Original research article

Clinical Microbiology Laboratory Adaptation to COVID-19 Emergency: An Experience at an Upcoming Government Medical College & Teaching Hospital in Almora, Uttarakhand and a brief Laboratory Situation Report.

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Abstract

Aim: For awareness and to reduce latency in adaptation to novel epidemics and pandemics as seen during ongoing severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic. To ensure early diagnosis and implementation of preventive public health measures there by reducing the burden of morbidity and mortality, if at all any such situation arises in future.

Materials and Methods: By using molecular methods RT-PCR and True Nat and rapid antigen testing the COVID – 19 testing is done for COVID -19in our microbiology laboratory, Soban Singh Jeena Govt.

Institute of Medical Sciences & Research, Almora The kits used were Covisure Genetix Biotech Asia Pvt. Ltd). RTPCR machine (CFX-96, BioRad). TruNat platform (MolBio Diagnostics Pvt. Ltd). COVISURE Realtime PCR kit approved by ICMR

TAT (the time from sample receipt in the laboratory to release of PCR result) has been calculated.

Results: 1,98,294 samples were tested for COVID – 19 Dept. of Microbiology, Soban Singh Jeena Govt. Institute of Medical Sciences & Research, Almora among which Cumulative Positive were 9323 and positivity rate was 4.7% by RT-PCR technique. 8660 tests were done by true nat for which 1564 were positive and the positivity rate was 18.1%. 5288 tests were done by Rapid Antigen Test, 184 were positive and 3.5% was the positivity rate for a time period of December 2020 to June 2022.

Conclusion: Although there was an initial lag phase in adaptation to COVID – 19 situation but later on due to self-motivation, co-ordinated and dedicated team work it has become possible to establish COVID – 19 testing facility in our upcoming teaching hospital in Almora. This proves that anything is possible with dedicated team work.

Keywords: SARS-CoV-2, early diagnosis, preventive public health measures, team work, mortality and morbidity and ICMR guidelines

Introduction

COVID–19 evolution briefly

In December 2019 a highly pathogenic HCoV, was recognized in Wuhan, capital city of Hubei province, China, causing serious pneumonia like illness and death. [1] On 12 January 2020 World Health Organization (WHO) named this new CoV as 2019-novel coronavirus (2019-nCoV). As per the International Health Regulations (IHR, 2005) on 30th January 2020, WHO declared the 2019-nCoV outbreak as a public health emergency of international concern (PHEIC). Later, Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses (ICTV) proposed to name the new coronavirus as SARS-CoV-2 and WHO officially named the disease as corona virus disease-2019 (COVID-19), both issued on 11 February 2020, and on 12th March 2020 WHO declared the COVID-19 outbreak as global pandemic. [2] By October 11, 2020, there were more than **37 million confirmed cases** with COVID-19 and **1 million deaths** were recorded.

Declaration by the WHO at the end of January 2020 lead to mandatory reformation of Microbiology diagnostic equipment for early diagnosis and for maximum direct detection of SARS – CoV-2 in patient samples. [3]

C o Vs are a large family of spherical, enveloped, non-segmented, positive sense, single stranded RNA viruses (+ ss RNA) characterized by spike proteins projecting from the virion surface and can be divided into four genera; including α−/ β−/γ−/δ-Co V, of which α-Co Vs and β-Co Vs are known to cause human infections [Figure 1]. [1,4,5] Previously, six Co Vs have been identified to cause infections in humans, among which α-Co Vs: HCoV-229E and HCoV-NL63, and β-Co Vs: HCoV-HKU1 and HCoV-OC43 have low pathogenicity and cause mild respiratory symptoms similar to common cold. The other two known β-Co Vs: SARS-Co V and MERS-Co V can cause severe and potentially fatal respiratory tract infections. [5,6] SARS-CoV-2 is the third identified H Co V to cause severe respiratory illness named as COVID-19 and has symptoms and incubation period resembling to that of SARS-Co V and MERS-Co V infections. [1,7]

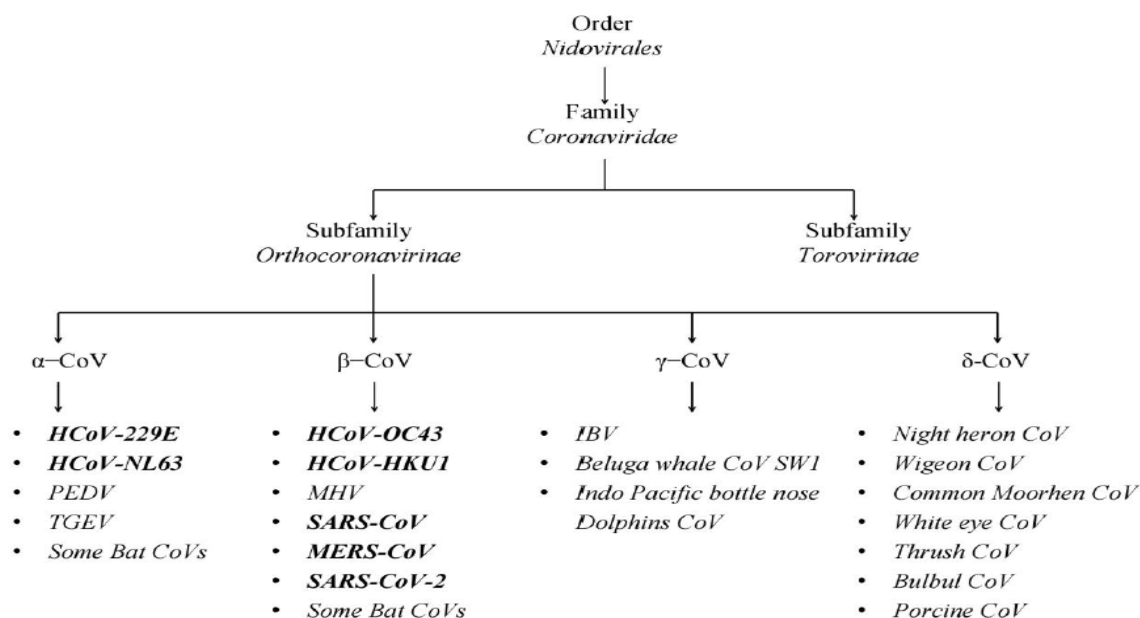

Figure 1: Classification of different types of Coronaviruses (Family: Coronaviridae; Subfamily: Orthocoronavirinae; Genus: α−/β−/γ−/δ-Co V). SARS-CoV-2 belongs to genus β-coronavirus.

Source: *Pal S, Juyal D, Jauhari S, Singh H, Prakash R, Thaledi S. SARS-CoV-2: Origin, Transmission, Prevention and Mitigation. Ann. Int. Med. Den. Res. 2020; 6(5): MB01-MB10*

SARS-CoV-2 belongs to genera β-CoV (subgenus sarbecovirus, subfamily Orthocoronavirinae), and preliminary analyses indicates that its genomic sequence is 96.2% identical to a bat CoV RaTG13 and 79.5% identical to SARS-CoV. [8,9] Its genome contains 2981 nucleotides, encoding for 9860 amino acids. Although its origins are not entirely understood, the genomic analyses suggests that SARS-CoV-2 probably evolved from a strain found in bats and an alternative intermediate reservoir such as turtles, pangolins and snakes are thought to be involved in its transmission to humans. [9,10] However it is still not certain that this intermediary exists. As the first cases of COVID-19 disease were linked to direct exposure to the wholesale seafood market of Wuhan, capital city of Hubei province, China, where live animals are sold, mostly for food and medicinal use (folk remedies) as per the Traditional Chinese Medicine, [1, 11] the animal to human transmission was presumed as the main mechanism. The practice of eating raw meat and the close contact between humans and animals are both considered as risk factors for the initiation of new H Co V outbreak and thus COVID-19 can also be considered as a zoonotic disease. Similar to SARS-CoV and unlike MERS-CoV, human to human transmission has been confirmed and was evidenced by the infection of healthcare professionals in Wuhan hospital, China, suggesting the virus to be highly contagious. [12] Soon it was apparent that the infection could be transmitted from asymptomatic people and also before any onset of symptoms. Uncontrolled spread from human-human has led to novel mutant covid -19 strains that are potentially more virulent as was evidenced during the second wave with Delta and Delta plus variant of the virus.

Brief genomics of SARS-CoV-2: SARS-CoV-2 is a unique β-Coronavirus with increased pathogenicity and transmissibility, is 50-200 nm in size with RNA sequence of 3000 bases in length and has 4 structural proteins that include spike (**S**), envelope (**E**), membrane (**M**), and nucleocapsid (**N**) proteins.

- N-protein: Holds the RNA genome
- S, E & M protein: Together create viral envelope
- Spike glycoprotein: Allows viral attachment to host cell membrane

SARS-CoV-2 rely on their spike (S) proteins for binding to the host cell surface receptor during host cell entry. [13] The viral S protein binds to the ACE2 receptors (present on the the airway epithelium and alveolar type 2 (AT2) pneumocytes, pulmonary cells) through the receptor-binding domain (RBD) in the S1 subunit, followed by the fusion of the S2 subunit to the cell membrane.

The genome of SARS-CoV-2 is comprised of a single-stranded positive-sense RNA. [14] The newly sequenced genome of the SARS-CoV-2 submitted in the NCBI genome database (NC_045512.2) is ~29.9 Kb in size. [15] The genetic makeup of SARS-CoV-2 is composed of 13–15 (12 functional) open reading frames (ORFs) containing ~30,000 nucleotides. The genome contains 38% of the GC content and 11 protein coding genes, with 12 expressed proteins. The ORFs are arranged as replicase and protease (1a–1b) and major S, E, M and N proteins, which follows a typical 5′-3′ order of appearance. These gene products play an important role in viral entry, fusion, and survival in host cells. [16] The whole genome of SARS-CoV-2 encodes about 7096 residues long polyprotein which consists of many structural and non-structural proteins (NSPs). The nucleotide content of the viral genome is held mainly by two non-structural proteins ORF1a and ORF1ab followed by structural proteins. Polyproteins pp1a and pp1ab are encoded by ORFs 1a and 1b, where polyprotein pp1ab is encoded by the ribosomal frameshift mechanism of the gene 1b. These polyproteins are further

processed by virally encoded proteinases and produce 16 proteins, which are well conserved in all CoVs belonging to the same family. [17]

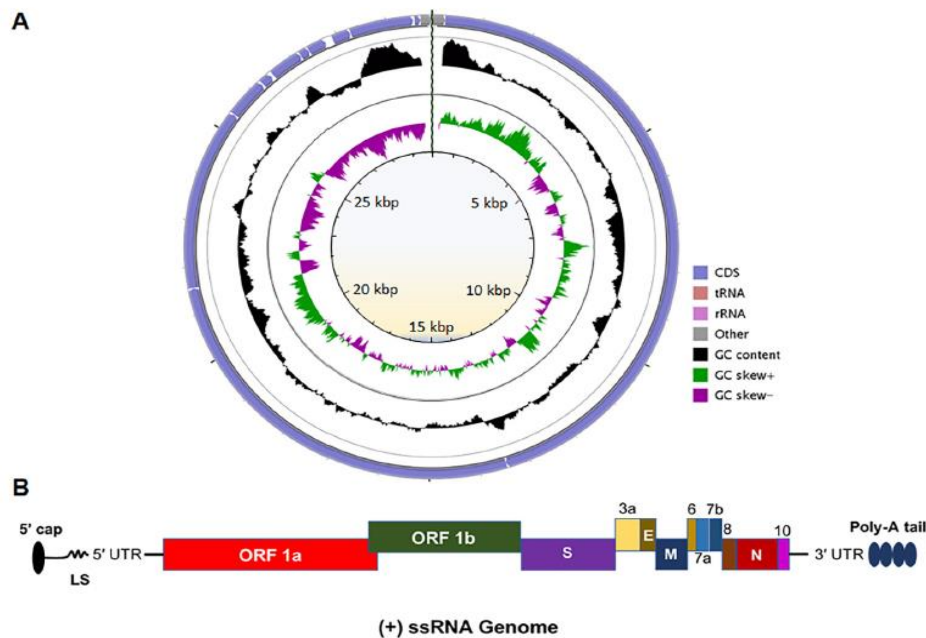

Figure 2: Genome architecture of SARS-CoV-2. **(A)** Representation of the reference genome of SARS-CoV-2 showing the protein-coding regions and GC content of the genome. (B) Representation of 5′ capped mRNA has a leader sequence (LS), poly-A tail at 3′ end, and 5′ and 3′ UTR. It consists of ORF1a, ORF1b, Spike (S), ORF3a, Envelope (E), Membrane (M), ORF6, ORF7a, ORF7b, ORF8, Nucleocapsid (N), and ORF10

Source: *Y.Z. Zhang, E.C. Holmes, A genomic perspective on the origin and emergence of SARS-CoV-2, Cell 181 (2020) 223–227*

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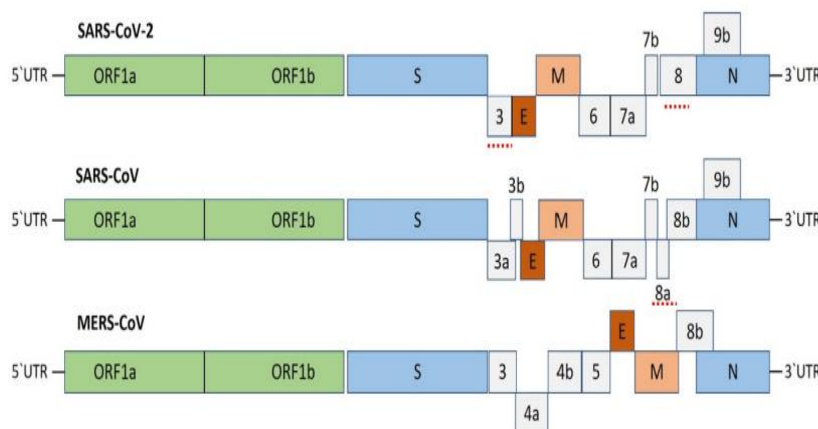


Fig. 4. Betacoronaviruses genome organization; The Betacoronavirus for human (SARS-CoV-2, SARS-CoV and MERS-CoV) genome comprises of the 5′-untranslated region (5′-UTR), open reading frame (orf) 1a/b (green box) encoding non-structural proteins (nsp) for replication, structural proteins including spike (blue box), envelop (maroon box), membrane (pink box), and nucleocapsid (cyan box) proteins, accessory proteins (light gray boxes) such as orf 3, 6, 7a, 7b, 8 and 9b in the SARS-CoV-2 genome, and the 3′-untranslated region (3′-UTR). The doted underlined in red are the protein which shows key variation between SARS-CoV-2 and SARS-CoV. The length of nsps and orfs are not drawn in scale.

We have recently witnessed a devastating pandemic by novelcovid19 that differed from other C o v s not only in transmissibility and disease severity but also has resulted in massive deaths and debility among humans including health care professionals. Considering the high transmissibility from human to human and the disease severity, precautionary periodic guidelines for containment measures as well as for laboratory diagnosis have been issued globally by WHO at the beginning of the pandemic itself. Consequently, general hospital wards are converted to dedicated COVID – 19 wards and Molecular diagnostic modalities were used on large scale in Microbiology laboratories for timely detection of the suspected cases. [18]

In India also, Central Government communicated to States/Union Territories to focus on undertaking aggressive measures to break the chain of transmission and directed that those who are found positive should be isolated/hospitalized and their close contacts be traced, tested, and treated without delay. States have been advised to monitor the situation closely and regularly so that the gains made so far in COVID management and containment are not lost. [19]

As Viral loads determine the asymptomatic cases transmissibility and infectivity, it has become mandatory even ordinary microbiology laboratories to adapt for any unparallel situations that may happen at any time during COVID – 19 Pandemic. [20,21] Laboratories situated in the remote and resource constrained areas (like our hospital) where lack of modern facilities and with logistic difficulties has faced adaptation problems initially when compared to National reference laboratories. Thereafter, our laboratory has been redesigned successfully for early detection of patients infected with SARS-CoV-2, to confirm COVID-19 cases by RT- PCR as well as by True Nat so as to identify and isolate patients as early as possible as per the latest guidelines issued by ICMR and also to determine the viral loads. In this study we are going to address the progressive adaptation of our microbiology laboratory in upcoming Government teaching hospital at Almora.

Molecular Methods
RT- PCR:

Sample collection: Nasopharyngeal Swab and/or Oropharyngeal Swab are collected in Viral Transport Medium (VTM) by a trained nursing staff in a **Qiosk** , thereafter the collected samples are processed in a COVID-19 testing facility. (BSL 2 +facility with BSL 3 practices)
Except for the above the samples are also received in our COVID-19 testing facility from fourteen different CHC/PHCs and collection centres in and around district Almora.
Decontamination and RNA extraction of the samples: Using viral lysis buffer the samples are decontaminated and are processed for **RNA extraction** in an Automated RNA Extraction platform (96 wells)

Master Mix preparation: The RTPCR master mix is prepared using the kit protocol (Covisure-Genetix Biotech Asia Pvt. Ltd)

Template addition: The extracted RNA (5 µl) is added to the Master Mix (20 µl) with a final volume of 25 µl and the prepared plate (96 well) is placed in a RTPCR platform (CFX-96, BioRad) for the run with one positive and one Negative control.

Results:

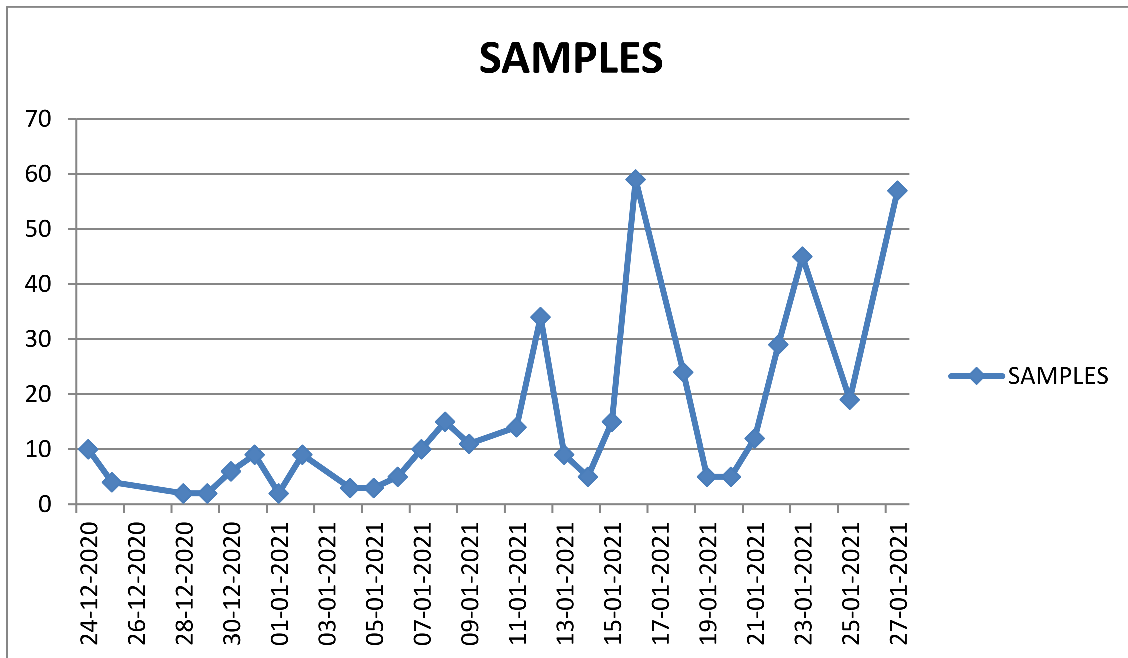
Once the amplification is complete in the automated PCR machine the graphs were analysed by the trained Microbiologist. The graphs showing the Sigmoid curve that will correspond to intensity of light emission by reporter molecules(dye) used as per the kit guidelines and with a Cycle Threshold (CT) value of 10-35 were considered as Positive.

TruNat

It's a Cartridge Based Nucleic Acid Amplification Test (CBNAAT) used for detection of SARS-CoV-2 RNA in the samples with a Turn Around Time (TAT) of 120 mins. The samples are collected in the separate VTM vials provided by MolBio and are loaded in a chip for extraction and amplification (TruNat Duplex test for COVID-19) in the TruNat platform (MolBio Diagnostics Pvt. Ltd). Samples showing amplification graph for Orf1a gene were considered as Positive.

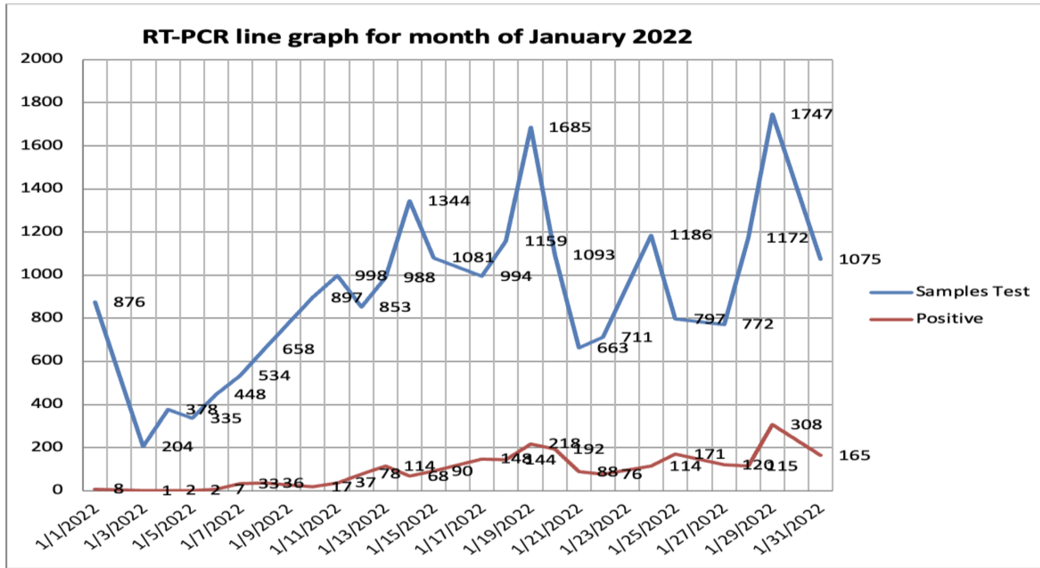
Results

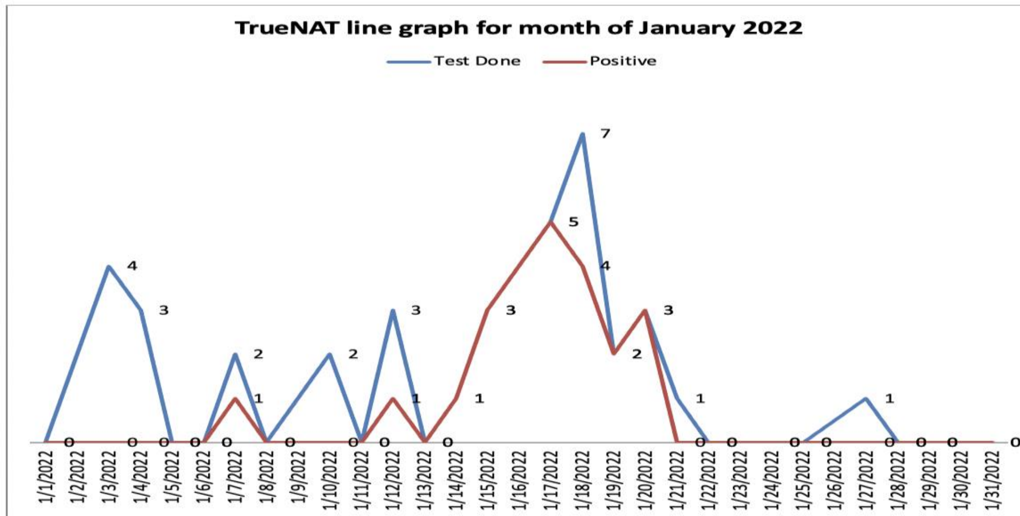
Initiation of COVID-19 RTPCR testing at GTB Hospital, Almora in December 2020

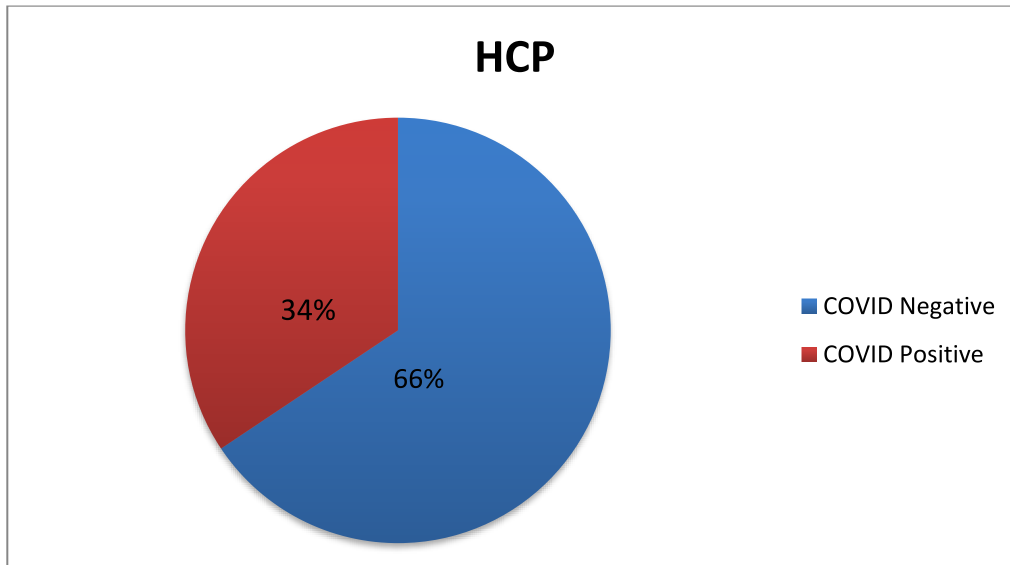


By January 2022, although our daily testing capacity was 1000 tests but as during the second wave there was sudden surge in no. of cases, we have even tested more than 1600 samples on 19.01.2022, and the total of samples tested in the month of January 2022 was 22,648. The peak incidence of COVID-19 positive cases in district Almora were seen in the month of January 2022, with a cumulative monthly positivity rate of 10.4% and was found to be comparatively lesser than the Average positivity rate of the country (16.4% approx.) during the same time period. We speculate that one of the reasons for the lower positivity rate in our district may be lack of approachable health care as well as diagnostic facilities in the remote villages. Needless to mention that our lab is the only COVID-19 testing centre receiving the samples from whole district of Almora.

Peak incidence of COVID-19 cases was seen in January 2022

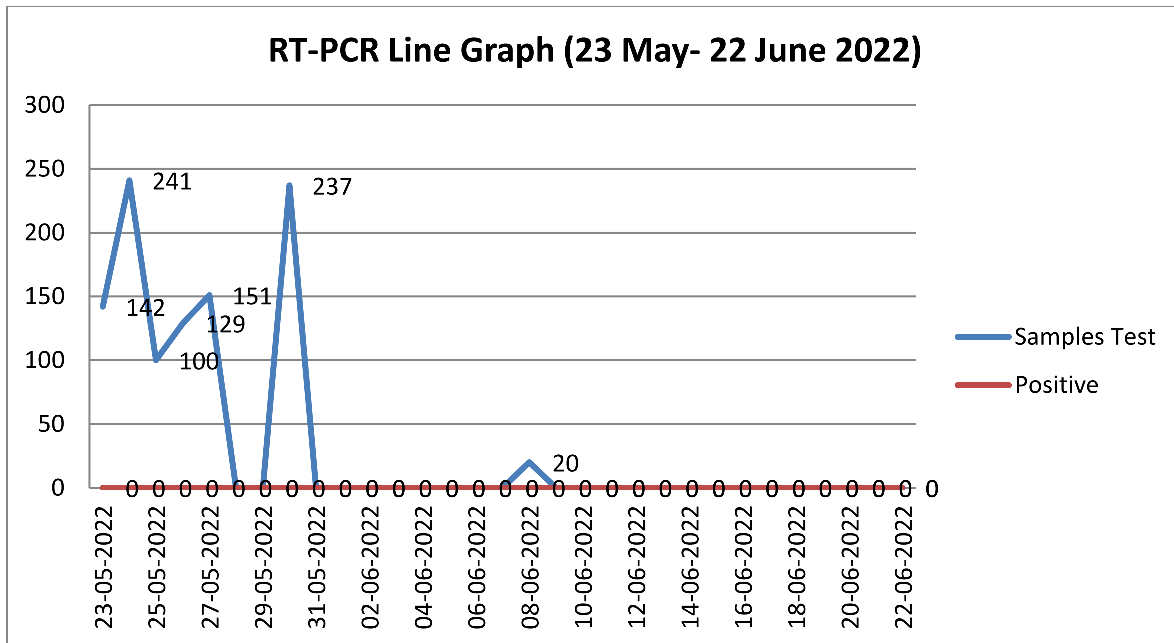






Our team included 32 health care personnel (3 trained Microbiologists, 6 laboratory Technicians, 1 Data Entry Operator, 3 Research assistants, lab attendant 1 Sweeper-1 and 17 Staff nurses). Out of the aforementioned 32 HCPs, 11 were symptomatically positive for COVID -19 and later successfully recovered from the infection.
To comply with workflow, the existing laboratory staff and the newly employed personnel have shown their skills towards a new, laborious and expensive diagnostic challenge. In particular, all of our laboratory staff are trained, energetic, enthusiastic and exclusively committed to the performance of SARS-CoV-2 testing.

Current Laboratory Scenario



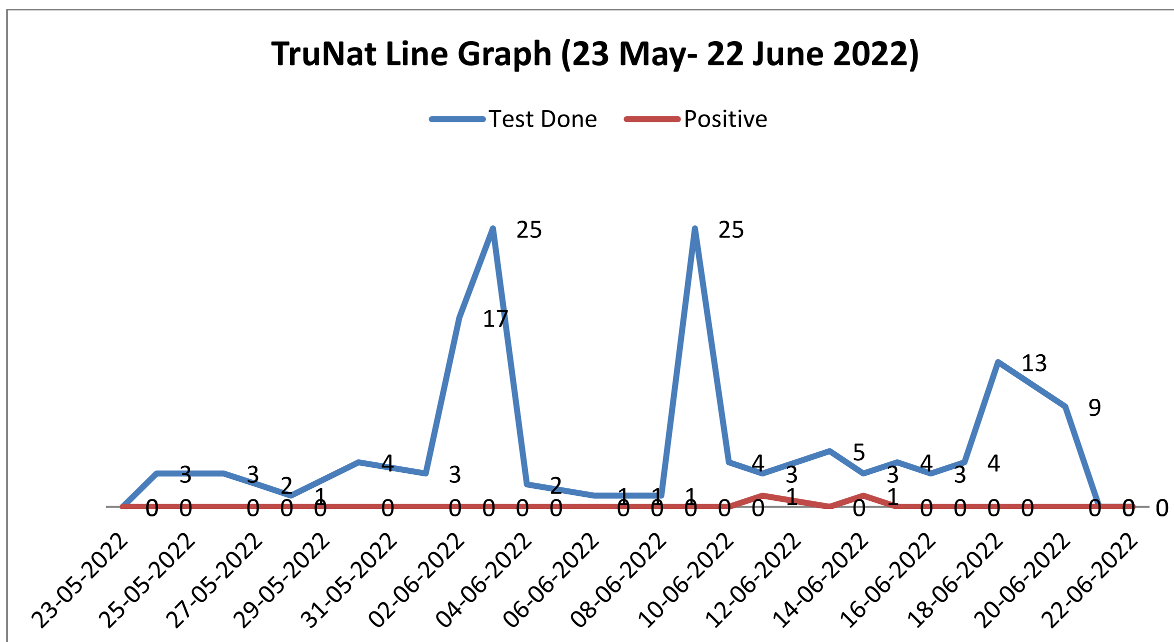


Table 1: Overall Scenario of the tests done at our lab, till Date

	RT-PCR	TruNat	Rapid Antigen Test (RAT)
Cumulative tests done	1,98,294	8,660	5,288
Cumulative Positive	9,323	1,564	184
Positivity Rate	4.7%	18.1	3.5

Discussion:

Because of occurrence of global health emergency due to SARS-CoV-2, the WHO at the end of January 2020 declared to modify Microbiology laboratory outfits worldwide to maximise the detection of SARS-CoV-2 in clinical samples. This is to intensify documentation of SARS-CoV-2 infections for epidemiological purposes and to sustain containment facilities as current COVID–19 disease differs from other corona virus infections, not only in severity but in human-to-human transmissibility as well.

Microbiology laboratory has played a key role in diagnosis as well as determining the transmission dynamics of this novel virus and has helped the public health authorities to formulate the rapid response guidelines to the pandemic situation. The role of government and scientific societies (National; MOHFW, ICMR and International; WHO, CDC) is not only to formulate guidelines for containment and limiting the further spread of the virus but also to improve global knowledge on the disease pathogenesis, possible therapeutic options and vaccination guidelines.

We have performed RNA detection in all the samples using COVISURE Realtime PCR kit approved by ICMR for the detection of E gene, RNAaseP, RdRp gene through FAM, VIC and CY5 channels. SARS – Co V -2 POCT has served as a complementary test to conventional polymerase chain reaction. There was change in the genes detected from time to time which we have adapted rapidly such as Orf1ab, N-gene and RNAaseP through FAM, ROX and HEX channels respectively and later on COVISURE multiplex Real time PCR Orf1ab, E-gene, RPP30 gene through FAM, HEX, ROX channels. TAT (the time from sample receipt in the laboratory to release of PCR result) has been calculated. This is directly proportional to time taken for pre-PCR steps or on laboratory working hours (12 h/day versus 24 h/day). Our laboratory has worked for two shifts, from 8:00 AM to 2:00 PM and 2:00 PM to 8:00 PM. Initially in the beginning phase during the first wave, the lack of man power, difficulty in getting timely laboratory supplies due to nationwide lock down, the TAT of our lab was 3-5 days which was later reduced to 24-48 hrs. Initially when the lab was established, we started with 4 samples on 24.12.2020 and the number gradually increased. With precise policy implementation of Indian public health system through ICMR and IDSP regular appropriate guidelines and with successful vaccination the epidemic has been controlled in our country. Recently in our laboratory we are recording zero positive cases by both RT-PCR as well as True nat with few positive cases occasionally.

In conclusion even though there have been all the aforementioned obstacles, the clinical microbiology laboratory can be remarkably responsive to emergencies like Covid -19 pandemic while providing solid example on how microbiology laboratory has to respond to pandemic situations however, we all know that we have to improve our response to outbreaks such as COVID – 19, if any happens in near future

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