# INCONSISTENCIES IN DETECTING RDRP GENE BY RDRP CONFIRMATORY ASSAY LEADING TO FALSE NEGATIVE COVID-19 RESULTS: A CASE REPORT FROM A DISTRICT REFERRAL HOSPITAL

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## *Abstract*

Reverse transcriptase polymerase chain reaction (RT-PCR) assays for coronavirus disease 2019 (COVID-19) should be interpreted with clinical, epidemiological history and exposure risk to avoid misdiagnosis. We report a cruise-ship worker with significant travelling history, presented with acute respiratory symptoms and radiographic evidence of viral pneumonia. Initial RNA-dependent RNA polymerase (RdRp) gene confirmatory assay was negative. Use of a more robust RT-PCR assay detected ORF1ab, N and S genes for COVID-19, and the diagnosis was supported by an IgM and IgG positive COVID-19 serology. Subsequent follow up samples which reported inconsistencies in detecting RdRp gene also raise the concern of reliability of RdRp gene as the confirmatory assay for diagnosis of COVID-19. Patient later had prolonged viral shedding beyond serological recovery, with a negative viral culture reflecting non-infectivity.

**Keywords:** *SARS-CoV-2, COVID-19, RT-PCR, False Negative, Prolonged Viral Shedding*

## *Introduction*

The COVID-19 pandemic is the defining global health crisis of our time and countries are racing to slow the spread of the virus by testing, isolating and treating patients, along with many other important management measures (1, 2). Several novel RT-PCR assays are available in a short space of time after the availability of the complete genomic mapping of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). With the lack of experience of long term use, some of the immature reagents could lead to inaccurate diagnostic accuracy (3). A journal reported an overall sensitivity rate of 70% for RT-PCR (4). A false negative result could potentially jeopardize the safety of public and healthcare workers, leading to failure of containment of the disease. Common causes of false negative RT-PCR results are such as: low viral load, particularly in the early illness stage or asymptomatic patients; incorrect sampling technique or inappropriate handling and storage of specimen (5).

Our case highlights the presence of false negative RT-PCR in a symptomatic patient due to failure to detect RdRp gene in his nasopharyngeal samples in the initial stage by in-house RdRp gene confirmatory assay. RdRp gene is a highly mutation prone gene and mutations accumulate as human to human transmission progresses (6), possibly causing false negative result (7), especially when commercial RT-PCR assays that were designed to detect mainly E and RdRp gene only were used. The in-house RT-PCR result was in conflict with the RT-PCR done in private laboratory, which was designed to detect other confirmatory genes. The presence of ORF1ab, S and N genes were reported, which confirmed COVID-19 infection in this patient. Additional antibody test also showed presence of COVID-19 IgM and IgG. His repeated in-house RT-PCR in latter days using the RdRp gene confirmatory assay also showed inconsistent results, which raise the concern of sensitivity and reliability of this assay that solely depends on presence of RdRp gene to confirm the diagnosis of COVID-19.

Prolonged viral shedding beyond development of serological immunity is another challenge we see in our patient. His symptoms resolved at day 9 of illness with seroconversion as confirmed by COVID-19 serology but SARS-CoV-2 was still detected from his nasopharyngeal swab at day 21 of illness despite clinical and serological recovery. Cases of prolonged viral shedding have also been reported in Singapore and Germany (8, 9). Isolation of viruses from the viral culture is required to show infectivity of the patient (10). A negative viral culture with presence of neutralising antibodies reflects a residual viral shedding (11), instead of ongoing or reinfection.

### *Case Presentation*

Our patient is a 32-year-old Malaysian man of Chinese ethnicity who has been working in a casino aboard a cruise ship for the past 4 years. His latest assignment lasted 7 months beginning July 2019 during which time he travelled to several countries including Japan, China, Thailand, Korea, Philippines and Australia. Apart from being obese (BMI 40.9 kg/m$^2$) he did not have any other concomitant medical illness and was a non-smoker.

Ten days after he returned from Sydney to Malaysia, he started to develop cough, runny nose and breathlessness. He denied having fever, sore throat, gastrointestinal symptoms, headache or anosmia. He was asymptomatic while he was on the ship and he was not aware of anyone from the cruise who was diagnosed with COVID-19.

He presented to a local private hospital at day 4 of illness for COVID-19 screening. He had a nasopharyngeal swab taken for COVID-19 RT-PCR at the hospital's outpatient screening centre. He came to Hospital Teluk Intan on day 5 of illness due to worsening of symptoms. Upon presentation, he was afebrile but lethargic and tachypnoeic, with a respiratory rate of 30 breaths per minute. His blood pressure was 134/90 mmHg, heart rate 85 beats per minute, and oxygen saturation was 91% on room air. On auscultation, he had fine inspiratory crackles over bilateral middle to lower zones. Cardiovascular and abdominal examination were unremarkable.

He had normal total white cell and absolute lymphocyte count (Table 1). Chest radiograph showed bilateral middle and lower zones, peripheral predominant interstitial opacities and consolidation (Figure 1). He was categorized as Patient under Investigation (PUI) for COVID-19 and was admitted to the designated isolation ward. He was commenced on intravenous amoxicillin-clavulanic acid, oral azithromycin and oseltamivir.

The first nasopharyngeal swab for RT-PCR which was done in the private laboratory was positive. This was taken on day 4 of illness utilizing Taqman SARS-CoV-2 assay kit version 2 (Applied Biosystems), Thermo Fisher Scientific by Life Technologies Corp, Pleasanton, CA, USA, which was able to pick up several confirmatory genes, such as ORF1ab, N and S genes. The same sample was tested with another commercial RT-PCR - LightMix Modular SARS and Wuhan CoV RdRp-gene kit by TIB MOLBIOL (version 09) (Roche Diagnostics GmbH, Mannheim, Germany), performed on Roche Cobas Z480 instrument by the private laboratory, which failed to detect RdRp gene. Upon admission to our hospital on day 5 of illness, a repeated nasopharyngeal swab was tested by designated government laboratory using Invitrogen SuperScript III platinum one-step qRT-PCR by Life Technologies Corp, Carlsbad, CA, USA which was designed to pick up RdRp gene and it was negative. In view of a strong clinical suspicion of COVID-19 infection and significant travelling history, nasopharyngeal swabs were repeated after 72 hours on day 8 of illness and sent to both government and private laboratories, which showed

**Table 1:** Blood investigations result of patient upon admission on day 5 of illness

| Date | 1/4/2020 | Normal value |
|---|---|---|
| TWC | 10.7 x 10$^3$ /µL | 4-11 x 10$^3$ /µL |
| Hemoglobin | 14.3 g/dL | 11.7-15.7 g/dL |
| Absolute lymphocyte count | 1.39 x 10$^3$ /µL | 1.0-4.8 x 10$^3$ /µL |
| Platelet | 218 x 10$^3$ /µL | 150-400 x 10$^3$ /µL |
| Sodium | 132 mmol/L | 136-146 mmol/L |
| Potassium | 3.8 mmol/L | 3.5-5.1 mmol/L |
| Urea | 3.9 mmol/L | 2.8-7.2 mmol/L |
| Creatinine | 88 µmol/L | 45-84 µmol/L |
| Arterial blood gas (under room air) | $PaO_2$ 78 mmHg<br>$PaCO_2$ 36 mmHg<br>pH 7.43<br>$HCO_3$ 23.4 mmol/L | $PaO_2$ 71-104 mmHg<br>$PaCO_2$ 35-46 mmHg<br>pH 7.35-7.45<br>$HCO_3$ 21-26 mmol/L |

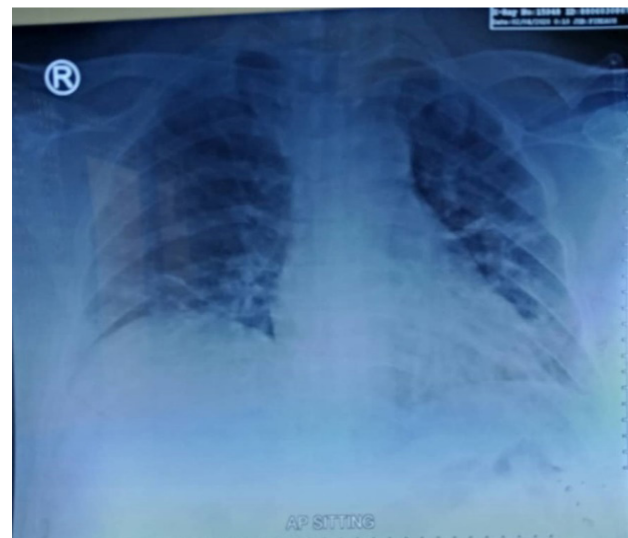


**Figure 1:** Chest X-ray of patient on admission (day 5 of illness), showing bilateral middle and lower zones peripheral predominant interstitial opacities with consolidation

similar results (Table 2). We concluded that he was infected with COVID-19 due to presence of IgM and IgG from his COVID-19 serology. He was tested again on day 15 of illness (2 weeks from onset of symptoms), followed by day 18, 21 and 23 of illness, according to COVID-19 Malaysia guideline recommendation on repeating nasopharyngeal swabs every 48-72 hours. The E gene was detected for all the samples but RdRp was only detected in samples taken on day 15 and 21 of illness. The day 18 sample was also tested using the commercial kit and the result was consistent with the two previous tests using the same kit. A respiratory panel screen (RP33) revealed no other viruses detected except for *Staphylococcus aureus*, which suggested a superimposed bacterial pneumonia.

**Table 2:** Summary of nasopharyngeal RT-PCR and COVID IgM/IgG serology results

| **Testing methods** | **Day of illness** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 4** | **Day 5** | **Day 8** | **Day 15** | **Day 18** | **Day 21** | **Day 23** |
| Invitrogen SuperScript III platinum one-step qRT-PCR by Life Technologies Corp, Carlsbad, CA, USA | | *E: NA<br>RdRp: –<br>(Negative) | *E: NA<br>RdRp: –<br>(Negative) | E: +<br>RdRp: +<br>(Positive) | E: +<br>RdRp: –<br>(Negative) | E: +<br>RdRp: +<br>(Positive) | E: +<br>RdRp: –<br>(Negative) |
| LightMix Modular SARS and Wuhan CoV RdRp-gene kit by TIB MOLBIOL (version 09) , Germany | E: +<br>RdRp: –<br>(Negative) | | | | | | |
| Taqman SARS-CoV-2 assay kit Version 2<br>ThermoFischer Scientic, USA | E: +<br>RdRp: –<br>ORF1ab: +<br>N: +<br>S: +<br>(Positive) | | E: +<br>RdRp: –<br>ORF1ab: +<br>N: +<br>S: +<br>(Positive) | | E: +<br>RdRp: –<br>ORF1ab: +<br>N: +<br>S: +<br>(Positive) | | |
| Healgen COVID-19 IgM/IgG rapid test Kit, Houston, USA | | | IgM: +<br>IgG: + | | | | |
| Viral culture<br>(Institute for Medical Research, KL) | | | | | | Negative | |

*E gene was not tested by the in house laboratory according to the laboratory protocol at that time
`-´=negative
`+´=positive
NA=not available

He was diagnosed as Group 4 (pneumonia requiring nasal prong delivering 3 litres of oxygen per minute) COVID-19 infection (12) and was commenced on hydroxychloroquine and lopinavir/ritonavir combination according to the COVID-19 management guideline (5th edition), Malaysia at that time (12). He was also continued on tablet amoxicillin-clavulanic acid to cover for superimposed bacterial infection. His symptoms improved on day 9 of illness, and he was subsequently weaned to room air. Patient had completed 5 days of hydroxychloroquine, as well as 1 week of tablet amoxicillin-clavulanic acid and lopinavir/ritonavir. Repeated chest X-ray on day 23 of illness showed resolution of pneumonia (Figure 2).

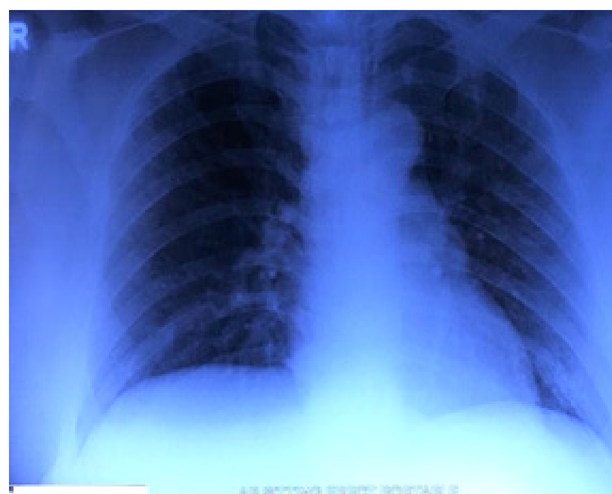


**Figure 2:** Repeated chest X-ray after resolution of symptoms on day 23 of illness showing resolution of interstitial opacities despite positive RT-PCR

Despite clinical improvement, his repeated RT-PCR on day 21 of illness remained positive. We concluded that he had prolonged shedding of residual viral genomic material as his viral culture which was sent on day 21 of illness was negative and he had developed IgG antibodies. He was

discharged on day 23 of illness after a negative RT-PCR and was instructed for 14 days home quarantine. No further RT-PCR was repeated.

## Discussion

According to Malaysia laboratory protocol which is adapted from World Health Organization (WHO) recommendation (13), E gene is used as the screening assay, and RdRp gene as the confirmatory assay. Target genes for diagnosis may vary by country. For instance, target genes for screening and confirmatory assays by RT-PCR are ORF1ab and N in Chinese laboratory protocol, while Germany screens RdRp, E and N (14). In another approach, N gene assay is used as screening, and ORF1ab is used as confirmatory assay (15). We might have potentially missed our patient's diagnosis of COVID-19 due to failure of detecting RdRp gene by in-house RT-PCR. Fortunately, Taqman SARS-CoV-2 assay kit version 2 was able to detect other confirmatory genes to conclude that he was infected by SARS-CoV-2. Cases with a strong clinical suspicion of COVID-19 infection and high risk of exposure that are similar to our patient's should hence prompt a clinician or pathologist to consider identification of additional confirmatory genes. An article from Korea also reported two false negative cases by RdRp gene confirmatory assay and both were found to be positive for SARS-CoV-2 by N gene confirmatory assay latter, leading to a suggestion that N gene rather than RdRp gene should be used as confirmatory assay to increase sensitivity of detection of COVID-19 (16). A study from Belgium reported failure of detection of E gene by SARS-CoV-2 E gene assay due to the presence of mutation of the SARS-CoV-2 genome (17). Hence, in our subject, the false negative RT-PCR result could potentially be due to a mutation of RdRp gene.

A risk-stratified protocol should also be planned and published as guidance for clinicians to manage individuals from different risk group. For low-risk exposure (18) individuals with a negative RT-PCR result, it is sufficient to rule out COVID-19 infection. However in high risk individuals, threshold to treat as COVID-19 infection should be lower even with a negative RT-PCR, or to consider repeating the RT-PCR assay to screen for additional confirmatory genes. Another method to improve RT-PCR sensitivity and to reduce rate of false negative result is to consider the type of sample to be sent. A sputum sample for RT-PCR should be sent in cases with lower respiratory tract infection as opposed to a nasopharyngeal or oropharyngeal sample as per recommended by WHO (5).

Patient also experienced prolonged viral shedding despite seroconversion and resolution of symptoms. According to European Centre for Disease Prevention and Control (ECDC), SARS-CoV-2 can be detected for 7 to 12 days in moderate cases and up to 2 weeks in severe cases (10). Another paper produced by China which was published in The Lancet on 9th March 2020 reported that the median duration of viral shedding was 20 days, with longest observed duration up to 37 days. The study also suggested that antiviral drugs have little effect in shortening the viral shedding period (19). To date, there is no evidence showing that prolonged viral shedding equates infectivity, unless it can be isolated from viral culture. Our patient had a negative viral culture from his nasopharyngeal swab prior to discharge, signifying a residual viral shedding.

## Conclusion

Failure of detecting RdRp gene as the confirmatory gene but with strong clinical suspicion and exposure history should prompt a clinician or pathologist to screen for additional confirmatory genes such as N, S or ORF1ab in order to conclude whether patient is infected by SARS-CoV-2. We should also refine the current protocol especially for high risk individuals, whereby the threshold to treat them as COVID-19 infected patients should be lower, even if the RT-PCR is negative. Prolonged viral shedding after recovering from COVID-19 infection are observed in certain individuals. In order to tell apart between residual viral shedding and infection, it is important to carry out virus isolation culture and neutralising antibodies level (10).

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## Consent

Written informed consent was obtained from the patient for publication of this case report and the accompanying images.

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## Competing interests

The authors have no conflicts of interest to declare.

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