Virol Curr Res
Volume 5:1, 2021

Virology: Current Research

Commentary

Open Access

Establishing PCR Testing in Nepal for COVID-19: Challenges and Opportunities

Ram Bahadur Khadka*

Department of Microbiology, Pokhara University, Pokhara, Nepal

Abstract

Polymerase Chain Reaction (PCR) invented by Kary Mullis (1983), has become the centrepiece of molecular detection of various infectious diseases including coronavirus disease 2019 (COVID-19). Many developing countries like Nepal faces various challenges and grab many future opportunities during and after establishment of molecular PCR laboratories throughout the country. This viewpoint describes the involvement of laboratory employees, development and adoption of new protocols or framework, deliberate partnership with national and international community is very efficient for the establishment of PCR laboratories. Beside this, continued alliance and nation leadership is crucial to generate a unified and sustainable PCR laboratory network in the country like Nepal. In future the established PCR laboratories can be utilized for the diagnosis of others pandemic diseases and can be used for multipurpose like in verification of infectious diseases; Oncology; Blood test; Genetic testing.

Keywords: COVID-19 • Nepal • RT-PCR • Viral RNA • DNA polymerase

Introduction

The American biochemist Kary Mullis developed molecular Polymerase Chain Reaction (PCR) techniques in 1983 to create numerous copies of a positive DNA phase. Although this device is commonly used in molecular biology and biotechnology laboratories, its utility is broad in various fields of medicine, forensic science, research, and molecular genetics. Despite many other tools or tests available, this tool in the current scenario is considered a gold standard test for the detection of SARS-CoV-2 throughout the world [1]. Real-time, nested, multiplex, quantitative, long-range, fast cycle, hot start, specific methylation, solid phase, and arbitrary priming are the types of PCR that help generate hundreds of thousands of copies of a small DNA phase and assist in the analysis of gene expression.

This molecular assay consists of annealed DNA, DNA polymerase, oligonucleotide primer, dexoirribonucleotide triphosphate, a buffer system based mainly on the use of primer-mediated enzymes. DNA polymerase synthesizes new DNA strands complementary to the template DNA. DNA polymerase can only load one nucleotide in the pre-current 3'-OH group. Therefore, you need a primer that consists of 3 main cyclic reactions: denaturation, annealing, and elongation. The additional nucleotides are then brought into the 3'-OH group of DNA polymerase [2,3].

Development of the RT-PCR Laboratory in Nepal

Nepal is a small landlocked Asian country nestled between China and India [3]. During the COVID-19 outbreak in Wuhan, China, there were around 200 Nepalese students. The first case of COVID-19 was reported on January 23, 2020 in a 32-year-old student who returned from Wuhan University of Technology and presented at Sukraraj Hospital for Tropical and Infectious Diseases, Kathmandu on January 13, 2020.

At that stage, our largest central government laboratory, the National Public Health Laboratory (NPHL) in Kathmandu, lacked reagents and test kits for identifying SARS-CoV-2 and due to this high-cost test in Nepal The patient's nasopharyngeal swab sample was sent to Hong Kong and later became the initially registered cases of COVID-19 in South Asia. But by January 27, the initial COVID-19 diagnostic test in Nepal was conducted at the NPHL using a level 2 biosecurity cabinet. The SARS-CoV-2 specific RT-PCR primer was made accessible in early February 2020 at the Kathmandu NPHL with the help of the World Health Organization (WHO) to perform approximately 100 tests per day with Kit for RT-PCR tests [4]. After 23 March, the second case was reported while about 610 samples were taken at the NPHL. Subsequently, the potential of the RT-PCR test quickly spread across the country in several states.

Due to the increase in cases, the test facility for COVID-19 diagnosis of the RT-PCR test has been extended to BP Koirala Institute of Health Sciences, Dharan on March 29 and Pokhara on March 31, but on March 6. In April, the Nepalese government set up another 10 workshops for all in seven provinces, including the major cities of Dharan, Janakpur, Kathmandu, Dhulikhel, Hetauda, Chitwan, Pokhara, Bhairahawa, Surkhet and Dhangadi. In addition to this, a group from the Central Microbiology Department of Tribhuwan University has added other laboratories in four provinces targeting surkhet, Bagmati, TU Teaching Hospital, Bhairahawa, Janakpur to discover and test more COVID-19 cases. Also due to the increase in active cases, the RT-PCR laboratory was established on 10 April at the Koshi hospital, Biratnagar. On April 11, Bir Hospital and Teku Hospital began testing for COVID-19 [5,6]. To date, 62 RT-PCR laboratories for COVID-19 testing are established in different province of Nepal (Table 1 and Figure 1).

Corresponding Author: *Khadka RB, Department of Microbiology, Pokhara University, Pokhara, Nepal, E-mail: rambahadurkhadka00@gmail.com*

Received: December 29, 2020; **Accepted:** January 12, 2021 ; **Published:** January 19, 2021

Table 1. Province wise distribution of RT-PCR Laboratories in Nepal.

S. No	Province	Number of laboratory
1	Province No.1	7
2	Province No. 2	6
3	Bagmati Province	32
4	Gandaki Province	3
5	Lumbini Province	8
6	Karnali Province	4
7	Sudurpashchim Province	6
	Government	42
	Private	24
	Total RT-PCR Laboratories	66

Figure 1. Total numbers of PCR laboratories in different provinces of Nepal.

Challenges

TInsufficient resources, funding and the absence of automated RT-PCR machines limit rapid RT-PCR testing in Nepal. Nepal, for the most part, reliance on external vendors severely limits the scalability of COVID-19 tests. Nepal must compete with the most profitable international locations for entry into COVID-19 in vitro diagnostics. The main challenge includes the absence of an adequate research base and the actual identification of the human potential of the health laboratory service. In addition to this, due to the absence of biosafety level 3 and also facilitating molecular laboratories in the country, problems arise for diagnosing COVID-19. In addition, with limited resources, the government of Nepal applies key strategies to solve challenging situations by establishing PCR labs in all provinces, decentralizing testing through strategic plans that could ensure the best of the lab, increasing variety and capacity, laboratory workforce, confirmed protocol for joint verification and troubleshooting guide PCR verification protocols to recognize new technology verification and validation strategies [7-9].

It is also a major challenge for the government to maintain or expand the currently established RT-PCR laboratories for possible contribution in current and future pandemics. Despite being one of the most delicate tests for finding viruses, RT PCR have some challenging situations and drawbacks. The performance of RT-PCR is based on the appropriate array of viral RNA which is usually isolated from the nasopharynx specimen of patients. This becomes an important task because the concentration of viral RNA (viral load) alters substantially in different patients or even in a same patient at some point in the direction of infection. This will regulate the amount of viral RNA that the response begins to evolve with, thereby raising the possibility of a terrible false result.

On the other hand, the efficacy of collecting the swabs differs between people (people other than fitness workers) by intensifying variability in the concentration of viral RNA and raise the possibility of biased RT-PCR results. Some analysts are also discussing whether or not specimens should be limited to nasopharyngeal swabs. What different samples can be accumulated that can increase the throughput of total detection based primarily on RT-PCR? Sputum has been shown with the help of a testing institution to be the appropriate standard in conjunction with nasal swabs.

Additionally, Bronchoalveolar Lavage Fluid (BALF) can also be examined and evaluated for critically infected patients. However, BALF need a specified suction tool that cannot be maneuverer without the help of a professional operator, making it an inappropriate opportunity to control large numbers of population. In addition to viral load, false positives or negatives can also arise from an erroneous confrontation of the main pattern with an infection with the marketers who save him an adequate extraction of RNA or act as inhibitors of the fluorescent sign at some point of the PCR Table 2. This can be avoided with the help of an expedited warning at some point in the fitting pattern and minimal transit time to the verification centre after the swab series. Minor changes in the primers and probes used for PCR, and the change in samples used as reference controls, could also be a resource to minimize false positives and negatives in the effects of RT-PCR [7-10]. Therefore, major challenges also exist with regards to how assays are implied practically and how the result of assays are interpreted and used.

Table 2. List of PCR laboratories in different provinces with name, numbers and location.

Province	RT-PCR laboratories and location	Total numbers
1	BP Koirala Institute of Health Science, Dharan	7
	Koshi Hospital, Biratnagar	
	Mechi Hospital Jhapa	
	Provincial Public Health Laboratory 1, Biratnagar	
	Noble COVID Hospital, Biratnagar	
	Neuro COVID Hospital, Biratnagar	
	Birat Medical College, Biratnagar	
2	Provincial Public Health Laboratory 2, Janakpur, Dhanusha	5
	Gajendra Narayan Sungh Hospital, Rajbiraj, Saptari	
	Narayani Hospital, Birgunj	
	Bardibas COVID-19 Laboratory, Mohattari	
	Gaur Hospital PCR Laboratory, Rautahat	
Bagmati	National Public Health laboratory (NPHL), Kathmandu	30
	Sukra Raj Tropical and Infectious Disease Hospital, Kathmandu	
	Bharatpur Hospital COVID-19 Laboratory, Chitwan	
	Vector Borne Disease Research and Training Center (VBDRTC), Hetauda	
	Provincial Public Health Laboratory, Dhulikhel, Kavre	
	Dhulikhel Hospital Dhulikhel, Kavre	
	Patan Hospital, Lalitpur	
	Bir Hospital, Kathmandu	
	Tribhuvan University and Teaching Hospital (TUTH), Kathmandu	
	Nepal Police Hospital Laboratory, Kathmandu	
	Shree Birendra Army Hospital, Kathmandu	
	Nepal APF Hospital, Kathmandu	
	Star Hospital Laboratory, Sanepa, Lalitpur	
	Nepal Korea Friendship Municipality Hospital, Bhaktapur	
	Central Diagnostics Laboratory and Research Center	

	Bidh Lab, Lalitpur	
	Suriya Health Care Pvt. Ltd., Lajimpat, Kathmandu	
	KMC Hospital, Kathmandu	
	HAMS Hospital, Kathmandu	
	B & B Hospital, Lalitpur	
	Nepal Medicity Hospital, Lalitpur	
	Chitwan Medical College, Bharatpur, Chitwan	
	Prasuti Griha (Paropakar Maternity and Women's Hospital), Thapathali, Kathmandu	
	Decode Genomics and Research Center, Sinamangal.Kathmandu	
	Intrepid Diagnostic Center Pvt. Ltd., Thapathali, Kathmandu	
	Kirtipur Municipality, TU Biotech Corona Laboratory,	
	B.P. Koirala Memorial Cancer Hospital, Bharatpur, Chitwan	
	COVID-19 Diagnostic Laboratory, Leprosy Mission, Nepal Aanandaban Hospital, Lele, Lalitpur	
	Nepal Medical College Molecular Laboratory, Kathmandu	
	Dr. Iwamura Memorial Hospital Laboratory, Kathmandu	
Gandaki	Pokhara Academy of Health Science. Pokhara, Kaski	2
	Provincial Tuberculosis Center Laboratory, Pokhara, Kaski	
Lumbini	Provincial Public Health Laboratory, Bhairahawa, Rupandehi	8
	Bheri Hospital, Nepalgunj, Banke	
	Lumbini Provincial Hospital, Butwal, Rupandehi	
	National Path. Lab. And Research Centre, Pvt. Ltd., Butwal	
	Rapti Academy of Health Science, Dang	
	Bageshwori Diagnostic and Polyclinic, Nepalgunj, Banke	
	Nepalgunj Medical college, Kohalpur, Banke	
	Gulmi COVID-19 Laboratory, Gulmi	
Karnali	Surkhet Provincial Hospital, Surkhet	4
	Karnali Academy of Health Science, Jumla	
	COVID-19 Test Laboratory, Dailekh	
	Chaurjahari Municipality PCR laboratory,Rukum (west)	
Sudurpaschim	Dadeldhura Hospital Laboratory, Dadeldhura	6
	Doti Hospital, Doti	
	Baitadi Hospital Laboratory, Baitadi	
	Seti Provincial Hospital, Dhangadhi	
	Bajhang Hospital Laboratory, Bajhang	
	Kamalbazaar Municipality PCR Laboratory	

Opportunities

Perspective Molecular diagnostics includes the dimensioning of DNA, RNA, proteins, or metabolites to find genotypes, mutations, or biochemical modifications within the body. The goal is to check for unique fitness states or to see if there are blood, tissue or bone disorders. Molecular diagnostics, basically the evaluation of DNA and RNA at the molecular grade, is a rapidly developing enterprise, which became feasible with the help of using experience in human genome development, which has spurred the rise of the diagnostic business. Any largely gene-based total cure that is developed could lead to more effective results in the affected person if seen with the help of a molecular diagnostic monitor. The molecular diagnostics company is supporting to pressure the In Vitro Diagnostic (IVD) company and the agencies involved [11].

Discussion

Molecular diagnostics has enormous business power due to the fact that these controls are more effective at detecting viruses and infectious diseases, and they do so with a degree of confusion sooner than was previously possible. Personalized Medication now includes all controls that modify or personalize the remedy for a selected disorder in an affected person. Under this concept, any molecular diagnostic test that improves the experience of a health professional about the condition, susceptibility or predisposition to infection of an affected person can be considered personalized medicine [12]. The technological knowledge of molecular diagnostics represents a business possibility for diagnostic agencies that must be at the forefront of a personalized or individualized medicine, where healing procedures are adapted to the unique genetic structure of each one. These checks allow physicians to examine treatment results with the help of monitoring the molecular homes of a disorder. Rising Technology Knowledge the clinical diagnostics market plays a key role in the ever-changing landscape of drug discovery and fitness assistance. The platform's new technology and increased expertise in the human genome are driving improvements in molecular diagnostics. Science and business are built on the factor that genetic controls can optimize drug therapy, and ancillary diagnostics are touted as a way to better describe an affected person's desire or await the final scientific results of a selected drug. The main molecular diagnostic packages consist of: Verification of infectious diseases; Oncology; Blood test; Genetic testing [13].

Conclusion and Recommendation

First, nations should institutionalize knowledge and sources to robotically acquire and analyze data according to the capacity and capacity of laboratory networks across the country. This will speed up the choice of the centers most likely to reuse or update cash services; calculation of faster routes for the transport of models or supplies; and discounts on geographic regions with unsatisfied fitness service calls. Due to limited resources, Nepal needs to be aware of sources that can help in the rapid, mainly evidence-based, review and optimization of laboratory networks to respond to fitness emergencies. Second, nations must apply the best national laboratory control guidelines to provide a more reliable routine supply of effects at any stage of the national laboratory network beyond just laboratory accreditation middle degree. Finally, Nepal needs to reduce its dependence on external understanding for diagnosis. This discount calls for alternatives to reconfigure the closed testing facilities that will be available and expand Nepal's national capacity to produce excellent diagnostics.

Due to the scarcity of monetary sources in growing countries, partnerships between the public, personal and corporate sectors must be maintained if a well-prepared laboratory is to be built. The financing of our bodies must address monetary inequality and adequate stability must be defended that emphasizes infrastructure and laboratory control. There has to be a focus on correct identity and the use of incredibly reproducible techniques. The investment our bodies have in achieving the long-term education and training of target laboratory employees. Therefore, it is very crucial to carefully decide how to link such new assays and assimilate them within national diagnostic algorithm of the country. The successful implementation of these assays is dependent on basic partnerships in the international laboratory sector and ensuring that fully quality assurance programs are built-in each provinces laboratory network of the country.

There must be progressive communication between the laboratory and the physicians, which could affect the accuracy of the remedy for the disorder.

Policy makers and fitness service employees must recognize that a correct and timely identity is critical to the prevention and repair of the disorder. It could be very important to have a well-maintained scientific microbiology laboratory that uses current technical knowledge to manage infections. Building a molecular microbiology laboratory to offer fast, accurate, and reliable controls will help the team of healthcare workers deliver more powerful drug regimens, thereby reducing mortality, the ordinary expense of healthcare sources. Health and improve the best physical care services in growing nations. Nation should carefully establish well strategy from now to conduct the established molecular based diagnostic laboratories in order to cope with different microbial diseases, for forensic investigation, diagnosis of malignant diseases like leukemia and lymphomas, tissue typing, research application various gene sequencing etc. in future days so that it could run smoothly and heighten the health policy of nation even after COVID-19 pandemic subsides.

References

1. Heather, VanGuilder, Kent E Vrana, and Freeman WM. "Twenty-Five Years of Quantitative PCR for Gene Expression Analysis." *Biotechniques* 44(2008):619-626.
2. Tan Yong, Wah. "Molecular Biology and Pathogenesis of Coronavirus: Virus and Host Factors Involved in Viral RNA Transcription (Doctoral dissertation)."
3. Dahal, Girdhari. "Foreign Relation of Nepal with China and India." *J Politic Sci* 29(2018):46-61.
4. Basnet, Sangita, Sameena Koirala, Basu Pandey, and Janak Koirala. "COVID-19 Containment Efforts of a Low-Resource Nation: The First Four Months in Nepal." *Cureus* 12(2020):e8946.
5. Shrestha, Ranish, Shrestha Sunil, Khanal Pratik, and KC Bhuvan. "Nepal's First Case of COVID-19 and Public Health Response." *J Travel Med* 27(2020):taaa024.
6. Covid 19 Dashboard
7. Plüss, David Nicolas. "Enhancement and Application of a Risk Assessment Technique for Research and Teaching Laboratories." *EPFL* (2015):1-230.
8. Zuckerman, Arie J. "Principles and Practice of Clinical Virology." *John Wiley Sons* 6(2009):1042.
9. Teles, Fernando S, De Távora Tavira LA, and Da Fonseca LJ. "Biosensors as Rapid Diagnostic Tests for Tropical Diseases." *Crit Rev Clin Lab Sci* 47(2010):139-169.
10. Lewis, Ricki. "Human Genetics: The Basics." *Taylor Francis* 2(2016):1-206.
11. Brown, Matthew P, Lai-Goldman M, and Billings PR. "Translating Innovation in Diagnostics: Challenges and Opportunities." *Genomic and Personalized Medicine* (2009):367-377.
12. Berg N, Powers N, Jones M, and Harrison C, et al. "*Accessibility of Your Research*?"
13. Clark, William R. "The New Healers: The Promise and Problems of Molecular Medicine in the Twenty-First Century." *Oxford University Press* (1997).

How to cite this article: Khadka Ram Bahadur. "Establishing PCR Testing in Nepal for COVID-19: Challenges and Opportunities." *Virol Curr Res* 5 (2021) : 123.