1   **Sequence analysis of SARS-CoV-2 in nasopharyngeal samples from patients with COVID-19**

2   **illustrates population variation and diverse phenotypes, placing the in vitro growth properties**

3   **of B.1.1.7 and B.1.351 lineage viruses in context.**

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5   **Running title:** Genetic diversity of SARS-CoV-2 and growth kinetics

6   **Keywords:** SARS-CoV-2, COVID-19, clinical samples, minor variants, growth kinetics

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28   **Abstract**

29   New variants of SARS-CoV-2 are continuing to emerge and dominate the regional and global

30   sequence landscapes. Several variants have been labelled as Variants of Concern (VOCs) because

31   of perceptions or evidence that these may have a transmission advantage, increased risk of

32   morbidly and/or mortality or immune evasion in the context of prior infection or vaccination.

33   Placing the VOCs in context and also the underlying variability of SARS-CoV-2 is essential in

34   understanding virus evolution and selection pressures. Sequences of SARS-CoV-2 in

35   nasopharyngeal swabs from hospitalised patients in the UK were determined and virus isolated.

36   The data indicated the virus existed as a population with a consensus level and non-synonymous

37   changes at a minor variant. For example, viruses containing the nsp12 P323L variation from the

38   Wuhan reference sequence, contained minor variants at the position including P and F and other

39   amino acids. These populations were generally preserved when isolates were amplified in cell

40   culture. In order to place VOCs B.1.1.7 (the UK 'Kent' variant) and B.1.351 (the 'South African'

41   variant) in context their growth was compared to a spread of other clinical isolates. The data

42   indicated that the growth in cell culture of the B.1.1.7 VOC was no different from other variants,

43   suggesting that its apparent transmission advantage was not down to replicating more quickly.

44   Growth of B.1.351 was towards the higher end of the variants. Overall, the study suggested that

45   studying the biology of SARS-CoV-2 is complicated by population dynamics and that these need

46   to be considered with new variants.

47 **Importance**

48 SARS-CoV-2 is the causative agent of COVID-19. The virus has spread across the planet causing a

49 global pandemic. In common with other coronaviruses, SARS-CoV-2 genetic material (genomes)

50 can become quite diverse as a consequence of replicating inside cells. This has given rise to

51 multiple variants from the original virus that infected humans. These variants may have different

52 properties and in the context of a widespread vaccination program may render vaccines less

53 ineffective. Our research confirms the degree of genetic diversity of SARS-CoV-2 in patients. By

54 isolating viruses from these patients, we show that there is a 100-fold range in growth of even

55 normal variants. Interestingly, by comparing this to the pattern seen with two Variants of

56 Concern (UK and South African variants), we show that at least in cells the ability of the B.1.1.7

57 variant to grow is not substantially different to many of the previous variants.

Introduction

SARS-CoV-2 emerged late 2019 in Wuhan, China and causes COVID-19 (1). This can be a fatal infection with severe immunopathology in the respiratory system (2). The virus has since spread worldwide and resulted in more than 2.5 million deaths (3) placing large burdens on healthcare infrastructures and global economies. Several vaccines have been granted emergency licensure and these appear to be driving down cases in countries with large scale vaccine roll outs. However, multiple variants have been identified worldwide and these have the potential for vaccine evasion and immune escape, leading to the label of Variants of Concern (VOCs).

SARS-CoV-2 has a single stranded positive sense RNA genome about 30kb in length. The first two thirds of the genome is translated to give the viral non-structural proteins (NSP1-16), which includes the viral RNA dependent RNA polymerase (NSP12). Several viral RNA synthesis processes occur during infection including replication of the genome and transcription of a nested set of subgenomic mRNAs (sgmRNAs). This latter process requires discontinuous transcription during negative strand synthesis (4). As a natural consequence, coronaviruses have high levels of recombination. This can result in both deletions and insertions and template switching as well as the formation of defective RNAs. An example of this is the probable insertion of the furin cleavage site in the spike glycoprotein (5). Although SARS-CoV-2 and other coronaviruses have some type of proof-reading capability (6), this is generally thought to help maintain their large genomes, without entering error catastrophe. Otherwise the accumulation of deleterious mutations would result in a rapid loss of fitness and extinction of a viral population (7). Additionally, potential genome modifications can result from nucleotide changes through the action of cellular proteins involved in RNA processing (8). SARS-CoV-2 accumulates mutations at

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80   roughly the same frequency as Ebola virus (9). These drivers of genetic diversity and the numbers

81   of people infected has led to multiple lineages and variants of SARS-CoV-2 being identified

82   worldwide.

83   The sgmRNAs encode for the main structural proteins, including the envelope protein (E)

84   protein, the membrane (M) protein, the nucleocapsid (N) protein and the spike (S) glycoprotein.

85   The S protein is a component of the enveloped virion and interacts with the angiotensin

86   converting enzyme-2 receptor (ACE-2) found on human cells. The S protein is also the major

87   source of neutralising epitopes and therefore under selection pressure in coronaviruses (and

88   SARS-CoV-2). Other viral proteins are involved in modulating the innate immune response.

89   Many variations in the coronavirus genome occur in the S gene (10-12) and this also has

90   been identified for SARS-CoV-2. For example, the D614G substitution in the SARS-CoV-2 S

91   protein, which emerged by March 2020, demonstrated improved transmissibility compared to

92   Wuhan variants, and proceeded to dominate worldwide subsequently. This mutation is most

93   often accompanied with another amino acid substitution in NSP12, P323L (13). In September

94   2020, a variant of concern, VOC 202012/01 (B.1.1.7 lineage) was detected in Kent in the UK which

95   possessed 23 mutations distinct from the Wuhan reference sequence, including the N501Y

96   substitution in the receptor binding domain of the S protein. This may increase the affinity of

97   spike protein to ACE-2 receptor (14). Initial data suggested this variant could be related to an

98   increased risk of hospitilisation and death (15). The variant has now spread to several countries

99   and modelling studies have suggested increased transmissibility (16). Preliminary experiments in

100   hamsters have identified increased viral shedding compared to the D614G variant (17). However,

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101   *in vitro* studies suggest that the B.1.1.7 VOC does not have any replicative advantage in primary

102   airway epithelial cells (18).

103   As variants are likely to continue to emerge on a background of incomplete vaccination

104   globally, understanding the significance of such variants both *in vitro* and *in vivo* is important to

105   provide biological mechanistic data rather than rely on *in silico* modelling to determine their

106   potential threat to vaccines or transmission advantage. To investigate the genetic and phenotypic

107   diversity of SARS-CoV-2 in patients and in the context of the emergence of the B.1.1.7 and B.1.351

108   lineage viruses and concerns around potential higher viral loads, the growth of these viruses was

109   bench marked against the Victoria isolate and clinical isolates from other samples taken during

110   the outbreak.

111   **Results**

112   Although consensus genomes for SARS-CoV-2 are reported on global databases from the

113   sequencing of clinical specimens, in reality the virus will exist as a population within an individual

114   and may also include defective RNAs. Likewise, in some pipelines, viral genomes or variants

115   containing out of place stop codons within ORFs will not be returned as consensus even though

116   they may be dominant. In this case, at a minor variant level, which could represent 49% of other

117   genomes within the same individual, the wildtype protein may be expressed, and counterbalance

118   any aberrantly functioning proteins. To investigate the sequence diversity of SARS-CoV-2 within

119   a patient and to compare the growth of these viral populations to recent VOCs, nasopharyngeal

120   swabs were taken from patients with COVID-19, sequenced and the genotypes and variants of

121   isolated viruses and their growth properties compared in cell culture (Figure 1).

122

123   **Sequence variation of SARS-CoV-2 in clinical swabs compared to Wuhan reference strain**

124   Nine swabs representing different time points in the outbreak in the UK contained

125   recoverable virus that could be isolated and grown. The virus population in these swabs was

126   sequenced and both consensus genomes and minor variants determined. Consensus sequence

127   variation was compared to the reference genome (NC_045512; Wuhan-Hu-1) to see how far the

128   isolates had diverged and with minor variants listed for the secondary and tertiary positions

129   (Supplementary Table 1). Most viruses demonstrated a few amino acid variations compared to

130   the reference sequence. For example, SCV2-006, a lineage B virus sequenced from the swab of

131   a patient from the Diamond Princess cruise ship (February 2020) had only one substitution

132   present, R203K in the N protein (Figure 2). In comparison, sequence analysis of SCV2-009, a virus

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133   isolated from a swab sampled from a patient in the UK in March 2020 (Figure 2) now possessed

134   the D614G and P323L substitutions in the spike glycoprotein and NSP12, respectively. These are

135   in contrast to the B.1.1.7 variant, which emerged later in 2020 and is characterised by the

136   presence of 23 amino acid differences from the reference genome. Analysis of the virus

137   population present in the nasopharyngeal isolate of SCV2-009 illustrated the diversity associated

138   with the virus. For example, taking the P323L substitution in NSP12, out of an amino acid

139   coverage of 202, 170 amino acids mapped to L, 12 to P and 9 to F. For the D614G substitution in

140   the spike glycoprotein, out of an amino acid coverage of 3452, 3360 amino acids mapped to G,

141   24 to S and 21 to V. This general pattern is reflected in other clinical isolates. For example, in

142   isolate SCV2-010, in NSP12, out of an amino acid coverage of 285, 273 mapped to L, 50 to I and

143   3 to P. In isolate SCV2-008, in NSP12, out of an amino acid coverage of 153, 130 mapped to L, 9

144   to P and 7 to F. This suggests, for NSP12, that at the minor variant level the reference sequence

145   amino acid is still present, but other amino acids such as F may be common (Supplementary Table

146   1), and subject to selection pressure. In some clinical swabs, for example in N at position 204, the

147   second most common feature is a stop codon. SCV2-011 and SCV2-018 were variants isolated

148   from clinical swabs taken from the same patient but three days apart, these did not vary at the

149   consensus between each other, but did at the minor variant level. SCV2-007 and SCV2-017 were

150   also variants isolated from clinical swabs taken from the same patient but three days apart and

151   did not vary at the consensus between each other in swabs, but did at the minor variant level.

152

153   **Comparison of sequence variation in stocks and after 72 hours in hACE2-A549 cells**

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154 In order to assess the biology of the viruses isolated from the clinical swabs and compare

155 their growth to B.1.1.7 and B.1.351, sufficient stocks had to be grown. To isolate SARS-CoV-2

156 from the clinical swabs, the nasopharyngeal sample was filtered and placed on VeroE6 cells with

157 antibiotics and antifungals until CPE was observed. The supernatant was collected from these

158 cells to generate sufficient stocks for infectivity assays and comparisons.

159 Growing virus for stocks may have introduced or selected for specific variants. One of

160 these, that has been characterised for SARS-CoV-2, is a deletion of the furin cleavage site in the

161 spike glycoprotein when grown in Vero E6 cell (19). Therefore, viral stocks were sequenced to

162 ensure they did not possess the deletion and to determine if variation occurred compared to

163 when the virus was sequenced directly from clinical swabs. Comparator viruses of known

164 provenance were obtained from collaborators. The comparator viruses were the B.1.1.7 ('Kent'

165 UK VOC) virus (termed SCV2-019 in this study) obtained at P4, the SARS-CoV-2/Victoria/01/2020

166 (an isolate from Australia) obtained at P3 (termed SCV2-021), and the B.1.351 virus ('South

167 African' VOC) (termed SCV2-022 in this study). These were grown in Vero/hSLAMS as a precaution

168 to prevent selection for the furin deletion. These were also sequenced to ensure they had the

169 variant defining mutations present. For these three comparator viruses, the sequencing showed

170 at the consensus level the furin cleavage site was intact and the other defining variations

171 separating these variants from the Wuhan reference sequence were present (Figure 3).

172 Analysis of the genome diversity between viruses sequenced in swabs from patients and

173 the virus stock used to infect cells indicated that most consensus variations from the Wuhan

174 reference sequence were still present (Supplementary Table 1). The minor variants at selected

175 positions were also still present. For example, in the stock preparation for SCV2-009, at position

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176   323 in NSP12, this was read with an amino acid depth of 517. The L was present at a depth of

177   499, P with a depth of 6 and F with a depth of 5, indicating that the consensus level amino acid

178   was still present with P and F at a minor level. For some stock viruses, variation from the

179   reference sequence was lost during preparation of the stock virus. The growth of these viruses

180   from the stocks was compared to a B.1.1.7. and a B.1.351 lineage virus and SARS-CoV-

181   2/Victoria/01/2020, obtained from near the start of the COVID-19 pandemic.

182

183   **Growth comparison of different SARS-CoV-2 variants to Variants of Concern (VOCs).**

184   In order to identify whether the B.1.1.7 (SCV2-019) and B.1.351 (SCV2-022) displayed a

185   growth advantage over less recent strains of the virus, three different cell lines were infected

186   with the viruses at an MOI of 0.01 over the course of 72 hours and the resultant supernatants, at

187   24, 48 and 72 hours titrated by plaque assay on Vero E6 cells. The three different cell lines were

188   Vero E6 (commonly used to grow viral stocks and initial isolates from clinical samples),

189   Vero/hSLAM (reported to prevent deletion of the furin cleavage site in the spike glycoprotein)

190   and hACE2-A549 cells. This latter cell line is based on A549 cells, which are respiratory epithelium

191   in origin, commonly used to study respiratory viruses in cell culture but overexpress the ACE2

192   protein. A549 cells mount an interferon response to virus infection.

193   In Vero E6 cells, eleven SARS-CoV-2 variants followed a similar pattern of growth, with

194   the exception of SCV2-021 (SARS-CoV-2/Victoria/01/2020) which grew at significantly reduced

195   levels compared to other variants by 72 hours post infection (mean 5.7×10^4 PFU/ml, p=0.006)

196   (Figure 4A). A similar pattern of growth was observed in all twelve variants in Vero/hSLAM cells,

197   but there was no significant difference (p>0.05) in the titres of any of the viruses produced by 72

11

198   hours post infection (Figure 4B). In contrast, in hACE2-A549 cells, there was more heterogeneity

199   observed between variants, with the range of viral titres being much lower (7.9 x 10^1 -3.01 x 10^4

200   PFU/ml) than that observed in Vero cells at 72 hours post infection. The B.1.1.7 variant (SCV2-

201   019) had the lowest titre at 24 hours post-infection in growth assays in hACE2-A549 cells, before

202   growing to reach a final titre 2.71 x 10^3 PFU/ml at 72 hours post-infection (Figure 4C). We

203   observed that between variants at the growth extremes at 72 hrs post-infection in hACE2-A549

204   cells there was an approximately <2 log difference between titres of SCV2-007 and SCV2-018,

205   despite the same amount of virus being used as the inoculum (MOI=0.01). For the VOC B.1.1.7

206   the growth at 72 hrs post-infection in hACE2-A549 cells was in the middle of the other variants

207   tested (2.71 x 10^3 PFU/ml) while the VOC B.1.351 had the second highest final titre of 2.62 x 10^4

208   PFU/ml. (Figure 4C). In addition, we note that the B.1.351 lineage variant had the highest viral

209   titres in both VeroE6 and Vero/hSLAMs at 24 and 48 hrs post-infection.

210   Comparing viruses grown from the same patient but sampled three days apart (SCV2-007

211   and SCV2-017, and SCV2-011 and SCV2-018 at day one and day three respectively, showed

212   differences in their growth in hACE2-A549 cells. (Supplementary Table 2). There was a 2-log

213   difference in the growth of SCV2-007 and SCV2-017, while there was little difference in the

214   growth of SCV2-011 and SCV2-018.

215

216   **The phenotype of the variants differed widely between cell lines, displaying mixed plaque**

217   **morphology and growth characteristics**

218   The phenotype of the plaques formed by each virus stock was observed in the three

219   different cell lines used at 72 hours post-infection. The appearance of the plaques from the

12

220   variants differed (Figure 5). SCV2-006, SCV2-011, SCV2-016, SCV2-018 and SCV2-022 had a larger

221   plaque phenotype after growth in Vero E6 cells, compared with SCV2-019 (B.1.1.7) and SCV2-021

222   (SARS-CoV-2/Victoria/01/2020). Equally, some variants displayed a mixed phenotype of both

223   large and small plaques in Vero E6 cells, as seen for SCV2-011, suggesting mixed viral species

224   were present (Figure 5). After growth in Vero/hSLAMS, SCV2-011 and SCV2-018 showed a mixed

225   phenotype after plaque assay. SCV2-019 (B.1.1.7) and SCV2-021 had the smallest plaque

226   phenotypes. After growth in hACE2-A549 cells, SCV2-006 and SCV2-022 (B.1.351) had the largest

227   plaque phenotypes, while SCV2-021 had the smallest plaque phenotype. SCV2-006 and SCV2-016

228   had mixed morphology of both large and small plaques. This illustrates the potential diversity

229   with a viral population.

230

231   **Genetic diversity of variants after passage in the three different cell types**

232   We hypothesised that differences in the phenotypic appearance of viruses and their

233   reproduction might reflect the presence of minor variants and stop codons in their underlying

234   sequences and this was investigated at 72 hrs post-infection in hACE2-A549 cells. All variants

235   have a consensus level genome but also minor variants. In SCV2-016, at 72 hrs post-infection

236   there was a stop codon at consensus level in ORF3A that was also present in the viral stock

237   (Supplementary Table 1). In SCV2-019 (the B.1.1.7. lineage virus) there was a stop codon in ORF8

238   at the consensus level at 72 hr post-infection, which was also present in the stock (Supplementary

239   Table 1). We note that both of these were low read depth, and other amino acids were present

240   at the minor variant level. Stop codons were also present in the variants at a minor variant level

241   (Supplementary Table 1).

242 **Discussion**

243   Sequence analysis of SARS-CoV-2 in clinical swabs from patients revealed a heterogenous

244 and diverse population from the Wuhan reference sequence. At the minor variant level, a

245 number of variants had genomes which contained premature stop codons. Examples of SARS-

246 CoV-2 genomes encoding non-functioning proteins have been previously identified in the human

247 population. For example, a cohort of patients in Singapore were identified with a deletion in ORF8

248 that was associated with a milder infection (20), although the variant disappeared either through

249 control measures or lack of fitness. This potential disconnect is not restricted to SARS-CoV-2. The

250 balance between consensus and minor variants and the presence of stop codons in virus

251 populations within individual patients has been shown to influence the activity of the Ebola virus

252 RNA dependent RNA polymerase and correlate with outcome in patients with Ebola virus disease

253 (21). Within an individual person with SARS-CoV-2, these mixtures of functioning and presumably

254 non-functioning viral proteins will potentially influence viral load.

255   Recent VOCs include SARS-CoV-2 variants from Nigeria (B.1.525). One of the differences

256 in this variant from the Wuhan consensus sequence is P323F in NSP12. This variation was also

257 identified in a cluster of patients in Northern Nevada in the USA (22). The analysis of variants in

258 this study, isolated earlier in 2020, indicated that an F at position 323 existed at a minor variant

259 level. Therefore, we hypothesize that if an F at this position was advantageous (e.g. altered RdRp

260 activity) then the variant would have been selected during passage. However, this was not the

261 case, and therefore we speculate that the emergence of an F at position 323 in NSP12 may be

262 through founder effect.

263   The growth of different variants, and a variant from near the start of the COVID-19

264   pandemic, were compared in three different cell lines to the growth of two VOCs and the

265   Australia Victoria variant. These VOCs were from the B.1.1.7 and B.1.351 lineages and represent

266   viruses that have an apparent transmission advantage in the general population and/or may be

267   less refractive to currently approved vaccines. There was an approximately 2 log difference in

268   growth at 72 hrs in the hACE2-A549 cells by the different variants. The growth of VOCs in this cell

269   line were within these limits. Extrapolating this observation to the perceived transmission

270   advantage of B.1.1.7 in the human population, would suggest this is not down to the VOC growing

271   to higher titres in cells in vivo compared with other variants, though we acknowledge that out *in*

272   *vitro* experiments may not correlate exactly with growth rates *in vivo*. With B.1.1.7, the same

273   heterogenous patterns of disease in humans as other variants, although we note that B.1.1.7 has

274   been associated with a decrease in Ct values from nasopharyngeal swabs and also an increase in

275   mortality in some populations. In contrast, different variants of the coronavirus infectious

276   bronchitis (IBV) can individually cause different spectrums of organ specific disease, and

277   therefore current variations in the genome of SARS-CoV-2 may not automatically equate to

278   radically different disease as observed with IBV. Due to the promiscuous nature of coronavirus

279   RNA synthesis, variants have and will occur all of the time. This emphasises the need for genotype

280   to phenotype studies to place newly emerged variants that have perceived differences in context.

281   Comparison of viruses isolated from the same patients at different time points revealed

282   intriguing differences. While the viruses SCV2-007 and SCV2-017 differed by 2-logs in hACE2-

283   A549 cells at 72 hours, there was little difference observed between the twin viruses SCV2-011

284   and SCV2-018. Notably, the SCV2-017 virus had picked up an additional mutation at 72 hours

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285   post-infection – a change from A in the reference genome at position 1120 in NSP3 to a V. It is

286   possible this may be responsible for the difference in growth of this virus to its founder, SCV2-

287   007, and could reflect viral adaptation to the immune response in this individual over the course

288   of infection (Supplementary table 2).

289   The analysis of virus in the nasopharyngeal swabs clearly paints a picture of a diverse

290   population of SARS-CoV-2. When studying isolates, even when grown in cell culture, that

291   population still continues. Thus, whilst lineage defining variations are present at a consensus

292   level, minor variants are present underneath that may have an impact on biology. This would

293   suggest that the study of specific genotypes requires either plaque purification or reverse

294   genetics. However, the study suggests that the viral population (consensus and minor variants)

295   should be taken into account when studying the transmission of SARS-CoV-2.

296   **Methods**

297

298   **Cells.** African green monkey kidney C1008 (Vero E6) cells (Public Health England, PHE) were

299   cultured in Dulbecco's minimal essential medium (DMEM) (Sigma) with 10% foetal bovine serum

300   (FBS) (Sigma) and 0.05mg/ml gentamicin at 37°C/5% CO_2. Vero/hSLAM cells (PHE) were grown in

301   DMEM with 10% FBS and 0.05mg/ml gentamicin (Merck) with the addition of 0.4mg/ml Geneticin

302   (G418; Thermofisher) at 37°C/5% CO_2. Human ACE2-A549 (hACE2-A549), a lung epithelial cell line

303   which overexpresses the ACE-2 receptor, were the kind gift of Oliver Schwartz [23] and were

304   cultured in DMEM with 10% FBS and 0.05mg/ml gentamicin with the addition of 10µg/ml

305   Blasticidin (Invitrogen). Only passage 3-10 cultures were used for experiments.

306

307   **Virus isolation.** The SARS-CoV-2/human/Liverpool/REMRQ0001/2020 isolate (Genbank ID

308   MW041156.1), was used at passage 3. The fourth passage of virus (here named SCV2-

309   006_stockP4) was cultured in Vero E6 cells with DMEM containing 4% FBS and 0.05mg/ml

310   gentamicin at 37°C/5% CO_2 and harvested 48 hours post inoculation. Virus stocks were aliquoted

311   and stored at -80°C.

312   Viruses named SCV2-007 to SCV2-018 were grown from nasopharyngeal swabs of

313   patients using the following method. One hundred microlitres of viral transport media from the

314   swab was mixed with 100µl DMEM with 4% FBS, 0.05mg/ml gentamicin, 25µg/ml plasmocin

315   (Invivogen) and 2.5µg/ml amphotericin B (Merck). These were then filtered using ultrapure MC

316   0.22µm filters (Merck) and the filtrate placed onto cells in a 24 well plate of Vero E6 cells for 1

317   hour. After one hour, the media was topped up with DMEM (2% FBS, 0.05 mg/ml gentamicin,

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318   25µg/ml plasmocin, 2.5 µg/ml amphotericin B). Cells were observed daily for cytopathic effect

319   (CPE) and the cell supernatant harvested once CPE was evident. This provided the first passage

320   virus. Stocks of these were then grown in Vero E6 as described above and frozen down in aliquots

321   at -80°C and named SCV2-007 to SCV2-018_stockP2.

322   The B.1.1.7 and B.1.351 isolates were used at passage 4. The fifth passage (here named

323   SCV2-019_stockP5 and SCV2-022_stockP5) were cultured in Vero/hSLAM cells with DMEM

324   containing 4% FBS, 0.05mg/ml gentamicin and 0.4mg/ml geneticin and harvested 72 hours post

325   inoculation. Virus stocks were aliquoted and stored at -80°C. SARS-CoV-2 Victoria/01/2020 was

326   passaged three times in Vero/hSLAM cells. The fourth passage stock (here named SCV2-

327   021_stockP4) was cultured in Vero/hSLAM cells DMEM containing 4% FBS, 0.05mg/ml

328   gentamicin and 0.4mg/ml geneticin and harvested 72 hours post inoculation. Virus stocks were

329   aliquoted and stored at -80°C (Supplementary table 2).

330

331   **Virus titration.** Viral titres of stocks were calculated using plaque assays. Briefly, confluent 24-

332   well plates of Vero E6 cells were inoculated with serial ten-fold dilutions of the stocks in duplicate

333   for one hour at 37°C/5% CO_2. Plates were overlaid with DMEM containing 2% FBS, 0.05mg/ml

334   gentamicin and 2% low melting point agarose (Lonza) and incubated at 37°C/5% CO_2 for 72 hours.

335   Plates were fixed using 10% formalin, the overlay removed, and plates stained using crystal violet

336   solution (Sigma). Virus titre was measured in plaque forming units per ml (PFU/ml).

337

338   **Virus growth kinetics.** Vero E6, Vero/hSLAM and hACE2-A549 cells were grown in 96 well plates

339   for viral growth kinetic experiments. For infection, media was removed from plates and virus

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340   inoculum added at an MOI of 0.01 in DMEM containing 2% FBS, 0.05mg/ml gentamicin and the

341   respective selective antibiotics for each cell line (6 wells per timepoint). Plates were incubated at

342   37°C/5% CO_2 for one hour. The inoculum was removed, and cells were washed once with PBS

343   (Sigma). The respective media with 2% FBS (100µl) was added to each well. The cell supernatant

344   was removed from wells and combined (0hrs post infection) and plates incubated further.

345   Supernatants were likewise removed at 24, 48 and 72 hours post infection. Approximately 250µl

346   of the supernatants were aliquoted directly into tubes containing 750µl Trizol LS (Fisher) to

347   inactivate the virus. All supernatants and inactivated supernatants were stored at -80°C until viral

348   titration and RNA extraction could be performed. All infections were performed at least three

349   times in independent experiments.

350

351   **RNA extraction and amplification of viral nucleic acids.** RNA from clinical samples was extracted

352   and DNase treated as described previously. Samples from patients were sequenced using the

353   RLSA approach (24). RNA from viral stocks and from 72-hour post infection cultures were

354   sequenced by Oxford Nanopore long read length sequencing on flow cells run on MinION or

355   GridION.

356

357   **Nanopore sequencing.** Sequencing libraries for amplicons generated by RSLA (24) or ARTIC were

358   prepared following the 'PCR tiling of SARS-CoV-2 virus with Native Barcoding' protocol provided

359   by Oxford Nanopore Technologies using LSK109 and EXP-NBD104/114.

360

361 **Variant calling.** The artic-ncov2019 pipeline v1.2.1 (https://artic.network/ncov-2019/ncov2019-

362 bioinformatics-sop.html) was used to filter the passed Fastq files produced by Nanopore

363 sequencing with lengths between 800 and 1600 for RSLA, and 400 and 700 for ARTIC. This

364 pipeline was then used to map the filtered reads on the reference SARS-CoV-2 genome

365 (NC_045512.2) by minimap2 and assigned each read alignment to a derived amplicon and

366 excluded primer sequences based on the RSLA and ARTIC V3 primer schemes in the bam files.

367 These bam files were further analysed using DiversiTools

368 (http://josephhughes.github.io/btctools/) with the "-orfs" function to generate the ratio of

369 amino acid change in the reads and coverage at each site of protein in comparison to the

370 reference SARS-CoV-2 genome (NC_045512.2). The amino acids with highest ratio and coverage

371 > 10 were used to assemble the consensus protein sequences.

372

373 **Statistics.** Viral titre data was log transformed and one-way ANOVAs performed with post-hoc

374 Bonferroni tests performed to determine if any significant difference at T=72 hours post infection

375 occurred between the SCV2-019 (B.1.1.7) and other viruses in different cell lines.

376

377 **Ethics and clinical information.** The patients from which the virus samples were obtained gave

378 informed consent and were recruited under the International Severe Acute Respiratory and

379 emerging Infection Consortium (ISARIC) WHO Clinical Characterisation Protocol CCP. Ethical

380 approval for data collection and analysis by ISARIC4C was given by the South Central-Oxford C

381 Research Ethics Committee in England (reference 13/SC/0149), and by the Scotland A Research

382 Ethics Committee (reference 20/SS/0028). Samples were use with consent from patients or

383 consultees. The ISARIC WHO CCP-UK study was registered at

384 https://www.isrctn.com/ISRCTN66726260 and designated an Urgent Public Health Research

385 Study by NIHR. Protocol, patient information sheets, consents, case report forms and process of

386 data and sample access request are available at https://ISARIC4C.net.

387

409   **Author Contributions**

410   TP, XD, RP-R, NR, CH, HG, BJ, JD, GLH, GS, ERA and EIP performed the experiments,

411   sequencing, bioinformatics and isolated virus. Data was analysed by TP, XD, RP-R and

412   JAH. MWC, LT, JPS and JAH supervised the project. MGS, JKB and PJMO established

413   the ISARIC4C consortium that was used to obtained some of the UK clinical isolates used

414   in the study. TP, XD, MWC and JAH wrote the manuscript, all authors provided editing

415   and final approval.

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587   and Identification of Deletions in the Viral Genome That Encode Proteins Involved in

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589

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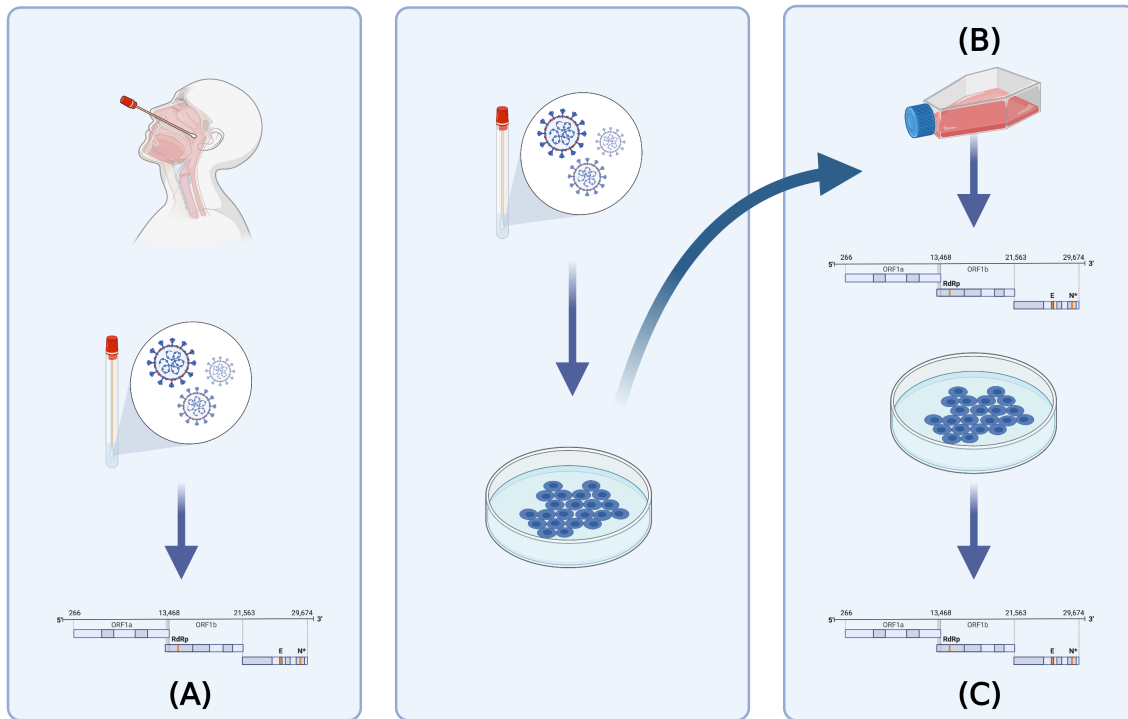
Figure 1. Testing strategy. (A) Nasopharyngeal swabs from patients with COVID-19 recruited to the ISARIC-4C study were sequenced using an amplicon based approach on the Oxford Nanopore MinION (P0). Virus was isolated from the same nasopharyngeal swabs(P1). (B) Viral isolates from the ISARIC-4C study, B.1.1.7, B.1.351 and Victoria isolates were grown up into stocks which were then sequenced. (C) Viral stocks were titrated and used to infected hACE2-A549 cells, and 72-hour post infection supernatants were sequenced.

597
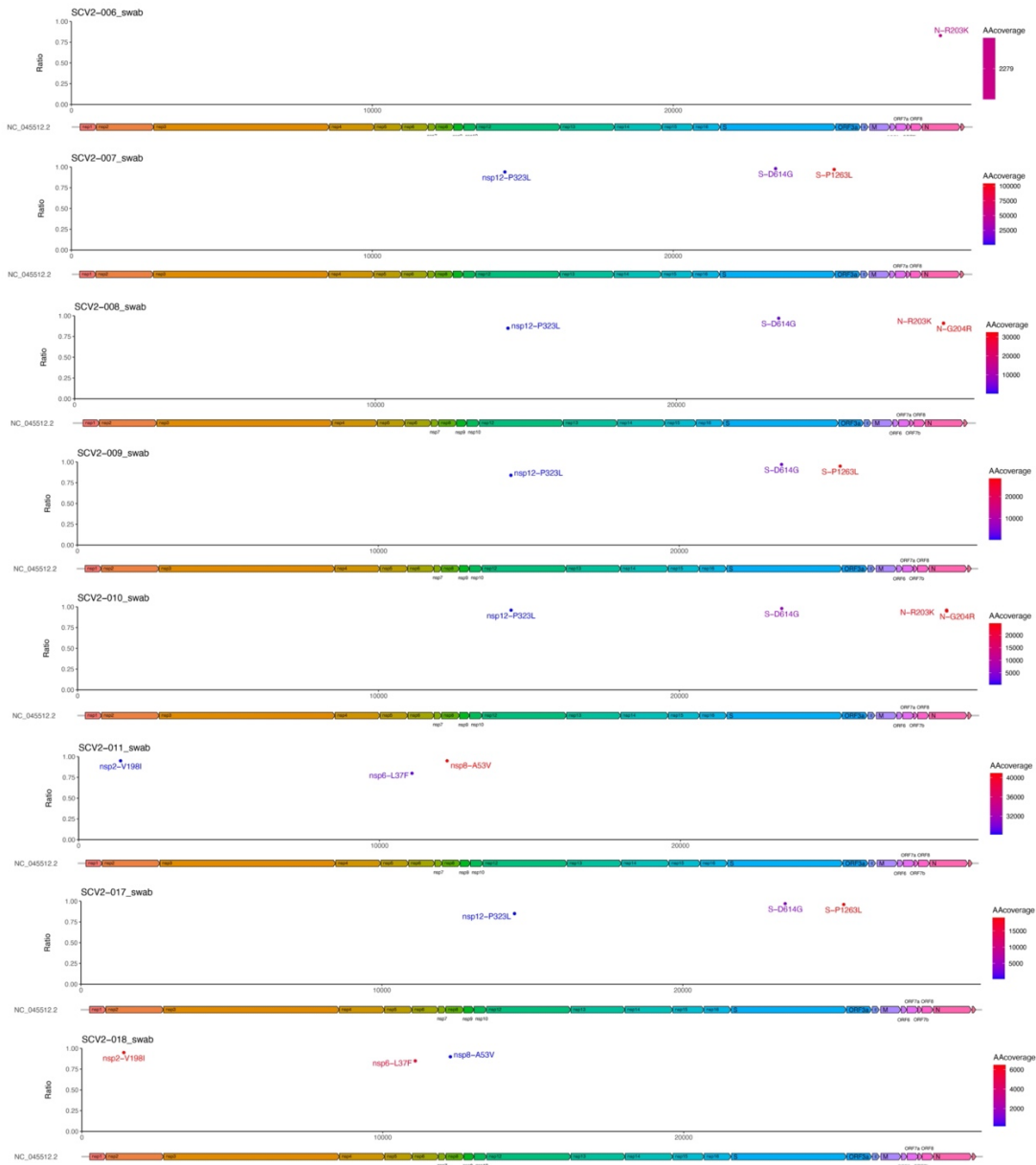
Figure 2. (A) Comparison of the consensus sequence SARS-CoV-2 in ISARIC4C swabs collected from patients in hospital wit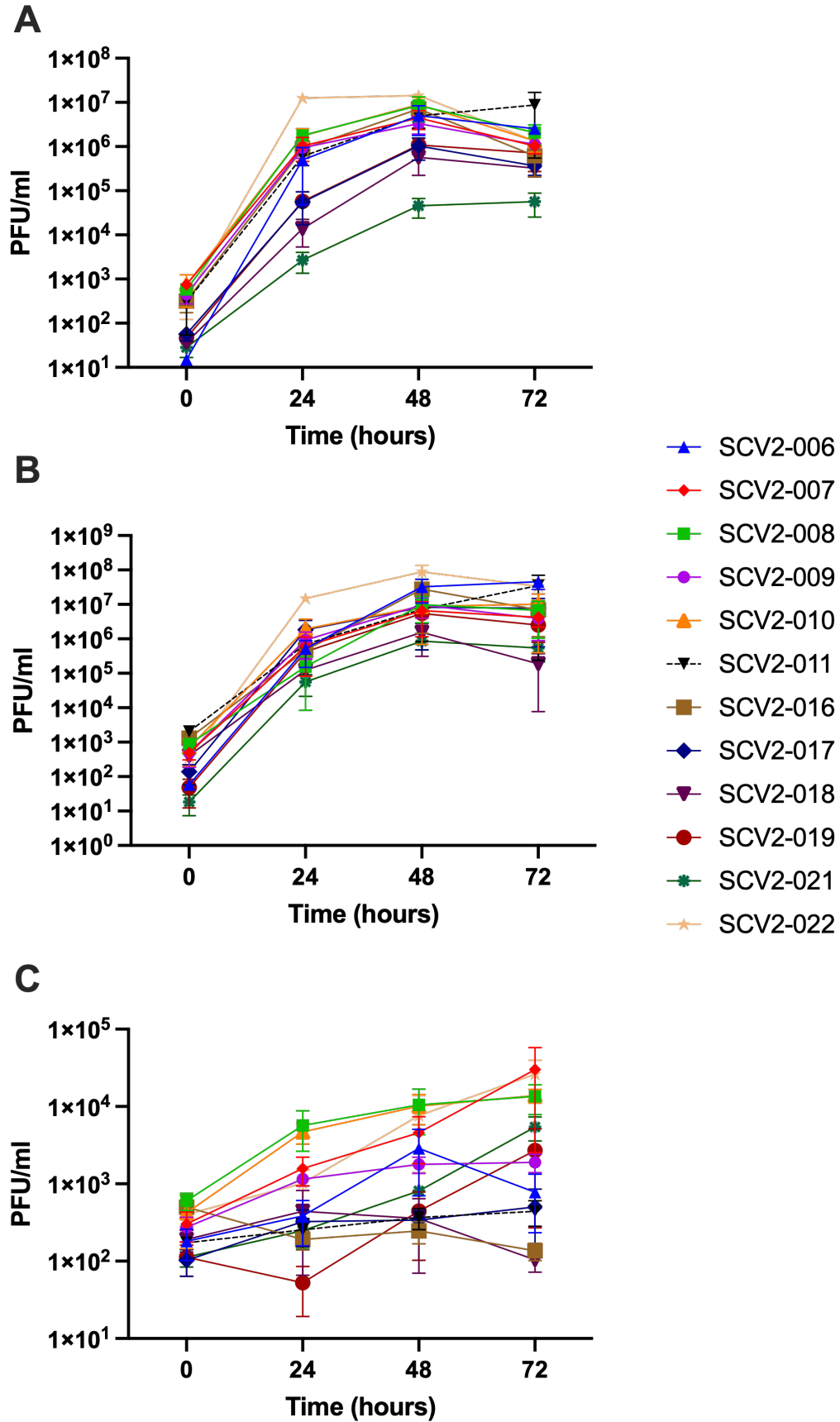h COVID-19. Variations from the Wuhan reference sequence are plotted with the location on the viral genome against the percentage (ratio) of reads where the variation is observed. Variants are coloured to demonstrate the number of reads achieved (>10).

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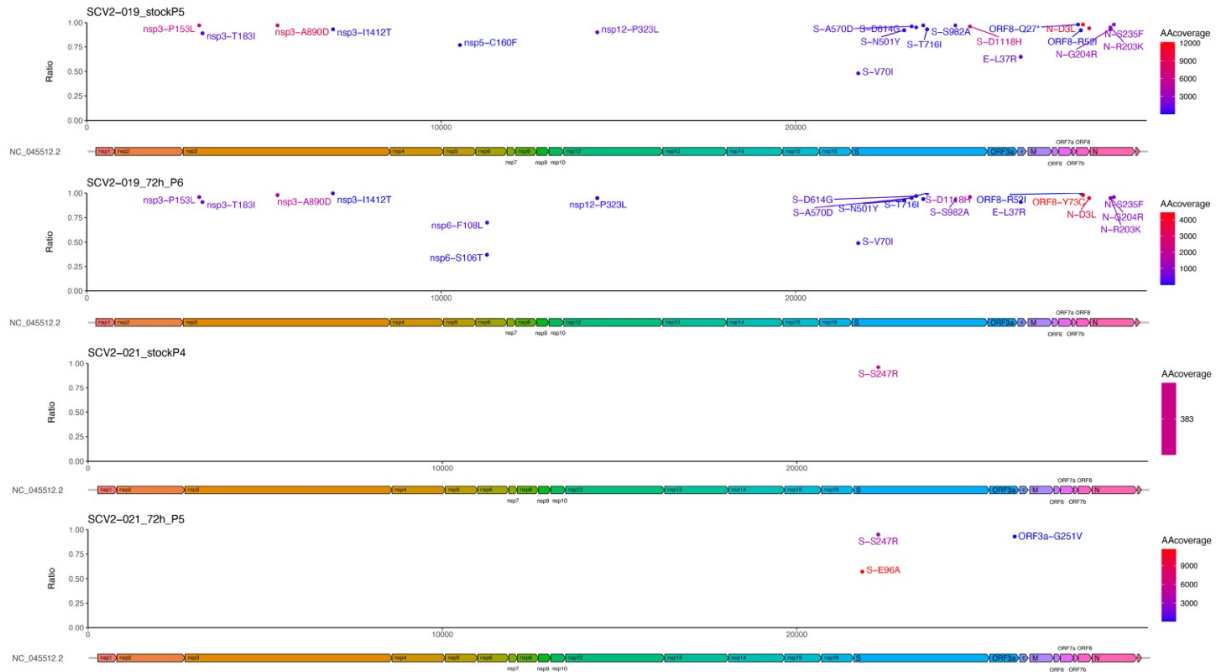
Figure 3. Comparison of the UK 'Kent' VOC (SCV2-019) and the Australian Victoria isolate (SCV2-021) with the Wuhan reference sequence. Variations from the Wuhan reference sequence are plotted with the location on the viral genome against the percentage (ratio) of reads where the variation is observed. Variants are coloured to demonstrate the number of reads achieved (> 10).

A

B

C

Legend:
- SCV2-006
- SCV2-007
- SCV2-008
- SCV2-009
- SCV2-010
- SCV2-011
- SCV2-016
- SCV2-017
- SCV2-018
- SCV2-019
- SCV2-021
- SCV2-022

608

609   **Figure 4.** Growth over time of 11 different viral isolates in three different cell lines compared to

610   the Variants of Concern SCV2-019 (UK 'Kent' VOC) and SCV2-022 ('South African' VOC).

611   Comparison viruses included the Australian Victoria variant (SCV2-021). (A) Growth of viruses in

612   plaque-forming units (PFU) per ml over times in Vero E6 African green monkey kidney cells. (B)

613   Growth of viruses in Vero cells expressing the human signalling lymphocytic activation module

614   (SLAM) gene (Vero/hSLAM). (C) Growth of viruses in human ACE-2 expressing A549 cells (hACE2-

615   A549). All experiments were repeated in triplicate using supernatant from 6 wells (n=3).

616
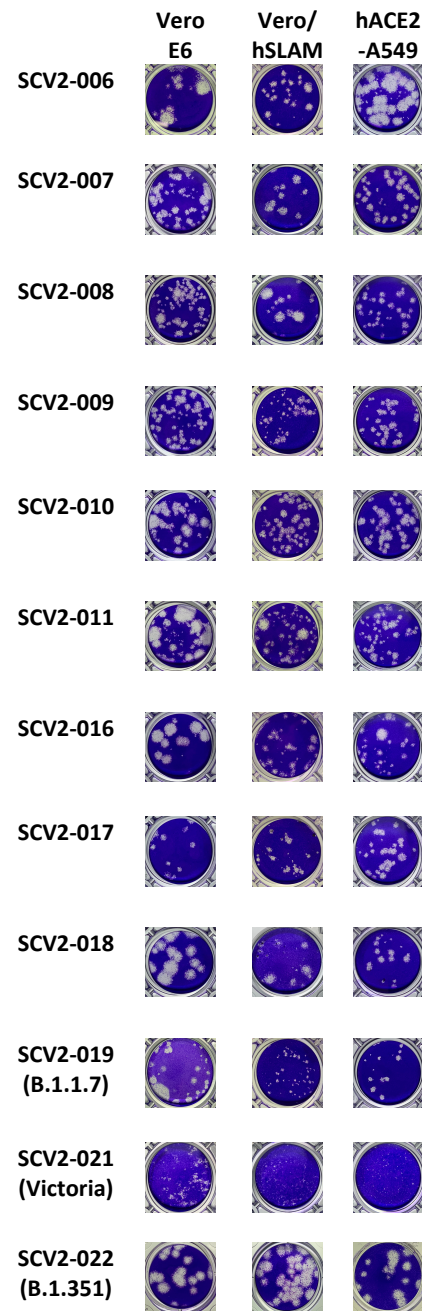
617   **Figure 5.** Phenotypic appearance of plaque assays from variants grown in three different cell

618   lines; (i) Vero E6, (ii) Vero/hSLAM and (iii) hACE2-A549 cells. Plaque assays were performed on

619   VeroE6 cells. Variants of Concern are SCV2-019 (UK 'Kent' VOC) and SCV2-022 ('South African'

620   VOC). Comparison viruses include the Australian Victoria variant (SCV2-021).