



## Original Research Article

## Suspected SARS-Cov-2 reinfections in health care workers from Assam, India: Are they true reinfections?

Gayatri Gogoi<sup>1</sup>, Mandakini Das<sup>2</sup>, Biswajyoti Borkakoty<sup>2,\*</sup>, Mondita Borgohain<sup>1</sup>, Anup Kumar Das<sup>3</sup>

<sup>1</sup>Dept. of Pathology, Assam Medical College and Hospital, Dibrugarh, Assam, India

<sup>2</sup>RVRDL, ICMR Regional Medical Research Centre, Dibrugarh, Assam, India

<sup>3</sup>Dept. of Medicine, Assam Medical College and Hospital, Dibrugarh, Assam, India



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## ABSTRACT

Frontline healthcare workers (HCWs) are repeatedly exposed to SARS-CoV-2 and chance of exposure to it are invariably high than any other category of population. In this study, we investigated suspected cases of SARS-CoV-2 reinfection among eight HCWs involved in COVID-19 healthcare duty in Dibrugarh, Assam. Diagnosis of SARS-CoV-2 was done by Real Time RT-PCR or Rapid Antigen Detection Test at AMCH, Dibrugarh and ICMR-RMRC, Dibrugarh. Cases who tested positive for SARS-CoV-2 by RT-PCR or RAT for the second time and with symptoms suggestive of COVID-19 were included in this investigation as suspected cases of reinfection. SARS-CoV-2 IgG Ab titre and immune status ratio was estimated using a commercial SARS-CoV-2 Ig Ab detection ELISA kit. All eight cases were asymptomatic in first episode of infection with a  $C_T$  value above 30 and were non-reactive for SARS-CoV-2 IgG Ab. The second episode was symptomatic and marginally severe in some cases with  $C_T$  value less than 30 and with positive SARS-CoV-2 Ab titre. Most asymptomatic cases with  $C_T$  value above 30 failed to elicit immune response during the first episode. This may suggest that SARS-CoV-2 in low amount might be harbored transiently as bystander in droplet particles before being expelled from the nasal cavity which can be detected by the highly sensitive Real Time RT-PCR test. They may be below the infectious dose that is necessary to cause a clinical or a sub-clinical infection and fails to illicit an immune response. It is therefore very important to critically analyze the suspected cases of reinfection to be labelled as true reinfections. In conclusion, not all resurgence of symptoms with positive SARS-CoV-2 result for the second time after recovery are true reinfections and may be labelled as retest positives rather than reinfections. Further, routine surveillance of SARS-CoV-2 Ab testing for HCW is recommended to ascertain their immune status as they are the frontline workers of managing COVID-19 patients and are highly exposed to SARS-CoV-2 infection, and have a much higher risk of re-infection than the general public.

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## 1. Introduction

COVID-19 which started in mid-December 2019 in Wuhan city of China, has evolved into a global pandemic, involving more than 213 countries with more than 51.8 million cases globally and over 1.28 million deaths.<sup>1</sup> These figures continue to change every single day with more cases and deaths being reported from different parts of the world.

As COVID-19 cases continue to rise worldwide, experts around the world are faced with a critical question: whether true COVID-19 re-infection occurs? This raises questions on the prospects of vaccine and its ability to protect the population from the disease. Cases with suspected or possible reinfection with SARS-CoV-2 have been recently reported in different countries.<sup>2-4</sup> In many of the reinfection cases, it is uncertain if the individual's reverse transcriptase Polymerase Chain Reaction (RT-PCR)

\* Corresponding author.

E-mail address: [biswaborkakoty@gmail.com](mailto:biswaborkakoty@gmail.com) (B. Borkakoty).

test remained positive for a long period of time following the first episode of infection or whether it represents a true reinfection.

Retest positive after 5 to 13 days of two consecutive negative tests on completion of quarantine protocol and recovered COVID-19 cases has been reported as early as February 2020 from Wuhan city, China [Lan L, 2020]. Cases of COVID-19 reinfections has been reported from many countries of the world including Hong Kong in August 2020, where in a 33 year old man immunocompetent man was tested positive twice for SARS-CoV-2 virus in about four and half months after the first infection. The reinfection was however less severe and the patient was asymptomatic.<sup>2</sup> Further cases of reinfection were reported from Nevada, USA in a 25year old man who was reinfected with two phylogenetically distinct strain of SARS-CoV-2 virus in a span of 48 days.<sup>3</sup> In late August and early September, news report of COVID-19 reinfections surfaced from different parts of India-Mumbai, Delhi and Hyderabad. Gupta et al., reported two cases of reinfection from Delhi, India, both in immunocompetent health workers posted in the COVID-19 unit at a tertiary hospital in India. The patient remained asymptomatic during both the episodes, however, genomic analysis revealed that it was reinfection caused by two different strains. Similarly, in Mumbai, reinfection was reported among four Health care workers who were SARS-CoV-2 RT-PCR positive in May/June and then again in July and reportedly both the episodes of reinfection were due to a different strain of the SARS-CoV-2 virus.<sup>5</sup>

Health-care workers (HCWs) who have been on the frontlines of managing COVID-19 patients are highly exposed to SARS-CoV-2 infection, and have a much higher risk of infection than the general public.<sup>6</sup> As on 2<sup>nd</sup> September 2020, according to Pan American Health Organization data, COVID-19 has infected some 5,70,000 health workers and killed 2,500 in the US alone. In India as of 29<sup>th</sup> August, approximately 87,176 infections have been documented among HCWs and around 573 have succumbed to COVID-19.<sup>7</sup> HCWs are exposed frequently to patients with varied clinical severity and possibly higher viral loads at different times points which poses a threat to HCWs for a COVID-19 infection than other group of populations.<sup>8</sup>

Infection caused by virus is mediated by the T lymphocytes which gets activated immediately when the pathogen presented by the antigen presenting cell is recognised. In the moment of activation, production of inflammatory mediators (IFN- $\gamma$ , TNF- $\beta$ , IL-1, IL-6, CCL2) and the production of perforin and granzyme B usually occurs. In more severe cases, high pathogenesis is observed caused by an intense inflammatory process (release of inflammatory mediators: IFN- $\alpha$ , IL-1 $\beta$ , IL-6, TNF- $\alpha$ , CCL2, CCL5, among others), responsible for the development of lung injuries, which culminates in respiratory failure, organ failure and death.<sup>9,10</sup> With a

constant stimulus caused by the virus infection, these cells continue to produce inflammatory mediators to reduce viral replication, however, this process causes tissue damage that evolves into an intensified pathogenesis.

Immunity plays an important role in preventing reinfections. The development of immunity to natural infection is a multi -step process.<sup>10</sup> The non-specific response is followed by an adaptive response where the body makes antibodies that specifically bind to the virus. B cells (Antibody response) produce antibodies that are specific to that virus. IgM antibodies can be detected as early as 3 days after infection and provides the first line of humoral immunity defence, after which high-affinity IgG responses are initiated and play a key role in long-term immune memory. Study by Hao. et al., 2020 reported that in COVID19 patients, IgM antibodies were generated 1 week after onset of symptoms and reached its peak in 2-3 weeks after which the level decreased. Meanwhile, IgG levels increased quickly beginning a little later compared with IgM and were maintained at a high level for 2 months. Therefore, the detectable levels of IgM and IgG antibodies could provide information regarding serological convention over the disease course, as the detection of IgM antibody indicates a recent exposure to SARS-CoV-2 and the detection of IgG antibody in the absence of detectable IgM antibody indicates prior virus exposure.<sup>11</sup>

In this report, we summarize the investigations on the case reports of eight HCWs who were involved in COVID-19 duty and were suspected to be re-infected with SARS-CoV-2 virus. Elucidating the characteristics and analyzing the suspected cases of reinfection is very crucial as it could impact our understanding as to which cases to be labelled as true reinfections.

## 2. Materials and Methods

### 2.1. Study design and participants

Eight HCWs from Assam Medical College & Hospital (AMCH) and ICMR-Regional Medical Research centre for NE Region, Dibrugarh who had tested positive for SARS-CoV-2 RT-PCR [RAT] in June to August 2020 and again tested positive by Rapid Antigen Test (RAT) or RT-PCR when they developed symptoms suggestive of COVID-19 in subsequent times were included in the study. Based on the RAT/ RT-PCR results and clinical presentation of the HCWs we suspected reinfection with SARS-CoV-2.

The Microbiology laboratory at Assam Medical College is a reference molecular laboratory for Dibrugarh and two other neighboring districts and is one of the tertiary care hospitals assigned for SARS-CoV-2 testing. Clinical data was obtained from infected HCW database of AMCH, accessed by first author and by direct interview with cases. The study was undertaken between 15<sup>th</sup> August 2020 to 31<sup>st</sup>

October 2020 to investigate and complete follow-up. Total 365 HCW were tested positive by RT-PCR and Rapid tests till 15<sup>th</sup> August 2020 at the said hospital.

The re-investigation plan of the suspected reinfections was decided with Regional viral research and diagnostic laboratory (VRDL), ICMR-RMRC Dibrugarh, which is a ICMR designated laboratory for COVID-19 diagnosis in Northeast India. Written informed consent was taken from all participants involved in the study.

## 2.2. Sample collection

Nasopharyngeal (NP) and oropharyngeal (O) samples were collected from eight HCWs in Viral Transport media (VTM) tubes and tested in the concerned laboratory.

## 2.3. Nucleic acid extraction

One of the aliquots was used for automated RNA extraction using MagMAX<sup>TM</sup>-96 Total RNA Isolation Kit, (Thermo Fisher Scientific, USA) as per the kit protocol.

## 2.4. SARS-CoV2 detection by Rapid Ag detection Kit

Detection of SARS-CoV2 was done by standard Q COVID-19 Ag, SD Biosensor, South Korea / India.

## 2.5. SARS-CoV-2 detection by Real Time PCR

All samples were tested by multiplex real time RT-PCR TaqPath<sup>TM</sup> COVID-19 RTPCR kit from Applied Biosystems (Thermo Fischer Scientific, USA) or TRUPCR<sup>®</sup>SARS-CoV-2 RT qPCR kit, Kilpest India Limited whichever was available in the laboratory during the investigation time period.

## 2.6. SARS-CoV-2 IgG Antibody testing

Estimation of SARS-CoV-2 Ig Ab titre level was done by SCoV-2 Detect<sup>TM</sup> IgG ELISA kit from InBios International, Inc. USA The immunological status ratio (ISR) was calculated from the ratio of the optical density (OD) obtained with the test sample divided by the calculated cut off value.

## 3. Results

All eight cases were laboratory confirmed SARS-CoV-2 positive for the first time by RT-PCR. All were asymptomatic in the first episode of infection except case no 5 with mild headache (Table 1). None of the cases had history of other concurrent chronic disease as comorbidity (Table 1).

Case 1 ZPS: He is a faculty posted in COVID-19 patient care duty in wards and ICU. After being positive on July 24, 2020 by Real Time PCR ( $C_T$  value-33/34) (Table 1). He was kept in isolation for 9 days and treated with vitamin

B complex, vitamin C and zinc oral form. His routine blood and biochemistry along with chest x-ray findings were normal. There was no history of post COVID-19 fatigue symptoms. Then he started COVID-19 emergency duty after mandatory negative RT PCR on 25/07/2020. He tested positive for second time on 14/09/2020 by RAT with symptoms of fever, cough, headache, malaise. His spouse and baby boy were also positive together with him. He was identified for investigation for suspected case of re-infection and blood for antibodies and swab for RT PCR collected on September 18, 2020 and tested in Regional VDRL RMRC[ICMR] laboratory. He was tested positive by Real Time PCR with a  $C_T$  value of 27/25. His blood serum sample was collected on the day of second suspected reinfection to check for previous infection and he was non-reactive for SARS-CoV-2 IgG Ab. After 28 days from second suspected reinfection, blood serum was collected and again tested for SARS-CoV-2 IgG Ab and it was positive with a titre OD value of 3.2 and ISR 12.9.

Case 2 PKB: He is a junior doctor posted in COVID-19 patient care duty in wards and ICU. After being positive on July 19, 2020 by Real Time PCR ( $C_T$  value 32/31) (Table 1) he was kept in isolation for 9 days and treated with vitamin B complex vitamin C and zinc oral form. He was asymptomatic and his routine blood and biochemistry along with chest x-ray findings were normal. There was no history of post COVID-19 fatigue symptoms. Then he joined hospital and re-joined duty. He was retest positive for second time 13/09/2020 by RAT with symptoms of fever, cough, headache, myalgia, rhinorrhoea, anosmia. He was identified for investigation for suspected case of re-infection and blood for antibodies and swab for RT PCR collected on September 18, 2020 and tested in Regional VDRL RMRC[ICMR] laboratory. There was no detection of antibodies and RT PCR was negative. Another follow up tests was done on 6/10/2020 and found increased antibody value with titre OD of 2.71 and ISR of 10.79.

Case 3 SCP: She is a staff nurse posted in COVID-19 ward of AMCH, Dibrugarh. On routine screening of health workers, she was found to be positive on August 13, 2020 by RT-PCR. She was kept in isolation for 9 days and as she was asymptomatic with normal routine biochemical test reports she was treated with Vit B complex, C and Zinc formulation. She joined hospital after testing negative by RAT and rejoined duty. She retested positive for the second time by RAT on September 22, 2020. This time she was mild symptomatic with fever, headache and muscle fatigue and weakness. She was kept in isolation in cabin and treated with Oral Doxycycline, Ivermectin and Dexamethasone. Blood serum collected on September 23, 2020 was non-reactive for SARS-CoV-2 IgG Ab and follow up test on October 21, 2020 showed a positive OD titre of 1.16 and ISR of 4.48.

Case 4 DBH: She is a medical doctor posted in Obstetrics & Gynaecology ward. On routine screening, she was found to be positive for SARS-CoV-2 on July 26, 2020 by RT-PCR. She was asymptomatic and her  $C_T$  value was 31/32. She stayed in isolation for 10 days and was RAT negative on August 4, 2020. She joined her duties and again was retest positive on August 28, 2020 by RAT after she complained of fever, bodyache and weakness. She was hospitalised and treated with Oral Doxycycline, Ivermectin and Dexamethasone. Her lymphocytes count was low (8%). Serum after first exposure was non-reactive for SARS-CoV-2 antibodies. Follow up after 20 days for SARS-CoV-2 IgG Ab was reactive with positive titre of OD 0.554 and ISR 2.2.

Case 5 SMC: She is a staff nurse involved in COVID 19 duty in wards and ICU. After being positive for SARS-CoV-2 virus on August 13, 2020 by RT-PCR ( $C_T$ -value 32/33), she was kept in isolation for 9 days and treated with vitamin C and Zinc oral. She had mild headache and her routine blood and biochemistry along with chest-X ray findings were normal. She was RAT negative on August 28, 2020. There was no history of post COVID-19 fatigue. She resumed her COVID-19 duty and she was again tested positive for second time on September 21, 2020 during routine screening of HCWs by RAT. She was symptomatic during the second episode with fever, weakness, breathing difficulty with SpO<sub>2</sub> level 90-91 followed by intensive care. She was treated with low molecular weight Heparin, Remdesivir and Methylprednisolone as per treatment regime for severely symptomatic patients (Table 1). She was investigated for suspected case of reinfection and blood for antibodies was collected after 20 days and was reactive for IgG antibodies with OD of 1.16 and ISR of 4.48.

Case 6 NPS: He is a scientist at ICMR-RMRC, Dibrugarh. He tested positive for SARS-CoV-2 on July 19, 2020, by Real Time PCR ( $C_T$ -value 33/34) after contact tracing with a known positive. He was asymptomatic and was in isolation and tested negative by RT-PCR on July 28, 2020. There was no history of COVID-19 related symptoms. He again tested positive ( $C_T$  value 21/22) on September 15, 2020 after attending for his family members who were positive for SARS-CoV-2. His blood was collected to check for antibodies against his previous infection and he tested negative for SARS-CoV-2 antibody. During his second episode, he was symptomatic and developed fever 101° F which continued for three days with SpO<sub>2</sub> level 93-95% and cough, malaise, anosmia. He was on antivirals, antibiotics, steroids. His lymphocytes count was 6%. He tested negative on September 28, 2020 by Real Time RT-PCR. He is currently having post viral fatigue symptoms with weakness, fatigue and malaise. He was identified for suspected case of re-infection and blood for SARS-CoV-2 antibodies was collected on October 6, 2020 tested positive for SARS-CoV-2 antibody with titre OD value 3.07 of and

ISR of 12.23 (Table 1).

Case 7 AT: He is a junior doctor posted in COVID-19 patient care duty in wards and ICU at AMCH, Dibrugarh. After being positive for SARS-CoV-2 virus on July 22, 2020 by RT-PCR ( $C_T$ -value 33/34), he was kept in isolation for 9 days and treated with oral vitamin C and Zinc. He was asymptomatic and his routine blood and biochemistry along with chest X-ray findings were normal. He was RAT negative on July 31, 2020. There was no history of COVID-19 related or post COVID fatigue symptoms. He resumed his COVID-19 duty and he was retest positive for second time on August 23, 2020 during routine screening of HCWs by RT PCR ( $C_T$  value 34/35). He was asymptomatic during the second episode and did not require any intensive care. All routine Blood, Biochemistry parameters were within normal limit. He was investigated for suspected case of re-infection and blood for antibodies and swab for RT PCR was collected on September 18, 2020 and was non-reactive for SARS-CoV-2 antibodies and RT PCR was also negative (Table 1).

Case 8 BKD: He is a junior doctor posted in COVID-19 patient care duty in wards and Fever Clinic. After being positive first time by Real Time PCR ( $C_T$  value 29/30) on August 13, 2020 (Table 1), and was asymptomatic, kept in isolation for 9 days and treated with oral vitamin C and Zinc. His routine blood and biochemistry along with chest X-ray findings were normal. He was isolated with another SARS-CoV-2 infected symptomatic doctor in a cabin. On August 20, 2020, he tested negative by RAT. Immediately next day he developed fever of 100 degree F along with cough and was RAT positive. He was hospitalized again till August 31, 2020. He is currently having post viral fatigue symptoms. He investigated as a suspected case of re-infection and blood for antibodies and NS/OS swab for RT PCR were collected on September 18, 2020. There were no detection of antibodies and RT PCR test was also negative. Follow up for Antibody detection was done after 25 days blood serum was tested for SARS-CoV-2 IgG Ab, however he was still non-reactive. (Table 1).

#### 4. Discussion

There is rising concern that patients who recover from COVID-19 may be at risk of reinfection. In this study, we report investigation on suspected reinfection cases with SARS-CoV-2 virus among eight HCWs in Assam. While the clinical presentation varied between the HCWs and between episodes in the same HCW, it was noteworthy that in all eight HCWs the first episode was asymptomatic or mildly symptomatic and the second episode was marginally more clinically severe than the first. Another strikingly similar observation was that the RT-PCR  $C_T$  value for the first SARS-CoV-2 positive episode was above 30 which is high meaning low viral load.

**Table 1:**

Case	Age / Sex	Co-morbidity	Symptoms	Date (+ve)	Type of Test	Date of (-Ve)	SARS-CoV-2 IgG Ab
Case 1 ZPS (first exposure)	36Y/M	Nil	Asymptomatic	23/7/20	RT PCR CT 32/33	04/08/20	Non-Reactive
Case 1 ZPS (second exposure)			Cough Fever Bodyache Weakness, palpitation	14/09/20	RAT	21/09/20	Reactive OD3.29 ISR 12.9
Case 2 PKB (first exposure)	27Y/B	Nil	Asymptomatic	19/08/20	RT PCR Ct 31/32	28/08/20	Non-Reactive
Case 2 PKB (second exposure)			Fever, Anosmia, headache weakness	13/09/20	RAT	20/09/20	Reactive OD2.7 ISR 10.79
Case 3 SCP (first exposure)	31y/F	Nil	Nil	13/8/20	RT-PCR CT-31/32	22/8/20	Non-Reactive
Case 3 SCP. (second exposure)			Fever, headache, weakness	22/09/20	RAT	30/09/20	Reactive OD 1.16 ISR 4.48
Case 4 DBH (first exposure)	26Y/F	Nil	Nil	26/7/20	RT-PCR CT-31/32	4/8/20	Non-Reactive
Case 4 DBH (second exposure)			Fever weakness, Bodyache	28/08/20	RAT	04/10/20	Reactive OD 0.554 ISR 2.2
Case 5 SmC (first exposure)	41y/F	Mild HT*	Headache	13/8/20	RT-PCR CT-32/33	23/8/20	Non-Reactive
Case 5 SmC (second exposure)			Fever weakness, Breathing difficulty in breathing SPO 90-91	21/09/20	RAT	6/10/20	Reactive OD 4.85 18.68
Case 6 (first exposure)	37 years	Nil	Asymptomatic	20/07/20	RT-PCR	28/07/20	Non-Reactive
Case 6 (second exposure)			Fever, headache, body pain, malaise, anosmia, cough, SpO2 level 94-95%	15 /09/20	RT-PCR Ct-value 21/20		Reactive OD 3.07 ISR 12.23
Case 7 AT (first exposure)	26 /M	Nil	Asymptomatic	24/07/20	RT PCR CT-35/36	01/08/20	Non-Reactive
Case 7 AT (second exposure)			Fever, Cough,	23/8/20	RT PCR CT value 34/35	31/08/20	Non-Reactive
Case 8 BKD (first exposure)	31 y/M	Nil	Asymptomatic	13/08/20	RT PCR Ct-30/31	22/08/20	Non-Reactive
Case 8 BKD (second exposure)			Fever, cough bodyache	23/08/20	RAT	31/08/20	Non-Reactive

For all the HCWs, the suspected second episode of reinfection was symptomatic with clinical manifestations that lasted for longer period than the first and with a RT PCR  $C_T$  value less than 30. The  $C_T$  value of RT-PCR correlates with viral load, and low  $C_T$  values (high viral load) might indicate infectiousness of the individual. Although  $C_T$  values can vary substantially between various test kits and laboratories, however a study by Singanayagam A et al.; 2020<sup>11</sup> reported that samples with  $C_T$  values greater than 35 were only 8% positive for cultivable virus. A good estimate of the virus can be obtained through viral plaque assays that measure the infectious virus.<sup>12</sup> However, these assays require biosafety level 3 facilities and are labour intensive, and the assays are not routinely done in clinical laboratories. This was a limitation in our study as we could not exactly determine the viral load of the cases. Secondly, another limitation of the study was that since we did not have access to the samples from the first episode of infection therefore Genetic sequencing of the virus could not be done.

Studies indicate that SARS-CoV-2 infection induces both a neutralizing antibody response, a cellular response with virus-specific T cells.<sup>13–15</sup> A person who recovers from COVID-19 appear to have memory B and T cells. However, not all individuals seroconvert, milder infections may have less robust immune response and antibody titers may decline with time.<sup>11,13–15</sup> In our investigation, we have noticed that all HCWs whose blood samples were collected at least 25 to 30 days of first infection did not show any antibody against SARS-CoV-2. This raises question firstly on the theory on reinfection and secondly on the immunity of the patients. It is true that person with weakened immune system may fail to develop antibody response but it is most unlikely in these cases as they are young individuals with no prior history of any disease. Further, the antibody titre of the HCWs after 28 days of suspected second episode showed positive antibody response with ISR as high as 12 to 15.

In this investigation of suspected reinfection cases, we have seen that Case 1,2,3,4,5,6 were asymptomatic SARS-CoV-2 positive during the first episode with no IgG Ab against even after 25 to 30 days of first infection. This may imply either the first test was a false positive or that the infectious virus particles might have been harboured in the nasal cavity which got detected in the nasal swab sample collected for SARS-CoV-2 diagnosis during routine surveillance by Real Time PCR or RAT. A negative IgG Ab titre implies that the infection failed to evoke any immune response or the antibody levels were so low that it was below the level of detection of the test. However, during the second episode of exposure, the SARS-CoV-2 IgG Ab titre levels and ISR were high. Case 5 and Case 6 had severe symptoms during the second episode. The Spo2 level in case 6 was 93-94 and in case 5 it was 90 -91 and required intensive care. Further lymphopenia was observed in case 5 and case

6. Lower lymphocyte count or lymphopenia is reported to be associated with increased mortality, ARDS, need for ICU care, and severe COVID-19.<sup>16,17</sup> The SARS-CoV-2 Ab titre in these two cases were significantly higher than the others. This is in sync with earlier reports where it has been reported that severe cases evoked higher antibody titres and mild cases had lower antibody titres.<sup>16,17</sup>

Another observation we have seen is that case 7 and case 8 who were both asymptomatic in first episode and mildly symptomatic in the suspected second episode failed to develop any immune response after the suspected second episode of SARS-CoV-2. The SARS-CoV-2 Ab test was negative in both episodes which makes them outlier in this scenario. The reason for negative result might be that they were false positive in both the episodes. Since they have no known co morbid conditions and immune associated disease the most likely explanation for these cases is that since they both are HCWs actively involved in COVID-19 duty, chances are that they might have harboured virus as a temporary bystander in droplet particles in their nasal cavity which got detected by the highly sensitive Real Time RT-PCR but it was in such low titre that it failed to cause a clinical or subclinical infection. Since in both the episodes they were asymptomatic or mildly symptomatic with normal biochemical test results, it is likely that these two cases were false positive in RT-PCR test or virus infecting dose in the nasal cavity was not sufficient to cause an infection.

There is a probability that a fraction of the asymptomatic cases may not evoke an immune response/ antibody response against the virus and they may remain susceptible to infection on further exposure. Therefore, asymptomatic SARS-CoV-2 positive cases need to be monitored for Ab titre as they may fail to develop immunity against the virus and might again get reinfected on further exposure.

As a significantly large number of health care workers are infected and are asymptomatic, without surveillance, it is difficult to ascertain the true extent of infection. Since HCWs are in close encounter with patients on a regular basis, it is interim to do surveillance of SARS-CoV-2 antibody among HCWs. As seen in our case investigation, those cases who are asymptomatic with a  $C_T$  value above 30 did not develop antibodies and are prone to re-infection. Chances of reinfection cannot be ruled out in the community,<sup>18–20</sup> but in asymptomatic cases with  $C_T$  value more than 30, the virus is more likely a temporary bystander virus particle detected in the nasal cavity which fail to evoke a clinical or sub-clinical infection and so did not elicit any immune response. Therefore, person who is retest positive for the second time cannot be labelled as re-infection for SARS-CoV-2.

## 5. Conclusion

While this study raises important questions, we are mindful that in the context of millions of infections, a few rare

or uncommon presentations are not unexpected. However, it is very essential to analyze the cases before labelling it as reinfection. Not all resurgence of symptoms with a second positive SARS-CoV-2 result after an interval of time are true reinfections. With that caveat, we suggest that reinfection with SARS-CoV-2 is possible, that the second episode may be more clinically severe and that this is worthy of worldwide attention and surveillance for its implications on the danger to HCWs on the frontlines of the pandemic. Frontline HCWs have more than threefold higher risk of SARS-CoV-2 infection than the general community. Therefore, we suggest routine surveillance of SARS-CoV-2 Ab level among HCWs engaged in frontline COVID-19 duty as part of safety protocol till vaccination of frontline HCWs.

## 6. Ethical Clearance

The study was carried out by RVRDL which is approved by the Institutional Ethical Committee of ICMR-RMRC, Dibrugarh to carry out investigations on outbreaks, pandemics related to viruses and other clinically important pathogens in Northeast India.

## 7. Source of Funding

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## 8. Conflict of Interest

None.

## 9. Acknowledgement

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## Author biography

**Gayatri Gogoi**, Assistant Professor

**Mandakini Das**, Research Scientist II

**Biswajyoti Borkakoty**, Scientist E

**Mondita Borgohain**, Professor and HOD

**Anup Kumar Das**, Professor and HOD

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