# Charting the challenges behind the testing of COVID-19 in developing countries: Nepal as a case study

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ABSTRACT

The infrastructure needed to detect Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection (COVID-19) that complies completely with WHO guidelines is lacking across many parts of the globe, especially in developing countries, including Nepal. We outline the problems faced by such countries and suggest that the national and international community should collaborate in the development and adoption of novel protocols for the rapid detection of COVID-19 according to locally available infrastructure, in order to fight against the outbreak.

## 1. Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory tract infection caused by betacoronavirus SARS-CoV-2 [1]. The World Health Organization (WHO) has characterized COVID-19 as a pandemic [2], and according to WHO Situation Report No. 97, about 2.8 million individuals globally have been confirmed to be infected and >190 thousands have died by 26 April, 2020 [3]. While most people with COVID-19 develop a mild illness, approximately 14% develop severe disease requiring hospitalization and 5% need intensive care unit support [4]. Inadequate health care facilities to provide treatment and life support to a potentially exponentially increasing number of patients due to an outbreak in a community, can in turn lead to more disease transmission from untreated cases.

The WHO strongly recommends testing of suspects for COVID-19 and the individuals who have been in contact with them in order to control the infection in a community [5]. However, due to the high infectivity of the virus and lack of appropriate resources (e.g., testing laboratories, kits), most of the world is struggling to contain the spread of virus and is facing an increasing number of fatalities. Risk of mortality is much higher in developing countries with poor scientific and health infrastructure such as Nepal, and these countries are struggling to test for and track the infection due to a lack of laboratory facilities and trained manpower [6]. Lack of good laboratory practices

and safety information, as well as social prejudices about the disease are other barriers to the fight against the virus in the developing world. In this manuscript, we outline these problems and discuss strategies that can be adapted for the rapid detection of COVID-19 in developing countries.

## 2. Lack of equipped diagnostics with appropriate instruments and testing kits

Swift collection and testing of biological samples from patients meeting the suspected case definition for COVID-19 are critical for proper monitoring and control of disease infection in the community. The WHO has recommended the testing of COVID-19 infection using a reverse transcription polymerase chain reaction (RT-PCR)-based protocol to target different viral genes [7]. However, these testing protocols require RNA extraction kits, costly RT (quantitative) -PCR machines and trained technicians to operate them. These resources are limited in countries with poor scientific infrastructure, such as Nepal, where there was only one laboratory equipped to test for coronavirus infection [8], with an increase to three planned for the end of March 2020 [9]. The establishment of fully equipped testing aboratories that fulfil WHO guidelines would require huge investment, expertise and time, which are currently limited by the COVID-19 crisis. Alternative testing protocols that allows COVID-19 testing with limited resources and available manpower in the country, will be highly useful in the fight against the COVID-19 pandemic.

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**Table 1**
Comparison of various LAMP-based protocols for SARS-CoV-2 detection.

| Study | Analytical sensitivity | Sensitivity comparison to qPCR | Description of procedure | Visual Aid |
|---|---|---|---|---|
| Zhang et al. (US) [12] | Approx. 264 copies (Negative at 48 copies, positive at 480 copies) | Better than qPCR | RNA extraction is required. Reverse transcription and LAMP in the same tube. Crude cell lysate spiked with RNA could be directly used. | Naked eye |
| Lamb et al. (US) [13] | 1.02 fg of synthesized dsDNA | Comparable | Patient samples[a] spiked with positive DNA could be directly tested without RNA extraction. Reverse transcription and LAMP in the same tube. Uracil-DNA glycosylase added to decrease cross-over contamination. | Naked eye or fluorescence under UV light |
| Yu et al. (China) [14] | 10 copies | Slightly less than qPCR | RNA extraction is done separately. Reverse transcription and LAMP are done in the same tube. | Naked Eye |
| Park et al. (South Korea) [15] | 100 copies | Not reported | RNA extraction, reverse transcription and LAMP done separately. Dye added into the LAMP reaction tube. | Naked eye or Fluorescence |

fg = femtogram, qPCR = quantitative polymerase chain reaction.

[a] Serum, urine, saliva; oropharyngeal and nasopharyngeal swabs.

We suggest that colorimetric loop-mediated isothermal amplification (LAMP) can serve as an alternative COVID-19 testing protocol in developing countries. This method requires a centrifuge (e.g. for RNA extraction, if needed) and a heating system (e.g. a water bath for nucleic acid amplification) as the two major instruments, and these can be easily arranged, even in countries with limited scientific infrastructure [10]. LAMP is a novel nucleic acid amplification technique that amplifies DNA with high specificity, efficiency and rapidity under isothermal conditions (usually 65 °C) [11]. Recently, multiple pre-print publications [12–15] (Table 1) have reported the successful use of LAMP-based protocols to test for SARS-CoV-2 RNA in serum, urine and saliva, as well as oropharyngeal and nasopharyngeal swabs (Table 2), both with or without the requirement of viral RNA extraction [12].

Researchers have also used computed tomography (CT) scanning to diagnose COVID-19, demonstrating good diagnostic sensitivity (97%) relative to RT-PCR [16]. CT can assist in making a fast diagnosis, which can be helpful in the rapid isolation and treatment of patients. However, CT technology has inherent problems that limit its use in COVID-19 testing, especially in developing countries. First, it requires deep cleaning of the scanning suites to avoid potential contamination between examinations. This drastically slows patient throughput, a high level of which is needed

in limited resource scenarios such as those found in developing countries. Second, and more importantly, contamination of the CT machine can potentially result in the infection of COVID-19-negative patients during scanning. Third, the trained radiologists required to examine the scans and diagnosis patients with COVID-19 are drastically lacking in developing countries.

As an example, Nepal has very few working CT scanners relative to its 29 million population [17–19]. Developing countries have the fewest CT facilities per million population in the world, as the establishment of such facilities is costly [6]. However, at this point in the crisis, CT facilities dedicated to COVID-19 patients are required to diagnose suspected patients and meet the need for diagnostic resources in developing countries.

## 3. Lack of trained manpower

Lack of trained manpower capable of performing the molecular biology experiments (e.g. viral RNA extraction and qPCR) required to test for SARS-CoV-2 and interpreting the results is another major limitation in the testing and confinement of COVID-19 in developing countries. For the few laboratory experts present in these countries, priority is given to other medical emergencies (e.g. heart attacks, road accidents). We suggest that training

**Table 2**
Standardization criteria for laboratories in Nepal.

| Category | E | D | C (n = 223)[d] | B (n = 45) | A (n = 8) |
|---|---|---|---|---|---|
| Min.Space Types[a] | 14 sq. m. (150 sq. ft.) >HP > Private Labs | 23.2 sq. m. (250 sq. ft.) >PHCC > Private labs | 37.2 sq. m. (400 sq. ft.) >50- bed hospitals > Private labs | 93 sq. m. (1,000 sq. ft.) >51–200 -bed hospitals > Sub-regional hospitals > Upto 50-bed specialist hospitals > Specific stand-alone labs | 186 sq. m. (2,000 sq. ft.) >200 bed- hospitals > Medical college labs > Referral Labs > Regional Hospitals > 50- bed specialist hospitals > Super-specific stand-alone labs |
| Human Resources[b] | >Total Min. 2 | >Total Min. 4 > At least one Technologist | >Total Min. 6 > At least 50% Technologists > At least one Masters | >Total Min. 12 > At least 50% Technologists > At least 3 Masters | >Total Min. 24 > At least 50% Technologists > At least 4 Masters (one per discipline) |
| Tests[c] | Haematology Biochemistry Microbiology eg. AFB Rapid Diagnostic Test Miscellaneous | Haematology Biochemistry Microbiology eg. AFB Rapid Diagnostic Test Miscellaneous | Haematology Biochemistry Microbiology Rapid Diagnostic Test Miscellaneous Histo/cytology | Haematology Biochemistry Microbiology Rapid Diagnostic Test Miscellaneous Histo/cytology Molecular Level | Haematology Biochemistry Microbiology Rapid Diagnostic Test Miscellaneous Histo/cytology Molecular Level Super-specialized services |

[a] Health posts (HP) are most peripheral government unit providing lab services in Nepal followed Primary Health Care Centres are.

[b] Technologists have Bachelors degree in Medical Lab Technology.

[c] Types and complexitis of tests in each section increase by lab categories.

[d] Lab numbers based on registered labs at NPHL.

and workshops directed to COVID-19 testing and organized at a national and international level will be helpful in generating the necessary skilled manpower. The invitation of trained volunteers from the international community could be another option; however, in the scenario of a global epidemic and lockdown, receiving such rapid help from the international community is unlikely. Alternatively, the available manpower could learn from the expertise of the international community by video, audio, and other user-friendly tools and checklists developed by WHO and other international organizations, to make the process fast and efficient [20,21].

## 4. Biosafety labelling

Biosafety is the practice of safe methods for managing biological materials in the laboratory environment that are potentially hazardous or detrimental to health, to reduce or eliminate the exposure risk of laboratory workers and the outside environment [22]. Usually, biosafety in a laboratory is achieved by strictly following good laboratory operating practices (e.g., the prohibition of food, drink and smoking materials in the laboratory setting, use of biohazard warning signs); by the use of physical containment facilities (e.g., biological safety cabinets with high-efficiency particulate air filters, laboratory coats); by good laboratory design (e.g. easy to clean areas with no carpets or rugs); and by training in performing research involving biological agents. Biosafety is a major concern in the developing world as the mishandling of biomaterial has the potential to evoke another episode of pandemic.

The WHO recommends that all the requirements of Biosafety Level-2 (BSL-2) or their equivalent are met at facilities handling COVID-19-related specimens and performing non-propagative molecular testing [23]. In many countries, including Nepal, there are very few laboratory facilities that meet the BSL-2 requirements for highly infectious diseases [24]. Establishing BSL-2-compliant facilities within a short time in an international lockdown situation would be difficult.

Additionally, essential medical supplies, such as the gloves, masks, aprons, syringes and swabs necessary to maintain the safety of medical workers, are insufficient in developing countries. Lack of these supplies may hamper testing for the virus and further the spread the infection via poorly protected healthcare workers. The Nepal Ministry of Health [9] has recently authorized thirteen laboratories for COVID-19 disease-testing using PCR technology in the country which, considering that the population of Nepal is 29 million, is the equivalent to each laboratory serving 2.2 million people. The avian influenza control project had planned to construct eight laboratories (one central veterinary laboratory, five regional veterinary laboratories and one national avian disease investigation laboratory and one foot-and-mouth disease/trans-border animal disease laboratory) with BSL-2 facilities by 2011 [25]. According to a guideline produced by the National Public Health Laboratory (NPHL) [26] in Nepal, which is considered the authority on medical laboratory testing in the country, laboratories are categorized into five grades (A, B, C, D and E, with A denoting the highest and E the lowest category) depending on the available resources and the diagnostic facilities provided (Table 2). Currently, there are 8 category A and 45 category B registered laboratories in Nepal in the private sector that could potentially be used to test COVID-19, with the expert help of national and international communities [27]. Most (55%) of these are outside the capital and have manpower that can be trained. There are approximately 125 hospitals in the government sector across Nepal where laboratories can be used for simplified diagnostic techniques such as LAMP [28]. According to the guidelines, laboratory facilities of all categories are required to have an adequate water supply, uninterrupted electricity, appropriate sterilization facilities, and facilities for proper storage for transportation of biological and clinical samples. Given these facilities and the incorporation of additional facilities for RNA extraction (some versions of LAMP may not require RNA extraction) and appropriate biosafety, LAMP can potentially be performed even in D or E category laboratories that have a water bath or heat block.

Collaboration between international and national organizations (e.g., private hospitals, universities, medical colleges) would be helpful in establishing biosafety facilities and for provision of the necessary supplies in these countries. For example, contamination risk during sample collection, transportation and preliminary laboratory processing (e.g., RNA extraction) can be reduced by concentrating financial and technical efforts on specific laboratories outside the capital city Kathmandu to which patient samples can be transported within hours. In fact, the first ever COVID-19 case in Nepal was diagnosed by government collaboration with a basic science research institute in Nepal [29]. Similarly, additional training in good laboratory practices with respect to the highly infectious SARS-CoV-2 and the orientation of LAMP technology could be organized by the international community or national experts, generating skilled manpower to test for the infection at the available laboratories with BSL-2 facilities.

## 5. Social prejudice

Various prejudices exist among health workers in developing countries regarding epidemiological characteristics, animal reservoirs, risk factors and treatment strategies, particularly with false news and views now being circulated on social media platforms. The development of such notions from this type of news is demoralizing to health workers and may negatively affect the fight to diagnose and treat COVID-19. A campaign broadcasting clear, research-based scientific information and guidelines through authentic government media (e.g., radio, television) will help reduce stigma and misinformation around the virus.

## 6. Other factors

As well as enhancing manpower and infrastructure for testing of samples with suspected COVID-19, safety of patient biomaterials during storage and transportation both within a country and across international borders should also be secured. A proper channel involving the local health workers, transport machinery and laboratory scientists should be established in the light of international experiences (e.g., China). Furthermore, hotline-based contact with countries that are successfully fighting the virus (e.g., China) will also help in the fight against the outbreak.

## Conflict of interest statement

The authors declare that there are no conflicts of interest.

## Author contributions

A. K. Giri and D. RSJB Rana were both responsible for the conceptualization, data curation and writing the original draft. A. K. Giri supervised the data and edited the manuscript.

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