ABSTRACT
Nepal reported the first COVID-19 case on January 23, 2020. A rapid increase in the number of COVID-19 cases was being reported in Nepal as of June 2020. Limited molecular laboratory capacity in a minimal resource setting is a challenge in the diagnosis of the ever-increasing cases. We share the experience and challenges in the rapid establishment of COVID-19 testing laboratory in Birgunj of Parsa district which is one of the SARS-CoV-2 transmission hotspots in Nepal. The initial step in establishing the COVID-19 molecular testing laboratory were i) identifying a suitable space ii) renovating it and iii) mobilizing materials including consumables, mainly from the hospital store and administration. A chain of experimental design was set up with distinct laboratories to standardize the extraction of samples, preparation of the master mix and detection. At the commencement of sample testing, laboratory contamination was among the primary challenges faced. The source of the contamination was identified in the master mix room and resolved. The established COVID-19 testing lab (Narayani PCR Lab) has tested more than 40,000 samples as of 31st August, 2020 and is now one of the preferred laboratory for CoVID-19 testing. The lessons learnt may benefit the further establishment of testing laboratories for COVID-19 and/or other epidemic/pandemic diseases in resource-limited settings.

Keywords: Biosafety; Contamination; CoVID-19; Molecular laboratory; Polymerase chain reaction (PCR)
The Narayani hospital located in Birgunj is a historic government institute, but with scarce human resources and infrastructure with respect to its population. The hospital laboratory was as good as inactive due to weak procurement systems for chemical reagents and maintenance of equipment. Additionally, the key equipment for molecular laboratory such as micro-centrifuges, refrigerators, biosafety cabinets, vortex, hotplate and micropipettes were not available.

At the face of rapid surge of COVID-19 cases, establishment of a COVID-19 molecular laboratory was planned. As a coordinated effort to combat the disease, a multi-disciplinary steering committee was formed by hospital administration to lead the laboratory establishment. The central team comprised of the Medical superintendent, head of the department of Pathology, chief medical technologist, quality officer, safety officer and the hospital store team leader. The team started identifying space for the laboratory along with mobilizing materials and consumables.

Rest Room
IT Section
Changing Rooms
Entrance to BSL-2 Lab
Aliquot Room
RNA extraction room
PCR Room
Master-mix room
Rest Room

Fig 1: Floor plan of COVID laboratory

ORGANIZING THE LAB

A separate setup for dirty (sample reception and extraction) and clean rooms (master mix) was done. Sample flow was organized in such a way that the dirty rooms did not contaminate the clean rooms (unidirectional workflow).

Experts from the molecular lab teams at NPHL helped with calibrating the RT-PCR machine. The central Information Technology (IT) team installed the software. However, on the second day of the machine calibration, a power fluctuation damaged the desktop. So, Uninterruptible Power Supply system was installed by the electrical repair unit after several requests.

During the first trial test, the biosafety cabinet filter was malfunctioning. Biomedical engineer helped to solve the
issues at hand by communicating with their colleagues and the representatives of Nepal Bio-medical Engineer’s association. After an intensive period of problem identification by the team, it was concluded that the cabinet needed replacement. It took almost 2 weeks to buy and install another biosafety cabinet. However, the glass window of the cabinet was broken during transportation. The maintenance team helped change the glass window the next day. In parallel, part of the team was mobilizing instruments such as pipettes, tips, vortex, centrifuges, hot water bath, ice-maker, distilled water to start the actual testing. Most of them were bought from the local suppliers.

**CONSUMABLES AND REAGENTS**

PCR reagents and Personal Protection Equipment (PPE) were made available by the hospital administration, through the aid from the Logistic Management Division, Teku as well as social organizations. (Fig 4)

**INITIATION**

The new laboratory was named Narayani Covid-19 PCR Laboratory. The team had received training and support from virology experts of NPHL. The first 23 samples for testing were collected from mass screening.

**SAMPLE COLLECTION AND TESTING**

Regular COVID-19 response of Narayani hospital involved investigation of suspected and confirmed cases, contact tracing and follow-up of the cases and their contacts. People staying in quarantine centers for 2 weeks or having a history of travel to COVID-19 affected countries or communities inside Nepal irrespective of the presence or absence of symptoms were identified along with documentation as per the protocol developed by the national reference laboratory. Oropharyngeal and/or nasopharyngeal samples were collected, stored and transported using standard Viral Transport Medium (VTM) reinforced by triple packaging, for SARS-CoV-2 testing in the laboratory.

The first PCR testing was done on Baisakh 20, 2077 (2nd May, 2020). At the end of the day, during result interpretation, we noticed no proper graph of internal control. The team had a quick communication with nearby laboratories. Fortunately, one of the experts from Provincial laboratory, Hetauda told us that they had the required buffer reagents left over from recent calibration and hence we sent the vehicle with cold chain box to bring it and the internal control reagent was prepared. The machine was retested with known positive sample.
MAJOR CHALLENGES FACED
The ambivalence in the results was a major issue in the beginning. There were no apparent flaws in the system; however, the flaw in the results could not be interpreted. Similar tests and results had to be repeated multiple times. There was a suspicion that the samples might have been contaminated with other samples. In order to address this issue, the core team started investigating for the potential suspects for the contamination.

The team ran the experiment again. However, the result was further frustrating as it showed that the contamination had not yet been removed. Our team concluded that this could be the result of the contamination of the entire laboratory for which the lab was closed for 24 hours to turn down faults and flaws. Following this, the team had to face extreme pressure from the public and the local government for the inability to cater test results in the given limited timeframe.

TROUBLESHOOTING: A. EXTRACTION ROOM
The entire extraction room, the safety cabinet and all materials including refrigerators were cleaned using hypochlorite solution and 70% alcohol to control contamination that may interfere with assay detection. The room was fumigated with aerial fumigator and neutralized. The safety cabinet was exposed to UV light for a couple of days.

TROUBLESHOOTING: B. AMPLIFICATION AND DETECTION ROOMS
In the amplification room, all the pipettes were calibrated as per the national standards and exposed to UV light; the door to the extraction room was closed and human movement was restricted, except for authorized persons. The results indicated that the source of the contamination was the master mix room and potentially the master mix cabinet, particularly the ill working internal airflow.

The team got a new laminar flow fitted with a Ultra-violet light from the hospital administration. The experiment was repeated, and the results were confirmed. The results were also re-confirmed in another laboratory.

PROGRESS
By mid-August, the laboratory was receiving continuous supply of samples from the different districts of Province 2 and was working in three shifts reaching a testing capacity of up to 600 samples per day. The laboratory adopted a sample pooling strategy with five samples pooled together and only pools that have tested positive were individually extracted while the negative pools were reported as negative samples. The laboratory core team was working on the expansion of human resources and providing training to other staff about biosafety processes and standard operating protocols. The new laboratory was now part of the national COVID-19 testing chain and was considered a preferred testing centre because of its convenient setup, reliable reporting and skilled staff. thousand positive samples.

LABORATORY LINKAGE
The laboratory coordinated the sample collection from health facilities, communities, and vulnerable groups. After sample processing, documentation was done using the Information management unit platform, results dispatched by email to Hospital administration, Epidemiology and disease control division (EDCD), Narayani COVID management team, District COVID crisis management centre (DCCMC) and then positive results were dispatched by the District police office, Parsa and District Public health office to all concerned authorities and negative results dispatched to patients via Short Message Service (SMS).

QUALITY CONTROL
NPHL acts as a reference lab for proficiency testing of COVID-19 PCR tests in Nepal. Five samples per lot were sent to our lab via courier. The samples were processed within 48 hours and the results were dispatched to NPHL within seven days of receipt of PT panel via email. NPHL reported the results as concordant or discordant within 15 days. Our lab results were declared concordant each time the samples were sent.

LESSONS LEARNED
Following lessons were learnt during the process: 1) Remodel and utilise existing spaces, 2) Cooperate with local and national health experts and equipment manufacturers to solve laboratory issues, 3) Optimally utilize existing human and equipment resources, 4) Create a contamination free space for COVID-19 tests.

CONCLUSION
Facing the challenges of the current pandemic and
best utilizing them as the opportunity, Narayani hospital laboratory has benefited from establishing a standard molecular laboratory which has utilized human resources and infrastructures within the hospital with empowerment, training and recruitment of the laboratory personnel and information managers. The experience from this scenario can be used in establishing other COVID-19 testing laboratories in minimal resource setting areas of Nepal in the present COVID-19 peak as well as possible future waves.

REFERENCES