Comparative Evaluation of Commercially available Rapid Diagnostic Test Kits for the use of Screening of Suspected Cases of Novel Corona virus infection in Nepal

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Acronyms

COVID-19	Novel Coronavirus
MERS	Middle East Respiratory Syndrome
rRT-PCR	Real-time Reverse Transcriptase Polymerase Chain Reaction
RDT	Rapid Diagnostic Tests HPLC
SARS	Severe Acute Respiratory Syndrome

Background:

Coronaviruses are a large family of viruses that mostly cause respiratory illness in humans. Recently identified corona viruses caused illness like Middle East Respiratory Syndrome (MERS), Severe Acute Respiratory Syndrome (SARS) and, most recently, Coronavirus Disease-19 (COVID - 19) [1,2]. The virus (which was initially called the novel Coronavirus 2019) has been named SARS- CoV2, and was first identified in the Hubei province in China [3]. The World Health Organization (WHO) has declared the COVID -19 as a global pandemic. More than 215 countries have been affected by the virus, with about 34, 89,053 individuals infected by it and the number of deaths has exceeded 241, 559 as of 3 May 2020 [4]. While various clinical features of the diseases have been documented (based on various individual case reports and case series), the complete clinical profile is yet to be understood. A recent study has shown the serial interval for COVID- 19 to be around 4 days, making silent transmission a potential threat for the spread of the disease [5]. Considering these facts, diagnostic tests play a vital role in identifying cases and preventing the further spread of the virus in the community.

The most widely used confirmatory test for COVID 19 is real-time reverse transcriptase Polymerase Chain Reaction (rRT-PCR). However, the rRT-PCR is a time intensive test and one that requires professional laboratory setting and skill manpower. Currently several countries have been using Rapid Diagnostic Tests (RDT) to screen for possible COVID 19 infection and epidemiological surveillance.

Rationale of the Study:

In Nepal, there are currently no RDTs that have been evaluated for efficacy against the most sensitive rRT-PCR test. In case of an outbreak, community screening of suspected cases needs to be done to establish the presence of COVID-19 transmission in the community. In this regard, RDTs would be an invaluable screening tool for a larger number of suspected cases, tracing contacts and preventing widespread community transmission of the virus, before final confirmation by rRT-PCR. This is especially important because early recognition of suspected patients allows timely initiation of appropriate Infection Prevention and Control measures. The

use of validated RDTs also helps in decreasing the burden on laboratories, in addition to early detection, case isolation and prompt management and treatment strategy.

The findings from this study will help the Ministry of Health and Population to develop policy regarding RDT kit use to detect and diagnosed cases of COVID- 19 in areas where RT-PCR is not practical or possible.

Research Objectives:

1. To evaluate commercially available RDTs for SARS-CoV2 (COVID 19) against the most sensitive and WHO/CDC standardized rRT-PCR.
2. To establish the laboratory based confirmation of the COVID19 cases among the clinically suspected referred cases.

Materials and methods:

Study Area:

The study was conducted in Sudoorpashchim Province of Nepal where there were an increasing number of COVID 19 suspected cases population who came from other countries to Nepal and a close contact community.

Study Design/ Sample Size

A hospital based quantitative cross-sectional study was conducted during ongoing outbreak of 2020 month of April. A total of 200 cases were enrolled in the study using consecutive sampling. Probable and suspected cases for COVID-19 admitted in hospitals (isolation/ quarantine for covid-19) were recruited for the study. In addition, a designated questionnaire was obtained from persons with travel history and close contact with laboratory confirmed positive cases living in quarantine for at least 10 days were enrolled in the study.

Case Definition:

A suspected case is a patient with acute respiratory illness with no other confirmed etiology to explain the symptoms and with a history of travel to/ from a territory that has reported local transmission of COVID-19, during 14 days prior to the onset of symptoms OR who has had contact with a confirmed or probable case of COVID -19 OR who requires hospitalization for his/her symptoms.

A probable case is a suspected case for whom the report from laboratory testing for the COVID-19 virus is inconclusive.[2]

A confirmed case is a person with laboratory confirmation of infection with the COVID-19 virus, irrespective of clinical signs and symptoms.2

Clinical Specimen

- 2-4 ml of blood samples were collected in gel tubes and serum were separated for the antibody detection of COVID-19.

- Subsequently, remaining serum samples were stored at -70°C to -80°C for future research purposes. All process for storage and handling of serum samples were carried out according to the standard protocol of WHO/ CDC guidelines.

- Specimen of respiratory system especially oropharyngeal swabs were collected with special precaution and well trained manpower according to WHO/ CDC guidelines for molecular test of rRT-PCR [7, 8, 9].

Rapid Diagnostic Test: Wondfo SARS-CoV-2 antibody test (Lateral Flow Methods) is an immunochromatographic assay used for rapid qualitative detection of IgM/IgG in human's whole blood serum or plasma samples against COVID-19 Infection. This is a medical diagnostic test that is easy to perform for preliminary or emergency medical screening of COVID-19 within 20 minutes. The test was performed according to Leaflets-Protocol provided from the manufacturer in the test kit packet.

Nucleic acid amplification tests (NAAT): Reverse transcriptase-polymerase chain reaction (RT-PCR) is used for the qualitative detection of nucleic acid in a given specimen (RNA virus). The test was performed as per WHO guidelines and protocol for laboratory testing of COVID-19. Real time polymerase chain reaction (RT-PCR) and RDTs was carried out in NPHL (National Public Health Laboratory) for evaluation/ validation (sensitivity and specificity) of test kit against SARS-CoV-2 infection.

Data analysis:

Data was entered in EXCEL cleaned, coded and analyzed using descriptive and analytical statistics.

Ethical Consideration:

Ethical approval was obtained from the Ethical Review Board of Nepal Health Research Council. Prior to enrollment in the study, all participants were informed as consent on aims, objectives and background of the study. Information was provided toward the risks and benefits of the current study. Similarly, a designated questionnaire and data were collected after obtaining returned informed consent. Anonymity and confidentiality of the participant were maintained

throughout the study. They have the right to withdraw from the study at any point, if they so wish, without any negative repercussions.

Results:

Background Characteristics

A total of 200 samples were assessed in this study. About 95% of participants enrolled were males and 5.5% were females. The mean age of the participants was 30.5 years. Among the participants 75% have a travel history of last-14 days and only 3.5% of participants don't have any travel history.

Table 1: Distribution of participants by age, sex, and travel history

Characteristics (N=200)	**N**	**Mean**
Age	196	30.5
Sex		**Percentage (%)**
Male	189	94.5
Female	11	5.5
Travel History (Last 14 Days)		
Yes	150	75
NO	50	25

Note. Out of 200 participants, 4 participants did not mention their age.

Sensitivity and Specificity

Compared to the reference standard (RT-PCR), the sensitivities of the Wondfo Kits were 50%, and the specificities were 99.5% (Table 2). Positive and negative predictive values of the test kit were 66.7% and 99% respectively. Similarly, 98.5% accuracy was found (Table 2).

Sensitivity: TP/ (TP + FN) × 100, 2/4× 100 = 50%

Specificity: TN/ (TN+FP) × 100,195/196 × 100=99.5%

Positive Predictive Value (PPV): TP/ (TP + FP) × 100, 2/3 × 100 = 66.7%

Negative Predictive Value (NPV): TN/ (TN + FN) × 100, 196/197 × 100 = 99%

Accuracy: TP+TN/TP+TN+FP+FN× 100, 197/200× 100 = 98.5%

Table 2: Comparative evaluation of RDTs and RT-PCR

RDTs (Test)	RT-PCR (confirmatory)		
	Positive	Negative	Total
Positive	2 (TP)	1 (FP)	3
Negative	2 (FN)	195 (TN)	197
Total	4	196	**200**

Findings from other countries

Exact reporting of sensitivity and specificity for the commercially available RDTs was not available publicly but announcement was made in their public domain and authenticate media. ICMR specified that a rapid diagnostics test (RDT) kit procured from Guangzhou Wondfo Biotech, Zhuhai Livzon Diagnostics in identification of Coronavirus has limited use. Although at the beginning this test showed good results in surveillance of the Coronavirus but over the period of time showed wide variation in the sensitivity of test as seen in various States of India. Therefore, ICMR decided returning those kits back to the suppliers and stopped its use in India [10]. Similarly, health officials from the Britain Suggested that RDT kit bought from the Wondfo Chinese Company were not delivering the effective results, so health officials defended the buying kit [11].

With the availability of limited data, WHO also does not currently recommend the use of rapid diagnostic tests for patient care, however conducting research and finding its potential utility is highly encouraged [12]. With the arise of unreliable results of the RDT kit manufactured from the WONDFO company, these company clarified to the Chinese state “product was intended only as a supplement for patients who had already tested positive for the virus"[13]. However, high accuracy (98.5%) of this test-RDT indicated that the test kit can be used for the emergency surveillance to define endemicity of COVID-19 infection in Nepal.

Conclusion:

Comparative evaluation of RDTs against confirmatory diagnosis of COVID-19 (RT-PCR) showed moderate sensitivity, but high specificity and desirable accuracy. The accurate diagnosis of people infected with the SARS-CoV-2 is essential to control the global spread of COVID-1. The existing clinical accuracy of rapid tests still needs to be stringently evaluated before they are authorized for the mass screening of COVID-19. Hence, RDTS could be used as complementary to the existing RT-PCR assays, which could lead to much better diagnosis of COVID-19 and provide additional information about the immune status of the cases and community as well as for rapid surveillance where there is no amenities of laboratory facilities for RT-PCR test.

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Annex:

Procedure for data collection

Inclusion Criteria

- Direct close contact with laboratory confirmed positive cases
- Patients with travel history admitted in hospitals for suspected COVID-19 infection (isolation ward / quarantine ward)
- Persons with travel history and are in quarantine for at least 10 days

Exclusion Criteria

- Seriously ill patients unable to provide samples.

Place: Quarantine place identified by local authority will be considered as a study place. Similarly, patients who are admitted in hospitals (isolation/ quarantine for covid-19) will be considered for the sample collection.

Sample collection: Sample collection process will be assisted by Provincial health directorate and other responsible authority (like EDCD and Ministry of social welfare-sudoorpaschim province).

Personal protective equipment (PPE): PPE will be arranged by Provincial health directorate and other responsible authority.

Accommodation and transportation: All the required arrangement will be facilitated by Provincial health directorate and other responsible authority.

Waste management: All forms of waste will be managed as defined in national guideline. Please consult with province label laboratory personnel for further details.

Sampling method:

Clinical Specimen: The sample was collected with the help of designated skilled lab personnel from each Province for COVID-19. Data collectors from Central have been assisted for all process.

Blood collection:

2-4 ml of blood samples was collected in gel tubes for serum separation, than serum was used for antibody test.

Storage and handling of blood samples:

All samples were stored at room temperature for 8 hours and tested immediately. In case of delay, the samples were stored at 2°C to 8°C till 7 days. Samples stored more than 7 days were excluded.

Respiratory specimen

Upper respiratory tract

Oropharyngeal swab (OP)

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.

Oropharyngeal swab (e.g., throat swab): Swab the posterior pharynx, avoiding the tongue.

Storage and handling of blood samples:

Store specimens at 2-8°C for up to ≤5 days after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below. In addition, remaining samples were stored at -70°C to -80°C for future study.