Possible Cases of SARS-CoV-2 Reinfection In Pekanbaru, Indonesia

Rahmat Azhari Kemal[1]*, Dewi Anggraini[2]

[1] Department of Medical Biology, Faculty of Medicine Universitas Riau, Pekanbaru, Riau, Indonesia.
[2] Department of Microbiology, Faculty of Medicine Universitas Riau, Pekanbaru, Riau, Indonesia.

Corresponding Author:
Rahmat Azhari Kemal, MD. Department of Medical Biology, Faculty of Medicine Universitas Riau. Jl. Diponegoro No.1, Pekanbaru, Riau 28133, Indonesia. Email: rahmat.azharikemal@lecturer.unri.ac.id

ABSTRACT

Confirmed and possible reinfection cases of SARS-CoV-2 have been reported from various countries. Here we present two cases of possible SARS-CoV-2 reinfection in Pekanbaru, Indonesia. A 26 years old female and a 27 years old male healthcare workers were first confirmed by PCR with high Ct-value (>35) while presenting no or mild symptoms, respectively. In more than one month since the last negative test results, both patients developed typical COVID-19 symptoms; fever and anosmia. RT-PCR results for SARS-CoV-2 were positive with Ct-value less than 30. The timeframe between 1st and 2nd episode, negative test result between episodes, and epidemiological risk factor strengthened the possibility of reinfection. However, we did not have whole genome sequence (WGS) or viral viability data to further confirm reinfection with different viable virus. The requirement of viral WGS data to confirm true reinfection cases calls for investment in whole genome sequencing platform in public health laboratories. We encourage standardized definition of SARS-CoV-2 reinfection case in order to be able to investigate and observe such cases.

***Keywords**: COVID-19, Indonesia, reinfection, SARS-CoV-2*

INTRODUCTION

Severe-acute-respiratory-syndrome coronavirus 2 (SARS-CoV-2) has caused the worldwide Coronavirus disease 2019 (COVID-19) pandemic since early 2020. While the medical and scientific community have speedily studied the virus, there are still many things to learn, including the possibility of reinfection. Investigation and observation of reinfection cases could provide better understanding on immunity to SARS-CoV-2.[1] Currently, at least there have been 4 cases supported by whole genome sequencing (WGS) data to confirm reinfection with genetically-distinct virus.[2–5] There are also several other possible reinfection cases without WGS data.[6–9]

To the best of our knowledge, there are no published reinfection cases from Indonesia.

We present two cases of immunocompetent, young persons that indicated the possibility of SARS-CoV-2 reinfection from Pekanbaru city, Riau Province, Indonesia.

CASE ILLUSTRATION

Case 1

The timeline of Case #1 is summarized on **Figure 1**. A 26-years old female healthcare worker was tested on August 4th, 2020 as part of contact tracing. One of her flat-mates was confirmed positive for SARS-CoV-2. The initial case was also a healthcare worker in the same hospital. Last shift of Case #1 in the hospital was a night shift on August 3rd, 2020. Naso-

oropharyngeal sample was taken on August 4th with positive result for SARS-CoV-2 (Ct-value of 35.50 RdRP) using AllPlex™ 2019-nCoV Assay (Seegene, South Korea). The patient did not have any complaints, and her lab results such as routine hematology (**Table 1**), blood gas analysis, and chest x-ray were within normal range. The patient was admitted to the hospital for isolation purpose on August 6th – 11th and remained asymptomatic. The patient was prescribed azythromicin, oseltamivir, paracetamol, omeprazole, acetylcystein, and vitamin D. Case #1 was tested negative twice on August 9th and 11th, thus declared to be recovered.

The Case 1 was tested on August 23rd, 2020 as another part of contact tracing while still presented no symptoms. The result of naso-oropharyngeal swab came back negative using Standard M nCoV Real-Time Detection Kit (SD BIOSENSOR, South Korea).

On November 3rd, Case 1 developed fever, cough, sneezing, and anosmia. The patient was on duty as nurse in COVID-19 ICU. Therefore, the patient's naso-oropharyngeal swab sample was taken in Influenza-Like Illness unit on November 4th. The RT-PCR (SD BIOSENSOR, South Korea) result was positive for SARS-CoV-2 with Ct-value of 22.19 (ORF1ab) and 21.78 (E). The patient was further hospitalized. Laboratory test showed neutropenia and lymphocytosis (**Table 1**) while chest x-ray was within normal limits. The patient had no comorbidities. The patient was prescribed levofloxacin, dexamethasone, enoxaparine, favipiravir, acetylcysteine, vitamin D, vitamin B, and curcuma.

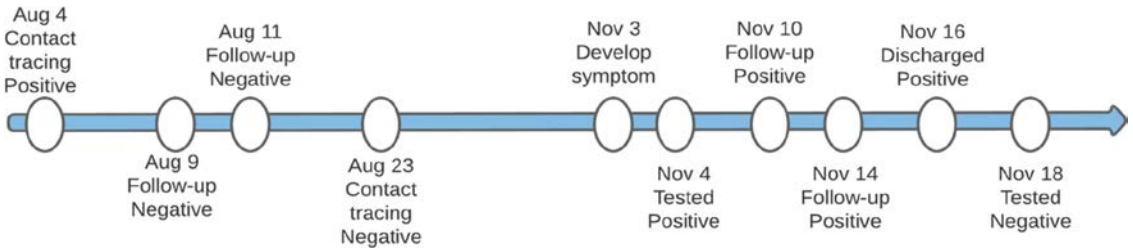

Figure 1. Timeline of Case #1

Table 1. Routine hematology result from first and second COVID-19 episode of Case #1.

Parameter	First COVID-19 Episode	Second COVID-19 Episode	Reference
Hemoglobin (g/dL)	13.1	13.8	11.7 – 15.5
Hematocryte (%)	38.9	39.9	35 – 47
Erythrocyte (mio/µL)	4.66	4.88	3.8 – 5.2
MCV (fL)	83.5	81.8	79 – 99
MCH (pg)	28.1	28.3	27 – 31
MCHC (g/dL)	33.7	34.6	33 – 37
Leucocyte (mio/µL)	9.3	5.8	4.8 – 10.8
Basophil (%)	0	0	0 – 1
Eusinophil (%)	1	1	2 – 4
Neutrophil (%)	58	39	50 – 70
Lymphocyte (%)	36	53	25 – 40
Monocyte (%)	5	7	2 – 8
Thrombocyte (mio/µL)	269	240	150 – 440
Absolute lymphocyte (mio/µL)	3.32	3.08	1 – 4
Neutrophil-Lymphocte Ratio	1.61	0.74	< 3.13
C-reactive protein	1.12	N/A	<5
D-Dimer	N/A	159.53	<500

The patient was followed up on November 10th and 14th. Both came back positive, with Ct-value of 33.78 (ORF1ab) & 31.66 (E gene) for the first follow-up and 35.29 (ORF1ab) and 33.82 (E gene) for the second follow-up. After symptom resolution, patient was discharged on November 16th with positive SARS-CoV-2 RT-PCR result of Ct-value 34.85 (ORF1ab) and 35.19 (E). All three follow-ups used the same RT-PCR kit (SD BIOSENSOR, South Korea). On November 18th, her naso-oropharyngeal swab was tested negative for SARS-CoV-2 RNA (GB SARS-CoV-2 Real-Time RT-PCR, GBC, Taiwan).

Case 2

The timeline of Case 2 is summarized on **Figure 2**. A 27-years old male self-reported malaise on September 15th, 2020. The patient's naso-oropharyngeal swab was tested for SARS-CoV-2 on September 18th using DiaPlexQ™ Novel Coronavirus (2019-nCoV) Detection Kit (SolGent, South Korea) resulting in positive result with Ct-value of 38.62 (ORF1a) and 38.08 (N). The same sample was re-extracted and retested using the same kit, resulting in same positive result (36.90 ORF1a, 36.74 N). His blood parameter and chest x-ray were within normal range, thus the patient conducted self-isolation at home. The patient had paracetamol, omeprazole, vitamin C, vitamin D, zinc, azythromicin, oseltamivir.

The patient reported going out for lunch with 3 people on September 10th. His contacts were additionally tested on September 19th. Two of them were negative, but one contact was tested positive (37.12 ORF1a, 37.70 N, DiaPlexQ™ kit). The contact reported sorethroat and cough starting on September 12th. Additional contact tracing from the abovementioned contact found another positive contact. The patient Case #2 was followed up on September 21st using the same test kit and his naso-oropharyngeal swab was tested negative. The patient continued self-isolation for additional 1 week. However, when tested for antibody using STANDARD Q COVID-19 IgM/IgG Combo Test Kit (SD Biosensor, South Korea) on October 26th, the patient was non-reactive for both IgM and IgG. The patient had no history of immunocompromised and was not taking any immunosuppressive drugs.

The Case #2 travelled inter-province on October 31st evening by car with a driver and

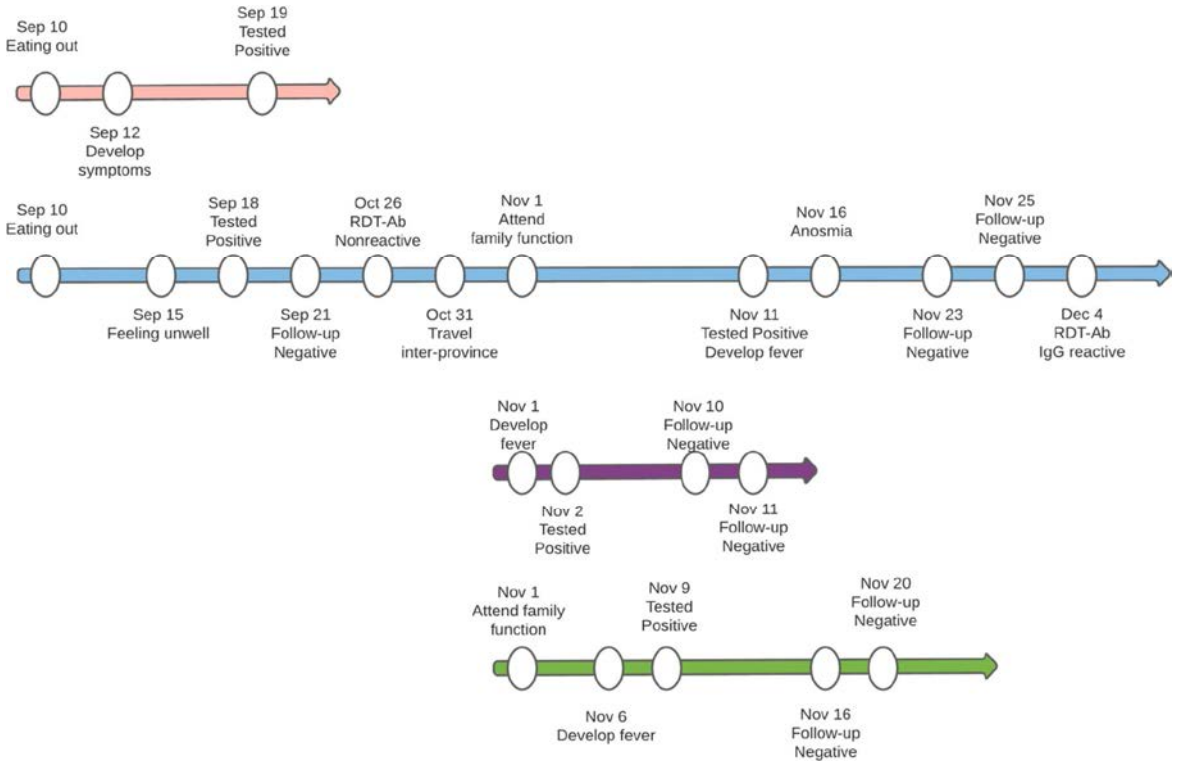

Figure 2. Timeline of Case #2 (blue) and of his contacts, friend (red), father (purple), and mother (green). RDT-Ab: rapid antibody test

arrived on November 1st morning to attend a family function. In the same morning, the patient's father developed fever and did not attend the family function. The father's nasopharyngeal swab was tested for SARS-CoV-2 (mBioCoV-19, BioFarma, Indonesia) on November 2nd with positive result (32.01 ORF1b, 35.21 RdRp). The patient's mother, who attended the family function on November 1st, developed fever on November 6th. Her nasopharyngeal swab was tested for SARS-CoV-2 (STANDARD M, SD Biosensor, South Korea) on November 9th with positive result (27.54 ORF1ab, 28.38 E).

Nasopharyngeal swab was taken from Case #2 for SARS-CoV-2 testing on November 11th morning as part of contact tracing while reporting no symptoms. However later in the evening, the patient developed fever (37.8°C) which on the following day reached 38.8°C. On November 13th, the SARS-CoV-2 test result came back positive (28.39 ORF1ab, 26.39 E, STANDARD M kit). Chest x-ray and blood analysis were not performed. The patient conducted self-isolation at home. The patient had paracetamol, omeprazole, vitamin C, vitamin D, zinc, azythromicin, oseltamivir. On November 16th the patient started to develop anosmia.

The Case #2's father was tested negative on November 10th and 11th, the mother was tested negative on November 16th and 20th, and the Case #2 himself was tested negative on November 23rd and 25th. All follow-up tests were conducted on nasopharyngeal swab sample using STANDARD M kit (SD BIOSENSOR, South Korea). The

Table 2. Possibility of reinfection based on CDC (2020) and Yahav (2020) criteria

Definition	Criteria	Case #1	Case #2
US CDC (2020)			
Suspected reinfection			
Characteristic clinical symptoms on 2nd episode	+	Fever, cough, sneezing, anosmia	Fever, anosmia
RT-PCR of 2nd episode	Ct < 33	22.19 (ORF1ab), 21.78 (E)	28.39 (ORF), 26.39 (E)
Timeframe from 1st episode	≥45 days	92 days	56 days
Close-contact	+	n/a	+
Viral RNA sequence	Different strain	n/a	n/a
Yahav et al. (2020)			
Confirmed reinfection			
True 1st episode	Ct value < 35	35.50 (RdRP)	38.62 (ORF1a) 38.08 (N) Same specimen retested: 36.90 (ORF1a) 36.74 (N)
Characteristic clinical symptoms on 2nd episode	+	+	+
RT-PCR of 2nd episode	Ct < 35	+	+
Negative test between 1st and 2nd episode	At least 1, ideally 2	3	1
Viral culture / subgenomic RNA*	+	n/a	n/a
Timeframe from 1st episode	>90 days†	92 days	56 days‡
Viral RNA sequence	Different strain	n/a	n/a
Clinical reinfection			
Characteristic clinical symptoms on 2nd episode	+	+	+
RT-PCR (Ct < 35)	+	+	+
Viral culture / subgenomic RNA[a]	+	n/a	n/a
Epidemiological risk factor	+	+	+

* Optional to provide evidence of replicating virus.
† Could be <90 days if recovery proven by negative PCR tests and current known COVID-19 exposure.
‡ Had one negative RT-PCR after 1st episode and close-contact with two laboratory-confirmed COVID-19 cases before 2nd episode.

Case #2 was tested on December 4th for using COVID-19 IgM/IgG Combo Test Kit (SD Biosensor, South Korea), resulting in IgG strong reactivity and IgM weak reactivity.

In most reported reinfection cases (**Table 3**), the first positive results were mainly asymptomatic or mildly symptomatic with high Ct-value and followed by nonreactive antibody test. Our Case #2 was nonreactive for both IgM and IgG within 6 weeks after the first, mildly symptomatic infection. To the extent of our knowledge, Case #2 had neither immunocompromised nor immunosuppressed condition which was shown by the presence of IgG after the second, symptomatic infection. Sensitivity and specificity of the rapid antibody test kit could play a factor. However, it could also be due to lower antibody response in mild cases compared to more severe cases.[12–13] Studies regarding antibody against SARS-CoV-2 and its persistence have also been contradictory. Several studies showed waning response while others showed lasting immunity. Non-hospitalised patients have been shown to have more rapid decline of antibody titer.[12] Ibarrondo et al. showed declining antibody with half-life of 36 days.[14] Jeewandara et al. also showed that 4 out of 13 mild COVID-19 patients had no detectable neutralizing antibody (NAb) at 40 days since illness onset.[13] On the other hand, Choe et al. showed that antibody against SARS-CoV-2 was still present at 8 months after asymptomatic or mild COVID-19.[15] Rodda et al. showed that not only antibody but also both memory B and memory T cell persisted at least 3 months after mild SARS-CoV-2 infection.[16] It is important to note that in both Choe et al. and Rodda et al. not all mild patients had seropositivity, with only 85% and 69.0-91.4%, respectively.[15–16] Another possible factor for reinfection is the low viral load

Table 3. Summary of several SARS-CoV-2 reinfection reports

Cases	Sex	Age (years)	1st Episode (RT-PCR)	2nd Episode (RT-PCR)	Timeframe (days)	Negative test between episodes	Epidemiological risk faktor	Viral RNA sequence
Pekanbaru Case 1	F	26	Asymptomatic 35.50 (RdRP)	Symptomatic 22.19 (ORF1ab) 21.78 (E)	92	3	Healthcare worker	N/A
Pekanbaru Case 2	M	27	Mild 38.62 (ORF1a) 38.08 (N)	Worse 28.39 (ORF) 26.39 (E)	56	1	Close-contact	N/A
Hong Kong (*2*)	M	33	Mild (Positive)	Asymptomatic 26.69	142	2	Travel abroad	Different clade
USA (*3*)	M	25	Mild 35.24	Hospitalized 35.31	48	2	N/A	Same clade, Genetically distinct
Belgium (*4*)	F	51	Mild 25.6 (N1) 27.2 (N2)	Milder 32.6 (N1) 33.2 (N2)	93	N/A	N/A	Different clade
Ecuador (*5*)	M	46	Mild 36.85 (ORF3)	Worse 30.82 (N)	63	1	N/A	Different clade
UK (*6*)	M	25	Mild (Negative, reactive antibody)	Milder (Positive)	>90	-	Close-contact	N/A
USA (*7*)	M	82	Hospitalized (Positive)	Hospitalized (Positive, high Ct- value)	55	2	N/A	N/A
Bangladesh (*8*)	M	40	Mild (Positive)	Mild (Positive)	53	1	Healthcare worker / Contact	N/A
Israel (*9*)	F	20	Mild (Positive)	Asymptomatic (Positive)	~90	2	Close-contact	N/A

during the first episode. Kim et al. showed in ferret model, lower viral load in the first episode resulted in lower NAb titer which correlated to reinfection when challenged with heterologous virus three weeks after primary infection.[17] In both of our cases, low viral load (indicated by high Ct-value) during the first episode and low antibody titer (showed by non- reactive rapid antibody test of Case #2) might had left them susceptible to reinfection after more than 45 days or 7 weeks from primary infection.

Our case report also highlights important public health messages. Our cases had considerably easier testing access therefore could be tested while presenting no or mild symptoms. It is possible that we are missing many reinfection cases with asymptomatic or mild SARS-CoV-2 infections due to limited access to testing. Widespread testing and data management might enable us to observe more possible SARS-CoV-2 reinfection cases. The current national report system, New All Record, has continuous data per personal ID number therefore it could be utilized to observe possible reinfection cases.

Additionally, investment in WGS platform, especially automated platform will surely be beneficial to confirm reinfection cases during current COVID-19 pandemic. Adaptation of routine whole genome sequencing in public health laboratory will support epidemiological analysis to detect, monitor, and control circulating or emerging pathogens in Indonesia.[18–19] Lastly, as shown by our case report, natural infection might result in varied immune response due to the varied viral load. We will require safe and effective vaccines as well as robust vaccination program to achieve herd immunity against SARS-CoV-2.

CONCLUSION

We presented two possible SARS-COV-2 reinfection cases from Pekanbaru, Indonesia. Both cases mostly fulfilled US CDC criteria for suspected reinfection, namely presence of typical clinical COVID-19 on 2nd episode, RT-PCR with Ct-value of less than 33 on 2nd episode, as well as timeframe of more than 45 days between 1st and 2nd episode. One of the cases, Case #2, had close-contact while Case #1 had epidemiological risk factor as healthcare worker.

However, in both cases, no viral RNA sequences or viral viability data were obtained. Both data are relatively laborious to obtain and not routinely conducted in public health laboratories, hindering many to observe and report (possible) reinfection cases. As reinfection cases could provide better understanding on immunity to SARS-CoV-2, definition and criteria of SARS- CoV-2 reinfection case are needed to capture and observe such cases. Combination of reinfection criteria from CDC[10] and Yahav et al.[11] could be adapted. Additionally, in order to fulfill the requirement of WGS data, investment in routine use of automated whole genome sequencing platform in public health laboratories is needed.

ACKNOWLEDGMENTS

Figures were designed by Fajri Marindra Siregar. We thank members of Biomolecular Laboratory for insightful discussion regarding this case report.

REFERENCES

1. Iwasaki A. What reinfection means for COVID-19. Lancet Infect Dis. 2020; (published online Oct 12, 2020). DOI: 10.1016/S1473-3099(20)30783-0
2. To KKW, Hung IFN, Ip JD, et al. 2020. COVID-19 re-infection by a phylogenetically distinct SARS-coronavirus-2 strain confirmed by whole genome sequencing. Clin Infect Dis. 2020; (published online Aug 25, 2020). DOI:10.1093/cid/ciaa1275
3. Tillett RL, Sevinsky JR, Hartley PD, et al. Genomic evidence for reinfection with SARS-CoV-2: a case study. Lancet Infect Dis. 2020;(published online Oct 12, 2020). DOI: 10.1016/S1473-3099(20)30764-7
4. van Elslande J, Vermeersch P, Vandervoort K, et al. Symptomatic SARS-CoV-2 reinfection by a phylogenetically distinct strain. Clin Infect Dis. 2020; (published online Sep 5, 2020). DOI: 10.1093/cid/ciaa1330
5. Prado-Vivar B, Becerra-Wong M, Guadalupe JJ, et al. COVID-19 re-infection by a phylogenetically distinct SARS-CoV-2 variant, first confirmed event in South America. SSRN. 2020; (published online Sep 8, 2020) [preprint]. DOI: 10.2139/ssrn.3686174
6. West J, Everden S, Nikitas N. A case of COVID-19 reinfection in the UK. Clin Med Dec. 2020. DOI: 10.7861/clinmed.2020-0912.
7. Duggan NM, Ludy SM, Shannon CB, Reisner AT,

Wilcox SR. Is novel coronavirus 2019 reinfection possible? Interpreting dynamic SARS-CoV-2 test results. Am J Emerg Med. 2021; (published online Jul 4, 2020). DOI: 10.1016/j.ajem.2020.06.079

8. Akram L, Khan MHUR, Iqbal, Akram A. A case report of recurrent COVID-19 infection of a physician in Bangladesh: Re-infection or persistence infection? Bangladesh J Infect Dis. 2020;7(Suppl 2):S57-S60. DOI: 10.3329/bjid.v7i00.50164
9. Nachmias V, Fusman R, Mann S, Koren G. The first case of documented Covid-19 reinfection in Israel. IDCases 2020; 22: e00970. DOI: 10.1016/j.idcr.2020.e00970
10. Centers for Disease Control and Prevention. Common investigation protocol for suspected SARS-CoV-2 reinfection. 2020; (published online Oct 27, 2020) https://www.cdc.gov/coronavirus/2019-ncov/php/reinfection.html
11. Yahav D, Yelin D, Eckerle I, et al. Definitions for coronavirus disease 2019 reinfection, relapse and PCR re-positivity. Clin Microbiol Infect. 2020; (published online Dec 4, 2020). DOI: 10.1016/j.cmi.2020.11.028
12. Röltgen K, Powell AE, Wirz OF, et al. Defining the features and duration of antibody responses to SARS-CoV-2 infection associated with disease severity and outcome. Sci Immunol. 2020;5(54):eabe0240. DOI:10.1126/sciimmunol.abe0240.
13. Jeewandara C, Jayathilaka D, Gomes L, et al. SARS-CoV-2 neutralizing antibodies in patients with varying severity of acute COVID-19 illnes. Sci Rep. 2021; 11(1): 2062. DOI: 10.1038/s41598-021-81629-2.
14. Ibarrondo FJ, Fulcher JA, Goodman-Meza D, et al. Rapid decay of anti-SARS-CoV-2 antibodies in persons with mild Covid-19. N Engl J Med. 2020; 383(11): 1085-1087. DOI: 10.1056/NEJMc2025179.
15. Choe PG, Kim K-H, Kang CK, et al. Antibody responses 8 months after asymptomatic or mild SARS-CoV-2 infection. Emerg Infect Dis. 2021;(published online Dec 22, 2020). DOI: 10.3201/eid2703.204543
16. Rodda LB, Netland J, Shehata L, et al. Functional SARS-CoV-2-specific immune memomry persists after mild COVID-19. Cell. 2021;184(1):169-83.e17. DOI: 10.1016/j.cell.2020.11.029
17. Kim YI, Kim SM, Park SJ, et al. Critical role of neutralizing antibody for SARS-CoV-2 reinfection and transmission, Emerg Microbes Infect. 2021. DOI: 10.1080/22221751.2021.1872352
18. Kwong JC, Mccallum N, Sintchenko V, Howden BP. Whole genome sequencing in clinical and public health microbiology. Pathology. 2015;47(3):199-210.
19. Köser CU, Ellington MJ, Cartwright EJP, et al. Routine use of microbial whole genome sequencing in diagnostic and public health laboratory. Plos Pathog. 2021;8(8):e1002824.