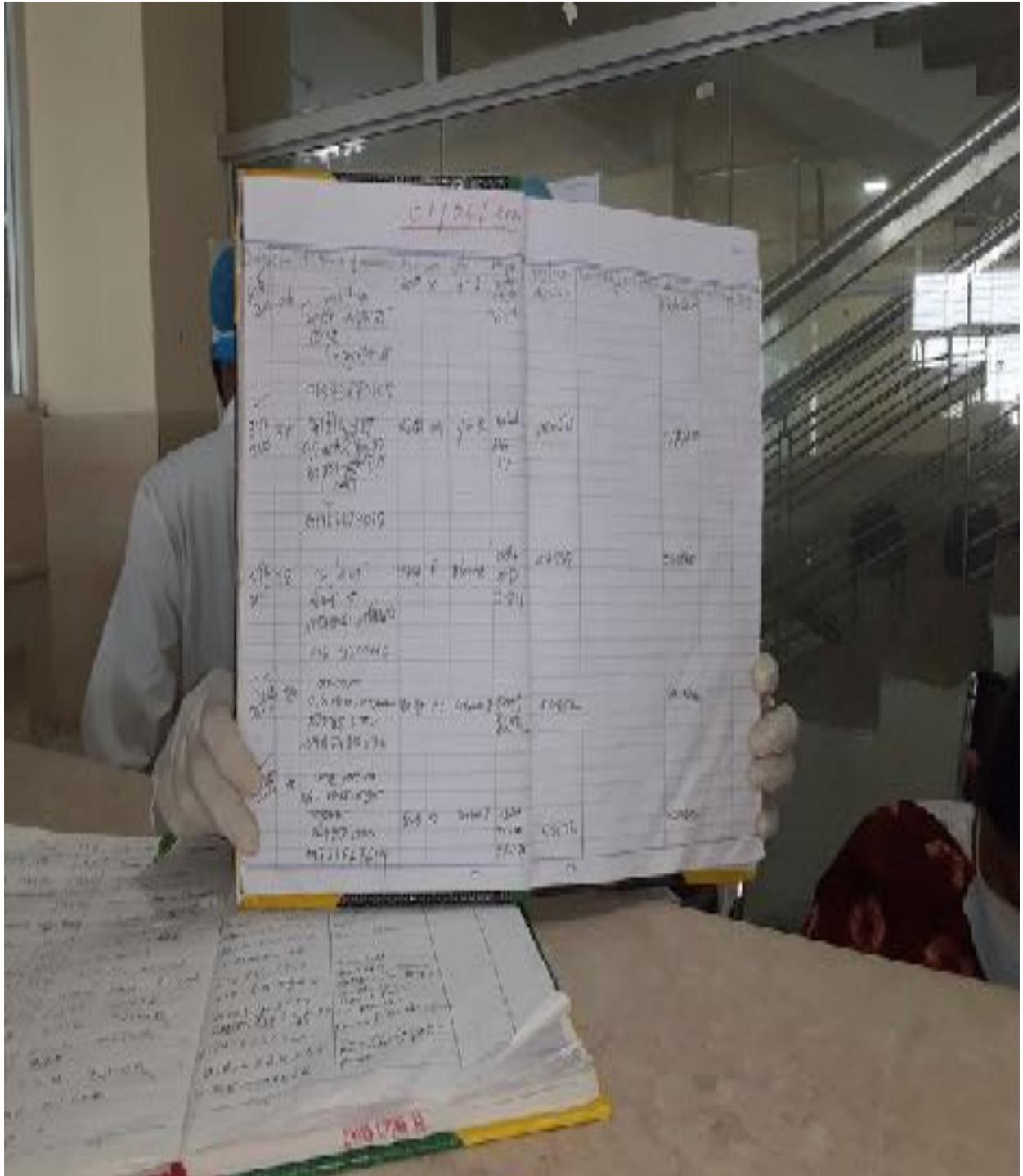
5. People waiting to give sample without physical distancing



6. Washing hand with antiseptic after collecting sample



7. Registration of sample providers (two different forms)
  1. Incomplete



2. Complete registration but without full address or contact number

Sl. No.	Plant Name	Local Name	Altitude	Locality	Collector	Number	Date
01	10-095	Chandrayan	16/5/2020	Alam	BR 585	27/7/2020	
02	10-045	Amr. Decidua	16/5/2020	Alam	BR 585	Do	
03	10-047	Chandrayan	16/5/2020	Alam	BR 585	Do	
04	10-048	Savage	16/5/2020	Alam	BR 585	Do	
05	10-049	ZARIA	16/5/2020	Alam	BR 632	27/7/2020	
06	10-050	Alam	16/5/2020	Alam	BR 701	27/7/2020	
07	10-115	Amr. Decidua	16/5/2020	Alam	BR 639	27/7/2020	
08	10-052	Alam	16/5/2020	Alam	BR 585		

9. Different types of sample collection tubes and swabs used



8. A wrong of sample collection from home



10. Sample collection in a wrong way in an upazila health complex of Chattagram



11. Sample collection in a wrong way in a district hospital



12. Sample collection in a wrong way through in a medical college hospital



13. A PCR laboratory (BSL-2) Cumilla



13. PCR Laboratory staff not wearing PPE adequately



14. A wash room staff without adequate PPE and with too many paraphernalia



15. A PCR laboratory staff with Dr. A M Zakir Hussain, survey Coordinator



16. Dr. A M. Zakir Hussain in NIPSOM PCR laboratory



17. A BSL-2 hood for mixing master mix



18. A BSL-2 hood for plating samples and a sample reader on the right side



19. A sample reader and recorder in a PCR Laboratory



20. A recorder of test result

