

## Confirmed Reinfection with SARS-CoV-2 Variant VOC-202012/01

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Dear Editor,

We have detected a confirmed case of reinfection with SARS-CoV-2 with the second episode due to the 'new variant' VOC-202012/01 of lineage B.1.1.7. The initial infection occurred in the first wave of the pandemic in the UK and was a mild illness. 8 months later, during the second wave of the pandemic in the UK reinfection with the 'new variant' VOC-202012/01 was confirmed and caused a critical illness.

A 78 year old man with a history of Type 2 Diabetes Mellitus, diabetic nephropathy on haemodialysis, chronic obstructive pulmonary disease (COPD), mixed central and obstructive sleep apnoea, ischaemic heart disease, with no history of immunosuppression, presented with fever during haemodialysis on the 2<sup>nd</sup> April 2020. There were no other symptoms and he was discharged home. He had a mild illness with an uneventful recovery. Combined nose and throat (NTS) swab tested positive for SARS-CoV-2 RNA. Testing was performed on the

Roche cobas<sup>®</sup> 8800 System, targeting the E gene and ORF1a gene targets. E gene cycle threshold (Ct) value was 26.8, ORF1a Ct value was 26.4. Our service has been routinely screening all haemodialysis patients under our care since the first surge of infections in London; a total of 22 routine NTS swabs were sent between the 5<sup>th</sup> May 2020 and the 1<sup>st</sup> of December 2020 and all tested negative for SARS-CoV-2 RNA. SARS-CoV-2 antibodies (using the Roche anti-SARS-CoV-2 IgM/IgG assay detecting antibodies targeting viral nucleocapsid 'N' antigen) were detectable on 6 occasions between the 4<sup>th</sup> June 2020 and 13<sup>th</sup> November 2020 with no evidence of antibody waning seen.

On the 8<sup>th</sup> December 2020 a routine repeat NTS was sent. Testing was performed on the Hologic Panther SARS-CoV-2 platform using the proprietary Aptima Transcription-Mediated Amplification (TMA) assay targeting ORF1a and ORF1b targets. The Relative Light Unit (RLU) value was 1348. A repeat sample was sent on the 14<sup>th</sup> December and tested with reverse transcription polymerase chain reaction (RT-PCR) using the Roche cobas<sup>®</sup> 8800 platform targeting the E gene and ORF1a

targets, with Ct values of 27.5 and 27.9 respectively. On the 14<sup>th</sup> December 2020, he presented to A&E with a 3 days history of shortness of breath (SOB) which had worsened overnight. He was brought in by ambulance *in extremis*, very short of breath (SOB) and unable to talk, with severe hypoxia, leading to emergency intubation. Severe Covid-19 pneumonia complicated by myocardial infarction with resulting trifascicular block and Atrio-Ventricular (AV) dissociation and pulmonary oedema was diagnosed. He was admitted to ITU treated with co-amoxiclav, clarithromycin, dexamethasone, and required cardiac pacing, haemodynamic vasopressor support, haemofiltration

Whole Genome Sequencing (WGS) of the viral genome was performed *in house* on stored aliquots of the samples collected on the 2<sup>nd</sup> April and the 8<sup>th</sup> December. Briefly, samples were sequenced with a multiplex PCR based approach according to the modified ARTIC protocol with version 3 primer set. Amplicon libraries were sequenced using Illumina MiSeq. Genomes were assembled with reference-based assembly and a bioinformatics pipeline with 10x minimum coverage cut-off for any region of the genome and 50% cut-off for defining single nucleotide polymorphisms (SNPs). The generated FASTA files were uploaded to the CoV-GLUE web platform for further analysis. 85.92% genome coverage was obtained on the sample dated 2<sup>nd</sup> April. Phylogenetically, the isolate belonged to lineage B.2, with no mutations observed in the S region. 95.6% genome coverage was obtained on the sample dated 8<sup>th</sup> December 2020. Phylogenetically, the isolate belonged lineage B.1.1.7 and accumulated 18 amino-acid replacements across the genome. The following amino-acid replacements were observed in the 'S' region: N501Y, A570D, D614G, P681H, T761I, S982A, D1118H. Also, deletions were present in the spike region: Y144 (21991-21993) and HV 69-70 (21765-21770).

The WGS results confirm reinfection with a different lineage 8 months after initial infection in the absence of significant immunocompromise. The reinfection was with the 'new variant' VOC-202012/01. This variant has been recently identified in the UK, and is rapidly spreading, especially in London and the South East of England and South Wales and may be responsible for a surge in new cases here<sup>1</sup>. The 'new variant' is characterised by numerous mutations in the spike region which

causes diagnostic escape in PCR assays using the 'S' gene as a target for amplification<sup>1,2</sup>. The numerous spike region mutations also raise questions about possible immune escape and/or vaccine evasion and likelihood of reinfection.

Reinfection has been confirmed before in a handful of cases worldwide, but confirmation of reinfection relies on WGS and so cases may be drastically underreported. Regular PCR screening of our dialysis cohort and access to in-house WGS allowed reinfection to be confirmed in this instance. The development of reinfection in this case may just reflect waning immunity after 8 months since primary infection in a high-risk individual with multiple comorbidities. Anti-SARS-CoV-2 antibodies were still present shortly before onset of reinfection, with no evidence of antibody waning. This may raise some concerns about immune evasion by this new variant, which is a concern with the high number of spike region mutations seen. We have no assay for SARS-CoV-2 antibodies recognising spike antigen, and neutralising antibody studies are pending. The antibodies detected recognise 'N' antigen, so drawing conclusions is difficult. The group of mutations identified in VOC-202012/01 appears to have significantly increased transmissibility compared to previously described mutations or haplotypes, but there is as yet no evidence of increased pathogenicity associated with these mutations<sup>1</sup>. In this case the initial illness was mild, and the reinfection with the new variant was critical/life-threatening. More severe illness on the second episode has been reported before in confirmed reinfections not caused by VOC-202012/01<sup>4</sup>. Rapid work on learning about immune, vaccine and diagnostic escape is needed, as are data on severity of illness caused by VOC-202012/01.

**None of the authors has any potential conflicts**

## References

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