# Virologic Characteristics of Cases of COVID-19 in Northern Vietnam, January – May, 2020

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**Abstract**

Background: Vietnam confirmed its first case of SARS-CoV-2 infection on January 23, 2020 among travelers from Wuhan, China and experienced several clusters of community transmission until September. Vietnam implemented an aggressive testing, isolation, contact tracing, and quarantine strategy in response to all laboratory-confirmed cases. We report the results of SARS-CoV-2 testing during the first half of 2020 in northern Vietnam. Methods: From January through May, 2020, 15,650 upper respiratory specimens were collected from 14,470 suspected cases and contacts in northern Vietnam. All were tested for SARS-CoV-2 by real-time RT-PCR. Individuals with positive specimens were tested every 3 days until negative twice. Positive specimens from 81 individuals were cultured. Results: Among 14,470 tested individuals, 158 (1.1%) cases of SARS-CoV-2 infection were confirmed; 89 were imported and 69 were associated with community transmission. Most patients (122, 77%) became negative after two tests, with 11 and 4 still testing positive when sampled a fourth and fifth time, respectively. Among 81 specimens with Ct values <30, SARS-CoV-2 was isolated from 29 (36%). Seven patients testing positive again after testing negative had Ct values >30 and negative culture. Conclusion: Early and widespread testing for SARS-CoV-2 in northern Vietnam identified very few cases which, when combined with other aggressive strategies, may have dramatically contained the epidemic. We observed rapid viral clearance and very few positive results following clearance. Large scale molecular diagnostic testing is a critical part of early detection and containment of COVID-19 in Vietnam and will remain necessary until a vaccine is widely implemented.

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**Methods:** From January through May, 2020, 15,650 upper respiratory specimens were collected from 14,470 suspected cases and contacts in northern Vietnam. All were tested for SARS-CoV-2 by real-time RT-PCR. Individuals with positive specimens were tested every 3 days until negative twice. Positive specimens from 81 individuals were cultured.

**Results** : Among 14,470 tested individuals, 158 (1.1%) cases of SARS-CoV-2 infection were confirmed; 89 were imported and 69 were associated with community transmission. Most patients (122, 77%) became negative after two tests, with 11 and 4 still testing positive when sampled a fourth and fifth time, respectively. Among 81 specimens with Ct values <30, SARS-CoV-2 was isolated from 29 (36%). Seven patients testing positive again after testing negative had Ct values >30 and negative culture.

**Conclusion:** Early and widespread testing for SARS-CoV-2 in northern Vietnam identified very few cases which, when combined with other aggressive strategies, may have dramatically contained the epidemic. We observed rapid viral clearance and very few positive results following clearance. Large scale molecular diagnostic testing is a critical part of early detection and containment of COVID-19 in Vietnam and will remain necessary until a vaccine is widely implemented.

**Keyword:** COVID-19; SARS-CoV-2; real-time RT-PCR, viral culture; containment.

**Introduction:**

SARS-CoV-2 is the cause of coronavirus disease 2019 (COVID-19), first reported in Wuhan, China in late December 2019. As of September 2020, SARS-CoV-2 was responsible for over 25 million cases and nearly one million deaths(1)

Vietnam is a country of 97 million people which, despite its lower-middle income status, managed to limit spread of SARS-CoV-2, requiring eight months to reach 1,000 cases and seven months to record its first fatality. The early days of the pandemic in Vietnam were marked primarily by importations from China whereas the second cluster of cases was characterized by importation from Europe (2–6).

Vietnam hosts two National Influenza Centers (NICs) including one at the National Institute of Hygiene and Epidemiology (NIHE). NIHE coordinates influenza surveillance in northern Vietnam and has played a critical role in responding to the COVID-19 pandemic. In its role as a reference laboratory throughout the country, NIHE received some of the earliest specimens from suspected cases of COVID-19. Our objectives were to describe the virologic characteristics of these specimens received during January through April 2020 and to explore differences in these characteristics among cases derived from different sources.

**Methods**

Vietnam established a National Steering Committee on Prevention and Control of COVID-19 on 28 January 2020, six days after the first cases of COVID-19 were identified in the country (2). Subsequent guidelines issued by the Steering Committee on 19 February 2020 called for collection nasopharyngeal and oropharyngeal (NP/OP) swabs from suspected cases and close contacts (F1) of confirmed cases (F0) and these guidelines were harmonized with those from WHO in March 2020 (7). Confirmed cases of COVID-19 underwent repeated sampling every three days during hospitalization until they recovered clinically and had at least three negative results by real-time RT-PCR for SARS-CoV-2.

*Realtime RT-PCR testing* : NP/OP swabs were placed into viral transport medium (VTM) and maintained at 4 °C during transport to the NIC at NIHE during 24-48 hours (8). RNA was isolated from NP/OP using

the viral RNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions in biosafety level 3 (BSL3) containment laboratories. Realtime RT-PCR was conducted using SuperScript III One -Step RT-PCR system with Platinum Taq High Fidelity DNA Polymerase (Invitrogen, Carlsbad, CA-USA) with target of E, RdRp and N genes following WHO recommendations. We defined confirmed cases as those with cycle threshold (Ct) values less than 37 for at least two target genes (E and RdRp or E and N) of SARS-CoV-2 (9).

*Viral isolation:* Vero E6 cells were maintained in Eagle's minimal essential medium (EMEM) containing 5% (v/v) newborn calf serum (NCS); 100ul of real-time RT-PCR positive samples were inoculated onto Vero E6 cells and incubated at 37 °C. Viral growth was monitored by daily observation of cytopathic effect. All experiments with SARS-CoV-2 viruses were performed in biosafety level 3 (BSL3) containment laboratories (10).

**Ethical considerations**

The Ethics committee of the National Institution of Hygiene and Epidemiology approved this study.

**Results:**

Characteristics of specimens received

During January 23 through May 25, the NIC received 15,650 NP/OP specimens from 14,470 suspected cases from 28 cities and provinces in northern Vietnam. Samples were submitted from two types of suspected cases: those from 6,420 persons entering Vietnam from abroad (China during the first cluster and other countries during the second cluster of cases) and 8,050 from persons who were contacts of suspected or confirmed cases. During the first cluster from January 23 to February 25, 2020, 1,741 specimens (11% of all specimens) were collected from persons travelling from Wuhan, China, their families, and their close contacts. During the second cluster of cases from March 7 through May 25, 2020, we received an additional 13,909 (88.9%) samples with nearly two-thirds received from four locations (Hanoi, 5,366; Ha Giang, 1,603; Thai Binh, 1,118; Lai Chau, 1,011). (Table 1).

Detection of SARS-CoV-2 by Real-time RT-PCR

For the entire study period, 15,650 samples from 158 (1.14%) suspected and confirmed cases tested positive for SARS-CoV-2 (Table 2). Eighty-nine (56%) of these were detected among suspected cases imported from other countries and the remaining 69 (44%) were detected among community contacts of confirmed cases. Thirteen cases were confirmed during the first cluster among persons returning from China or their close contacts. Among the 158 confirmed cases, 143 (91%) were Vietnamese nationals and 96 (61%) were female, although we observed significant differences in the distribution of gender among imported cases (44/89, or 49% female) compared with cases detected among community contacts (52/69, or 75% female, $P<0.0009$ by chi-square). The median age was 41years (IQR: 3 months – 88 years old) for community contacts and 33 years (IQR :10 years -74 years old) for imported cases. Eleven (12%) of the 89 imported cases were only detected upon their second sampling while in quarantine.

Based on Ministry of Health guidelines, laboratory-confirmed cases required follow-up testing until at least two subsequent tests were negative. Most cases required three or four subsequent tests to meet this criterion, but we also observed some cases undergoing collection of 10-15 subsequent specimens (Table 3).

Correlation between Ct Values, Date of illness / Days since first positive sample, and Viral Culture results:

We analyzed the cycle threshold (Ct) values of 158 confirmed cases with SARS-CoV-2 infection with serial sampling during hospitalization until two consecutive negative results were obtained. The proportion of cases that tested positive declined with the number of times they were sampled. Among the 652 samples collected, 167 (26%) had Ct values less than 30 and 105 (63%) of these were identified at the first sampling. Among cases who were sampled a third and fourth time, only 12/124 (10%) and 6/60 (10%) cases, respectively, had Ct values less than 30 (Table 3).

We identified 99 positive specimens with Ct values greater than 30 including seven cases who tested positive again after having tested negative ("re-positives"). Cases with Ct values greater than 30 followed a pattern similar to those with Ct values less than 30: 84 (85%) cases were positive in the first three samples only and an additional 10 (11%) cases remained positive in one of the next three samples. One case was sampled 15 times with no positive results after the tenth sampling.

A total of 81/158 (51%) confirmed cases had appropriately stored samples with sufficient volume to be inoculated on Vero-E6 cells, from which we obtained 29 (36%) SARS-CoV-2 isolates. Of these, 20 samples had detectable cytophathic effects between 72 and 96 hours and an additional nine isolates were harvested after a second blind passage. We identified 28 samples with Ct values of less than 20 and, of these, 18 (64%) yielded culturable virus (Table 4). An additional 20 cases had Ct values of 20-25 and, from these, we were successful in culturing virus from 10 (50%). The additional nine isolates recovered during the second passage had Ct values of 25-30, suggesting a low load of viable virus. No viral isolates were recovered from samples with Ct values >30 (n=20).

**Discussion**

During the first five months of the COVID-19 epidemic in Vietnam, we characterized all upper respiratory specimens received by NIHE coming from cities and provinces in the north of Vietnam. Just over one percent of all samples received yielded positive results by real-time RT-PCR and yet, by the end of May 2020, fewer than 400 cases had been identified in total in Vietnam, with no deaths and with no community transmission after 14 April 2020.

Rapid scale-up and decentralization of testing was a key component to Vietnam's response to minimize entry and transmission of SARS-CoV-2. Another component of Vietnam's response was its implementation of centralized quarantine for all persons entering the country from affected countries since early February 2020 (4). Travelers in quarantine were required to provide upper respiratory specimens for testing upon arrival and before the end of the 14-day quarantine period. Among these travelers in quarantine centers, we identified 89 laboratory-confirmed cases, 78 (88%) of which were positive on their first sampling and 11 during quarantine. Lastly, quarantining and testing of first- and second-degree exposed community members was implemented to stop further transmission of virus from primary cases who had not been detected on entry and subsequent cases. These data suggest that centralized quarantine coupled with testing upon arrival and quarantine based on exposure rather than symptoms is an effective measure for preventing exposure of communities and transmission of SARS-CoV-2 imported from other countries.

Although viral culture remains the gold standard for confirmation of viral infection, because of shorter turn-around time and higher sensitivity, detection of SARS-CoV-2 by real-time RT-PCR is the accepted gold standard for detecting SARS-CoV-2 for purposes of isolation and contact tracing. Semi-quantitation of viral nucleic acids using Ct value can be used to select samples for virus isolation (2,10–12). We observed a strong correlation between Ct values and cell culture positivity rate (table 4). This suggests viral load data may be used as a rough proxy for the infectivity of infected patients.

There is concern about prolonged viral nucleic acid detection in samples from patients recovered from COVID-19. The large majority of these samples, both in the literature and in our collection, have high Ct values and so far culture attempts have not been successful. This observation supports the hypothesis that prolonged shedding or re-positivity of samples is not associated with continued replication, but rather an indicator of removal of damaged lung tissue containing intact stretches of viral RNA by coughing or ciliary transport.

Among these 158 confirmed COVID-19 cases, we also identified seven individuals with positive real-time RT-PCR results after two consecutive negative results within 15 days (Table 3). We were unable to culture virus from these specimens, all of which had Ct values greater than 33, suggesting that these cases represent viral remnants rather than infectious virus. These findings are consistent with findings from South Korea and China (13–15). Positive real-time RT-PCR results can be confusing for patients and hospital staff who understandably wish to prevent continued transmission, either among patients and healthcare workers or among the general community. Our findings should provide reassurance that patients with positive real-time

RT-PCR results with Ct values >30 more than 10 days after onset or first positive result and after having had a negative result are at extremely low risk of transmission. These findings also support a strategy of testing based on signs of clinical recovery, rather than a "test-of-cure" strategy.

Our study had several limitations. First, the specimens we received were collected as part of the national strategy for prevention and control of COVID-19 without accompanying systematic clinical metadata and we were thus unable to stratify between asymptomatic, mild and severe cases. Second, we were not able to systematically assess the possible duration of viral shedding because most of our cases were detected upon arrival, through contact tracing, and by the quarantine process. Thus, sampling times were determined by disease control staff in the field, rather than in the context of a rigorously designed study. Third, the source of specimens for viral isolation was upper respiratory tract specimens only. We did not receive any sputum or tracheal aspirate fluids, which may have different characteristics in terms of Ct values or culturable virus.

In summary, we describe here the virologic and epidemiology characteristics of cases of laboratory-confirmed COVID-19 in northern Vietnam from two clusters of cases during the first months of the pandemic. We determined that most cases that will be laboratory-confirmed are confirmed within the first few samplings. We also determined that most cases that are positive very late in their clinical course are extremely unlikely to represent active infection but, rather, remnants of viral RNA.

**Conflict of interest**

The authors declare there are no conflict of interest about this work

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