

1 **Increased transmission of SARS-CoV-2 lineage B.1.1.7 (VOC**
2 **2020212/01) is not accounted for by a replicative advantage**
3 **in primary airway cells or antibody escape**

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23 **Abstract**

24 Lineage B.1.1.7 (Variant of Concern 202012/01) is a new SARS-CoV-2 variant which was first
25 sequenced in the UK in September 2020 before becoming the majority strain in the UK and spreading
26 worldwide. The rapid spread of the B.1.1.7 variant results from increased transmissibility but the
27 virological characteristics which underpin this advantage over other circulating strains remain
28 unknown. Here, we demonstrate that there is no difference in viral replication between B.1.1.7 and
29 other contemporaneous SARS-CoV-2 strains in primary human airway epithelial (HAE) cells. However,
30 B.1.1.7 replication is disadvantaged in Vero cells potentially due to increased furin-mediated cleavage
31 of its spike protein as a result of a P681H mutation directly adjacent to the S1/S2 cleavage site. In
32 addition, we show that B.1.1.7 does not escape neutralisation by convalescent or post-vaccination
33 sera. Thus, increased transmission of B.1.1.7 is not caused by increased replication, as measured on
34 HAE cells, or escape from serological immunity.

35 Introduction

36 In late 2019, SARS-CoV-2 emerged into humans from animals and rapidly led to a global
37 pandemic. In September 2020, a new variant of SARS-CoV-2, lineage B.1.1.7 (Variant of Concern
38 202012/01) emerged in the UK (Rambaut et al., 2020). B.1.1.7 is distinguished by a large number of
39 mutations and a long phylogenetic branch length separating it from its closest sequenced isolates. The
40 genetic distance from other viruses has prompted suggestions that B.1.1.7 may have evolved during
41 extended infection of an immunocompromised host (Rambaut et al., 2020). In late 2020 and early
42 2021, B.1.1.7 spread rapidly to become the dominant lineage in the UK. This is likely accounted for by
43 increased transmissibility measured by an increase in the effective reproduction number (R_t) of 0.4-
44 0.7 (Volz, Mishra, et al., 2021). B.1.1.7 has now been detected in 88 other countries, and has become
45 predominant in several of these (O'Toole et al., 2021). Moreover, recent reports suggest that B.1.1.7
46 infection results in an approximately 70% higher hazard of death compared to other strains (Challen
47 et al., 2021; Davies et al., 2021). To effectively control SARS-CoV-2 and to assess the risk of future
48 variants, it is vital to understand the phenotypic characteristics and the underpinning mutations which
49 have resulted in the higher transmissibility and pathogenicity of the B.1.1.7 lineage.

50 The B.1.1.7 lineage is characterised by 23 mutations across the viral genome (Rambaut et al.,
51 2020). The spike glycoprotein (S) harbours 9 of these including N501Y, Δ 69-70, Δ 144 and P681H.
52 N501Y lies in the receptor binding domain (RBD) and has been shown to enhance binding of S to its
53 receptor ACE2 (Starr et al., 2020; Supasa et al., 2021). This mutation gives the B.1.1.7 UK variant the
54 alternative designation 20I/501Y.V1 and has also been observed in several other lineages including
55 the B.1.351 (501Y.V2) South African variant and the Brazilian P.1 (20J/501Y.V3) variant (Faria et al.,
56 2021; Tegally et al., 2020). Two deletions, Δ 69-70 and Δ 144, map to the N-terminal domain (NTD).
57 Deletions around position 144 have been observed during extended replication *in vitro* and within-
58 host evolution in immunocompromised patients, and have also been linked to escape from NTD-
59 targeting antibodies (Andreano et al., 2020; Choi et al., 2020; Kemp et al., 2021; McCallum et al., 2021;

60 McCarthy et al., 2020). Δ 69-70 has arisen in multiple lineages and its effect is unknown but may
61 compensate for a putative fitness cost of substitutions in the RBD (Kemp et al., 2020). P681H is at the
62 S1/S2 cleavage site and could affect the efficiency of furin cleavage. We and others have previously
63 shown that efficient cleavage of S at this site enhances transmissibility and pathogenicity of SARS-CoV-
64 2 (Johnson et al., 2021; Peacock et al., 2020; Zhu et al., 2021). B.1.1.7 also harbours mutations of
65 interest in other genes including a premature stop codon in ORF8, an accessory gene that likely
66 enables immune evasion (Zhang et al., 2020), and a 3 amino acid deletion in NSP6, one of several
67 proteins associated with virus regulation of the innate immune response (Xia et al., 2020).
68 Interestingly, truncation of ORF8 and the identical deletion in NSP6 are also present in several other
69 emerging SARS-CoV-2 variants suggesting a profound degree of convergent evolution even outside
70 the S gene, however their phenotypic effects remain undefined.

71 The appearance and rapid spread of the B.1.1.7 variant beyond the UK is clearly not a result
72 of chance, for example a founder effect, but due to a transmission advantage conferred by its
73 particular genetic constellation. However, transmission is a multifactorial phenotype and it is not yet
74 clear which of the B.1.1.7 mutations, or specific combination of mutations, contribute to the
75 modification of viral traits which support its increased transmissibility. Improved transmissibility may
76 owe to a combination of factors including more rapid viral replication within a host increasing the
77 amount or duration of virus emitted from an infected host, increased environmental stability of
78 infectious virus, more efficient entry into host cells, improved innate immune evasion that would
79 increase the chance of an exposure leading to infection, or the ability to overcome convalescent and
80 post-vaccination sera thereby increasing the size of the population susceptible to infection.

81 Epidemiological data to support some of these potential explanations for increased
82 transmissibility are thus far mixed. The results of analyses of Ct values or number of mapped
83 sequencing reads as a proxy for viral load are currently conflicting making it difficult to conclude
84 whether there is a replicative advantage for B.1.1.7 in-host (Golubchik et al., 2021; Kidd et al., 2020;

85 Walker et al., 2021). Another recent study showed similar peak viral burden but an increased duration
86 of infection with B.1.1.7 infection, albeit with a small sample size (Kissler et al., 2021).

87 The question of whether the B.1.1.7 variant escapes pre-existing immunity is also unclear. In
88 vitro passage of SARS-CoV-2 in the presence of neutralising antibodies can give rise to spike mutations
89 which evade antibody immunity (Andreano et al., 2020; Weisblum et al., 2020) making surveillance
90 for such escape mutations in circulating viruses and measurement of their effects *in vitro* a priority. In
91 an intense burst of research around this question, convalescent and post-vaccination sera have been
92 used in neutralisation assays with B.1.1.7 live virus isolated from infected patients, recombinant
93 viruses generated by reverse genetics and virus pseudotypes (PV) with some or all of the B.1.1.7
94 mutations in S, but results have been inconsistent. In some studies, PV bearing B.1.1.7 and wildtype S
95 showed equivalent (<2-fold) neutralisation by convalescent sera (Collier et al., 2021; Rees-Spear C et
96 al., 2021) or sera raised against vaccines (Muik et al., 2021; Wu et al., 2021). However, other studies
97 found a modest decreased susceptibility (up to 4-fold) of B.1.1.7 PV to convalescent sera (Hu et al.,
98 2021; Wang et al., 2021) or vaccine sera (Collier et al., 2021). Sera raised against BNT162b2 vaccine
99 did not show decreased ability to neutralise a recombinant virus with 3 key B.1.1.7 S mutations; $\Delta 69$ -
100 70 + N501Y + D614G (Xie et al., 2021). Against authentic SARS-CoV-2, convalescent and BNT162b2
101 vaccine serum titres were equivalent against a mouse-adapted strain with N501Y and the parental
102 strain (Rathnasinghe et al., 2021), whereas a full B.1.1.7 isolate showed a two-fold reduction in
103 neutralisation by BNT162b2 vaccine sera (Diamond et al., 2021). Others have reported a 2-fold
104 reduction in neutralisation of authentic B.1.1.7 virus by both convalescent and BNT162b2 vaccine sera
105 with some convalescent sera which weakly neutralise the WT virus falling below the threshold of
106 detection against B.1.1.7 (Skelly et al., 2021). Taken together, the evidence to date shows that the
107 B.1.1.7 variant is equivalently or slightly less well neutralised by polyclonal sera and does not yet
108 present a substantial risk of escape from pre-existing or vaccine-induced antibody immunity.

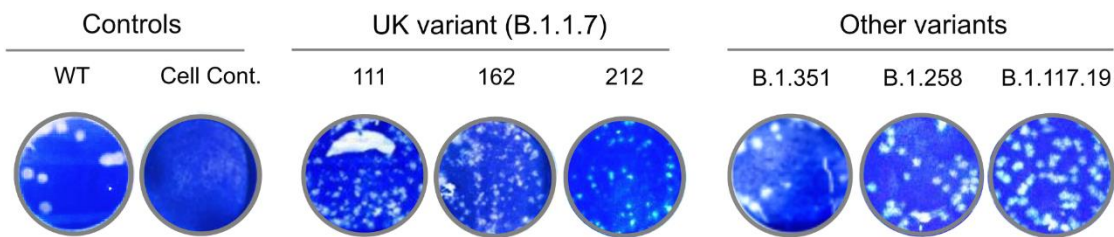
109 However, other than investigating its antigenicity, no studies have yet reported on the
110 virological characteristics of the B.1.1.7 variant which might contribute to its emergence and spread.
111 In this study, we experimentally characterised a panel of B.1.1.7 isolates. We tested whether B.1.1.7
112 shows enhanced replication compared to contemporaneous strains in Vero cells or in primary human
113 airway epithelial cells grown at air liquid interface. We investigated whether B.1.1.7 lineage viruses
114 have differences in their furin cleavage efficiency. Finally, we also tested whether different isolates of
115 B.1.1.7 escape neutralisation by sera from convalescent and vaccinated patients. Collectively our data
116 suggest that neither immune escape nor increased replication capacity account for the rapid
117 emergence and increased transmission of B.1.1.7, and instead point to an increase in furin cleavage
118 of spike that may enhance infectiousness of the variant.

119 **Results**

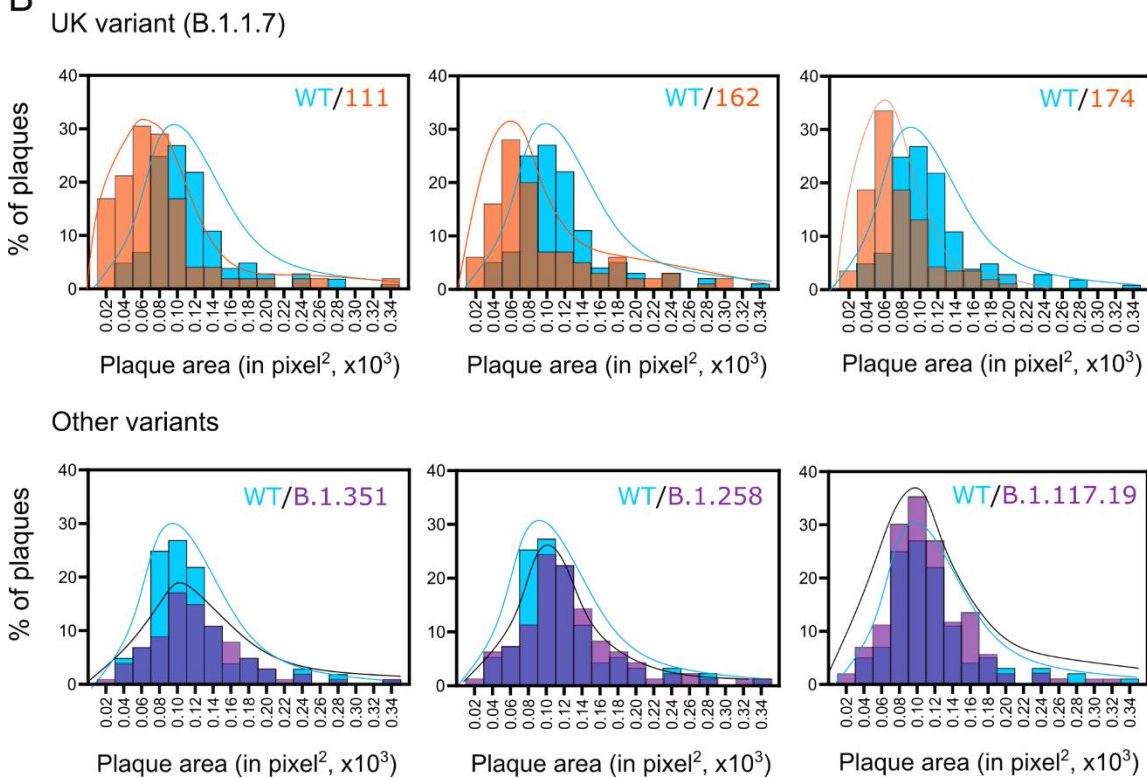
120 **SARS-CoV-2 lineage B.1.1.7 replicate poorly and displays a small plaque phenotype in Vero cells**

121 To characterise SARS-CoV-2 lineage B.1.1.7 (Variant of Concern 202012/01), we assembled a
122 panel of isolates including five B.1.1.7 lineage viruses derived from four independent patients,
123 together with historic and contemporaneous isolates from other SARS-CoV-2 lineages. Full sequence
124 names and lineage information are provided in Methods. For each isolate, we verified the sequence
125 and established the genome content and infectivity of the Vero cell passage 2 stock. During viral
126 titration, we noticed that the B.1.1.7 isolates displayed a small plaque phenotype in Vero cells
127 compared to non-B.1.1.7 viruses (Figure 1A). The mode area of plaques produced by the historic WT
128 SARS-CoV-2 isolate (IC19) collected from a patient in March 2020 and containing D614G, was ≥ 0.10
129 $\text{pixel}^2(\times 10^3)$, whereas the B.1.1.7 variants produced plaques of $\leq 0.06\text{-}0.08 \text{ pixel}^2(\times 10^3)$ (Figure 1B, top
130 panel). Plaques of contemporaneous non-B.1.1.7 isolates; B.1.258 with $\Delta 69\text{-}70$ and N439K spike
131 mutations, and B.1.117.19 carrying an A222V mutation (Figure 1B, lower panel) did not significantly
132 differ from WT IC19.

A



B



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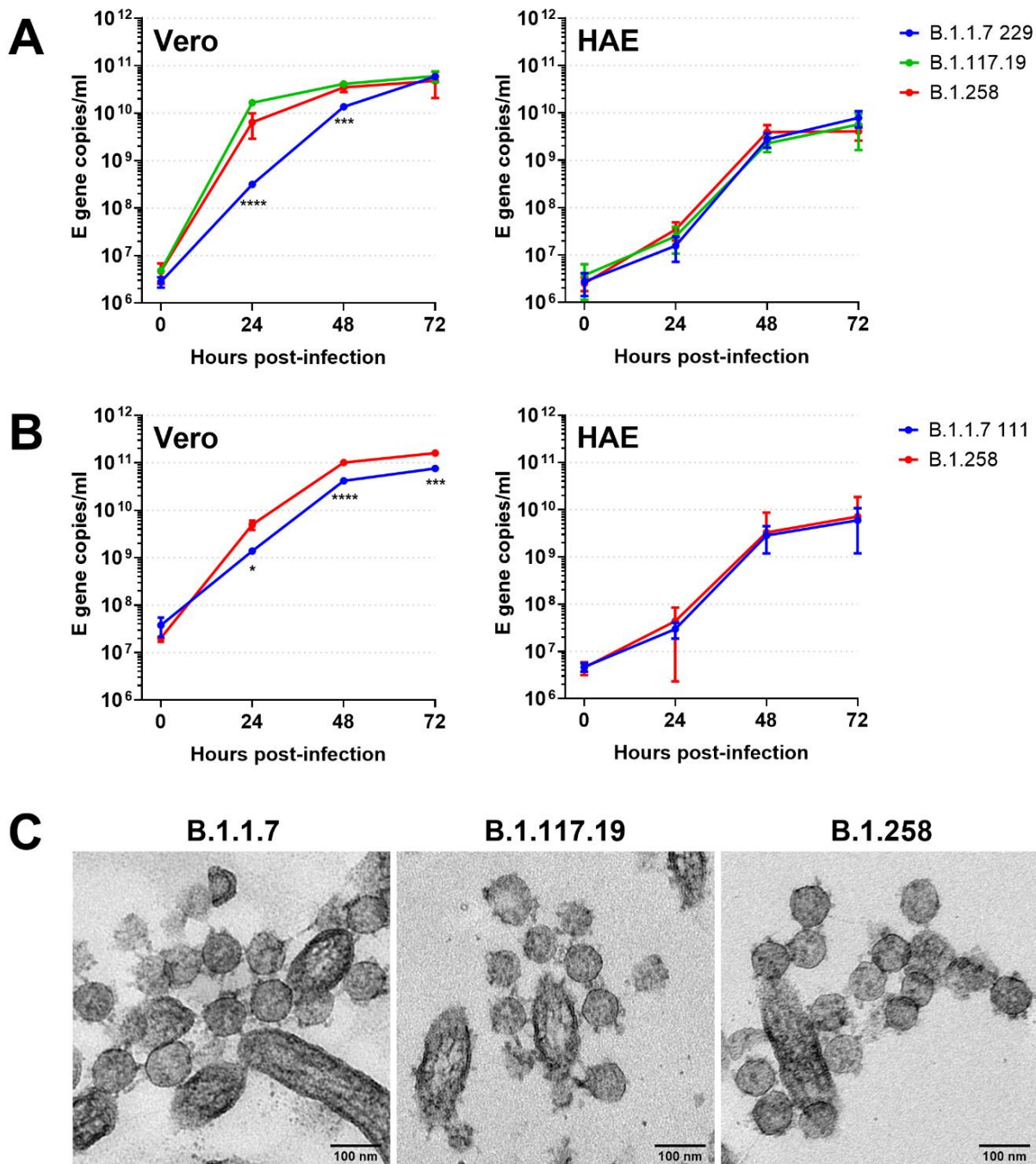
Figure 1 - Plaque sizes of SARS-CoV-2 lineage B.1.1.7 isolates. Vero cells were infected with WT SARS-CoV-2 (IC19) or the indicated B.1.1.7 or other contemporaneous variants. Cells were overlaid with agar at 37°C for 72 h and plaques were visualised through crystal violet staining. (A) Representative images of virus plaques. (B) Histograms of plaque sizes quantified using ImageJ. A minimum of 300 plaques per virus were measured.

139 **Increasing prevalence of SARS-CoV-2 lineage B.1.1.7 is not accounted for by a replication advantage**
140 **over non-B.1.1.7 viruses in a human airway epithelial cell model**

141 To investigate whether increased transmissibility of the B.1.1.7 variant could be explained by
142 more rapid replication kinetics, we infected primary human airway epithelial (HAE) cells or Vero cells
143 at a standardised multiplicity of infection. In a first experiment, we normalised the virus inputs based
144 on infectivity as measured by plaque assay on Vero cells. We infected Vero or HAE cells at 0.01 plaque
145 forming units (pfu)/cell and quantified viral replication by assaying virus in Vero cell media or in
146 extracellular washes obtained from the apical surface of infected HAE cells collected at different time
147 points after infection, by qPCR for E gene (Supplementary 1). Levels of B.1.1.7 genomes were higher
148 at all timepoints relative to the pair of non-B.1.1.7 viruses tested (B.1.117.19 and WT IC19). The
149 increase genome level at time 0 is consistent with the high genome:pfu ratio in the B.1.1.7 stock. The
150 increased genome copies of B.1.1.7 at later timepoints in HAE cells might also be accounted for by the
151 higher input since titration on Vero cells had underestimated the infectivity of the stock. In contrast,
152 in Vero cells, despite its higher input levels at time 0, B.1.1.7 showed a growth defect relative to
153 B.1.117.19 and IC19 isolates.

154 As calculating viral titre on Vero cells had underestimated B.1.1.7 infectivity, we next
155 normalised inputs based on genome copies as measured by E gene qPCR. HAE or Vero cells were
156 infected with 1×10^4 genomes/cell of B.1.1.7 alongside contemporaneous B.1.258 and B.1.117.19
157 isolates and again virus released from infected cells was quantified by qPCR (Figure 2A). In Vero cells,
158 the B.1.1.7 isolate again displayed a significant growth defect. However, in HAE cells, the three isolates
159 showed no significant difference in growth kinetics. To further confirm the differential growth
160 phenotype of B.1.1.7 in different cell types was consistent, we infected Vero or HAE cells with a
161 different B.1.1.7 isolate alongside the B.1.258 strain (Figure 2B). Again, a significant defect was seen
162 for B.1.1.7 replication in Vero cells but there was no difference in replication kinetics in HAE cells.

163 Transmission electron microscopy of fixed HAE sections at 72 hours post-infection showed no obvious
164 differences in the morphology of B.1.1.7 virions compared to those of the other isolates (Figure 2C).



165

166 **Figure 2 – Comparative replication kinetics of SARS-CoV-2 lineage B.1.1.7 isolates in Vero and**
167 **primary human airway epithelial (HAE) cells. Triplicate wells of Vero or primary human airway**
168 **epithelial cells were infected with SARS-CoV-2 isolates at a multiplicity of 1×10^4 genomes/cell and**

169 *replicating virus released in media or washed from HAE apical surface was quantified by E gene qPCR*
170 *at time points post-infection. (A) B.1.1.7 isolate 229 replication in Vero cells relative to non-B.1.1.7*
171 *B.1.117.19 and B.1.258 isolates (left hand panel) and in HAE cells (right hand panel). (B) B.1.1.7 isolate*
172 *111 and B.1.258 isolate replication in Vero cells (left hand panel) and in HAE cells (right hand panel).*
173 *Statistical differences measured by ANOVA on log transformed data. *, $P < 0.05$; ***, $P < 0.001$; ****,*
174 *$P < 0.0001$. (C) TEM images of extracellular virions at the HAE apical cell surface at 72 hours post-*
175 *infection.*

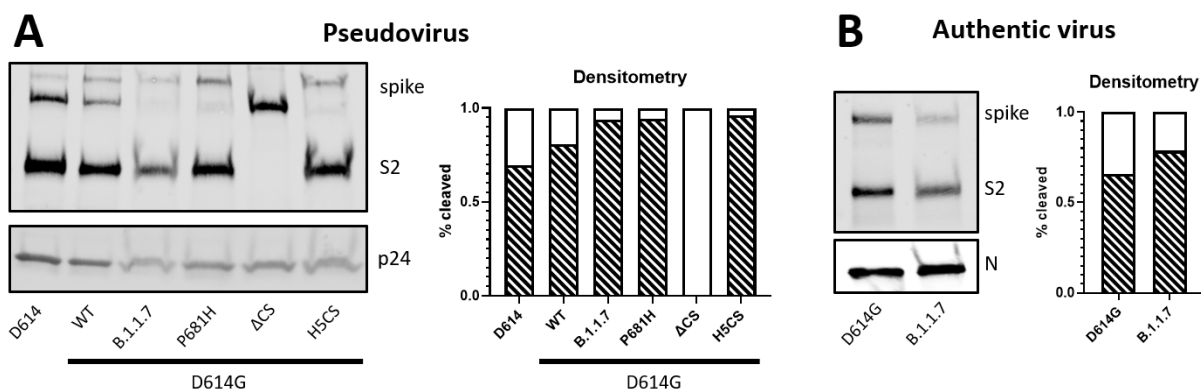
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177 **The P681H substitution in the B.1.1.7 spike confers an optimised S1/S2 furin cleavage site**

178 We next investigated whether the phenotype of attenuated replication in Vero cells we
179 observed for B.1.1.7 could be explained by differences in the efficiency of cleavage of its spike (S)
180 surface protein since one of the lineage defining mutations, P681H, is close to the furin cleavage site.
181 We and others have previously shown that efficiency of S1/S2 cleavage in producer cells can modulate
182 the entry efficiency of SARS-CoV-2 into different cell types (Hoffmann, Kleine-Weber, & Pöhlmann,
183 2020; Johnson et al., 2021; Peacock et al., 2020). Deleting the furin cleavage site enhances entry into
184 cell lines that lack TMPRSS2 protease expression (e.g. Vero) but attenuates entry into TMPRSS2-
185 expressing cell lines (e.g. HAE or Calu-3 cells). We hypothesised that the loss of replication we
186 observed in Vero cells might be accounted for by increased furin cleavage of the B.1.1.7 S, resulting in
187 further virus instability. To test this, we generated a lentiviral pseudotype (PV) bearing full B.1.1.7 S,
188 or S with P681H alone, and assessed the efficiency of cleavage by western blot (Figure 3A). The B.1.1.7
189 S showed increased cleavage compared to WT D614G S, more akin to that of D614G S containing a
190 highly optimized polybasic furin cleavage site from an H5N1 avian influenza haemagglutinin (H5CS),
191 as previously described (Peacock et al., 2020). Of the 9 mutations present in the B.1.1.7 S, P681H alone
192 was sufficient to confer the optimised cleavage in line with its proximity to the S1/S2 cleavage site. To
193 confirm the cleavage phenotype for authentic SARS-CoV-2 virus, we assessed S cleavage efficiency of

194 B.1.1.7 isolate 229 and WT D614G (IC32) virus by western blot (Figure 3B). Again, increased cleavage
195 was observed for B.1.1.7 S compared to WT D614G S.

196



197

198 **Figure 3 - SARS-CoV-2 lineage B.1.1.7 spike cleavage.** (A) Lentiviral pseudotypes bearing various SARS-
199 CoV-2 spike (S) glycoproteins with the indicated mutations were concentrated by ultracentrifugation
200 and the proportion of cleaved S quantified by densitometry on western blot. ΔCS - furin cleavage site
201 removed; H5CS – influenza H5 haemagglutinin polybasic cleavage site. (B) S cleavage of concentrated,
202 authentic SARS-CoV-2 D614G and B.1.1.7 lineage viruses and quantification by densitometry.

203

204 **SARS-CoV-2 lineage B.1.1.7 is susceptible to human convalescent sera from individuals infected in**
205 **the first pandemic wave**

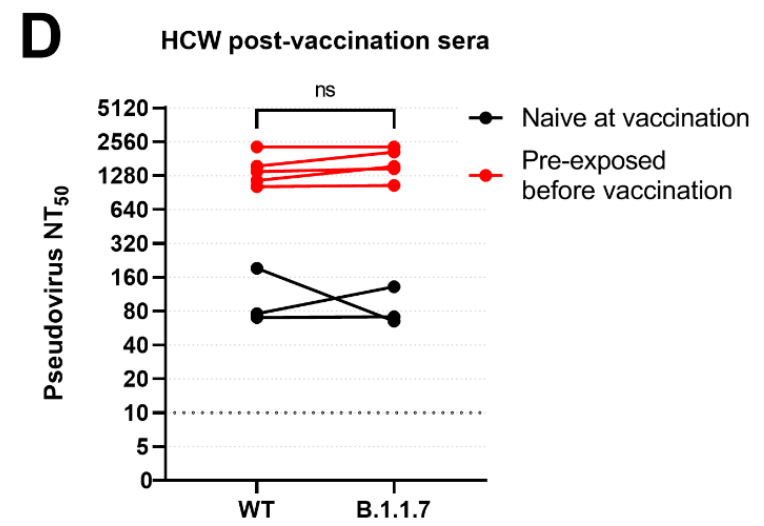
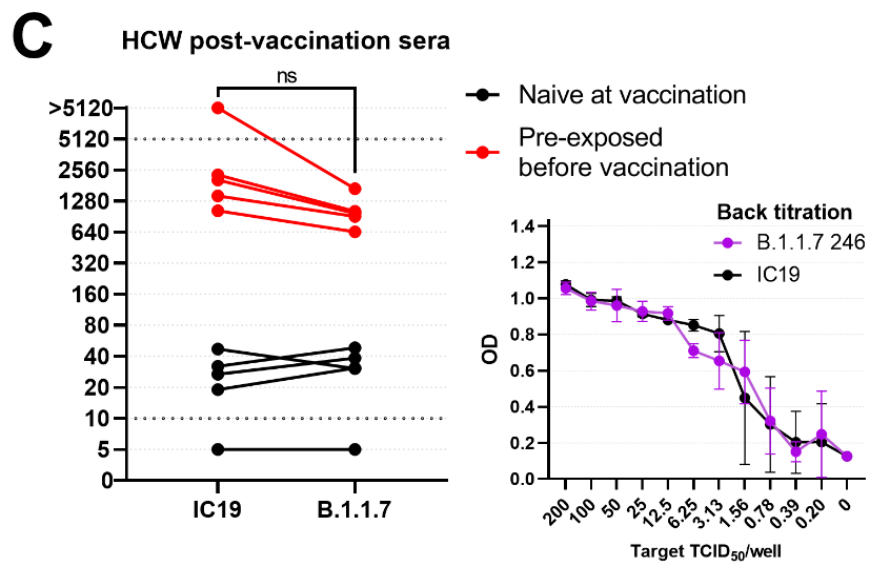
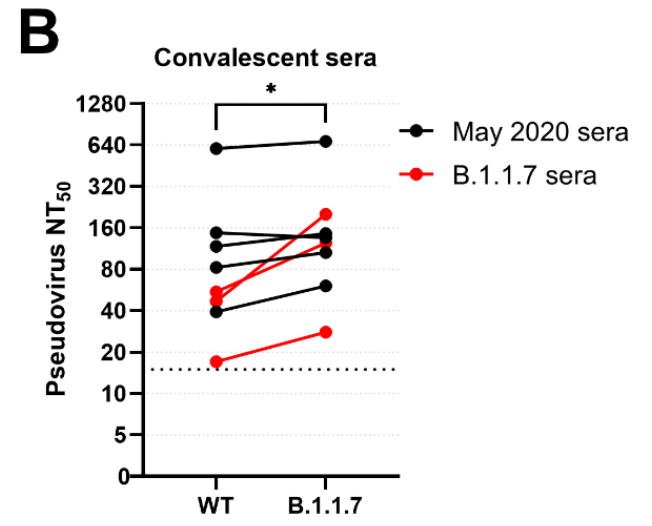
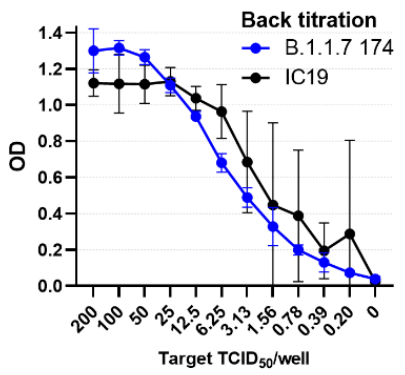
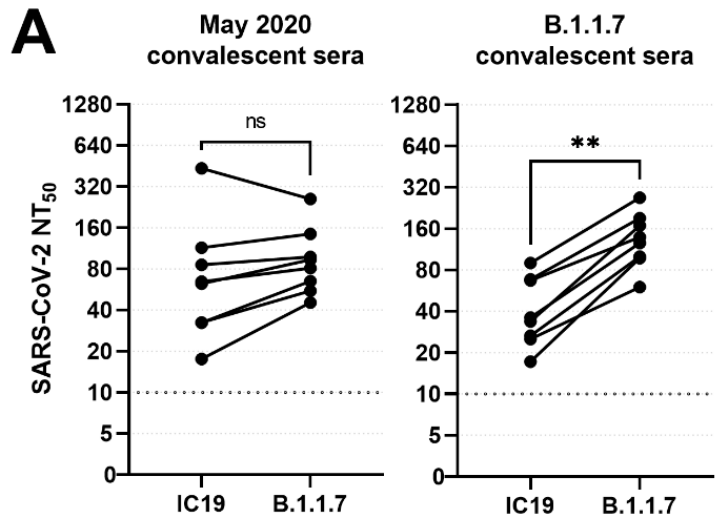
206 The potential escape of newly arising SARS-CoV-2 variants from neutralising antibodies raised
207 against previous strains is a global concern, particularly with vaccine efforts accelerating in many
208 regions. To test whether B.1.1.7 lineage escapes from neutralising antibodies, we carried out live virus
209 neutralisation assays to assess sera neutralisation of B.1.1.7 isolate 229 with WT IC19 as a comparator.
210 Neutralisation was carried out with sera collected from healthcare workers (HCW) in May 2020
211 collected at least 21 days since a mild or asymptomatic SARS-CoV-2 infection confirmed by PCR (n=8),
212 and sera from individuals in December 2020/January 2021 with previous sequence-confirmed B.1.1.7

213 infection (n=8) (Figure 4A). All sera were tested against approximately 100 TCID₅₀ of the two virus
214 isolates in the same assay and NT₅₀ values were calculated. Virus inputs for the assay were
215 approximately equivalent based on the back titration carried out concurrent with the neutralisation
216 assay (Figure 4A). The median titres of the May 2020 sera against B.1.1.7 and IC19 were 87 and 63
217 respectively, with 6/8 (75%) showing a less than 2-fold difference in their ability to neutralise the two
218 viruses. The remaining two May 2020 sera showed 2.0-fold and 2.6-fold increased ability to neutralise
219 B.1.1.7. Overall, no significant reduction in titre of May 2020 sera was observed against the B.1.1.7
220 isolate indicating a lack of antibody escape. Sera collected following infection with B.1.1.7 had a
221 median titre of 132 against the B.1.1.7 isolate and 35 against IC19. All 8 (100%) of these sera showed
222 a greater than 2-fold (2.0-fold to 5.7-fold) increased ability to neutralise the homologous B.1.1.7 virus
223 over IC19. This suggests that antibody responses mounted against SARS-CoV-2 lineage B.1.1.7 viruses
224 retain efficacy against previously circulating strains but that this response is reduced perhaps owing
225 to an immunodominant response to B.1.1.7-specific epitopes. To complement the findings of the
226 authentic virus neutralisation assay, lentiviral PV bearing the S glycoprotein of either WT or B.1.1.7
227 virus were tested with a subset of the May 2020 and B.1.1.7 sera (Figure 2B). The same trend was
228 observed with May 2020 antisera showing no significant difference in neutralisation, but B.1.1.7
229 convalescent antisera more efficiently neutralising B.1.1.7 spike-bearing PV.

230 **BNT162b2 vaccination induces equivalent neutralising antibody responses against B.1.1.7**

231 To investigate whether antibody responses elicited by currently distributed vaccines were also
232 likely to be protective against B.1.1.7, sera were taken from HCW on the day of receiving their first
233 dose of the Pfizer/BioNTech BNT162b2 vaccine (day 0) and approximately 21-25 days post-
234 vaccination. Day 0 sera were tested by Fortress lateral flow immunoassay (LFIA) for the presence of
235 SARS-CoV-2-specific antibodies to segregate HCW into individuals who were pre-exposed to virus
236 (n=5) and those who were naïve on the day of vaccination (n=5). The post-vaccination sera were then
237 assessed for neutralising antibodies against B.1.1.7 isolate 246 and IC19 (Figure 4C). Neutralising

238 antibody titres post-vaccination were significantly higher in sera from pre-exposed (red lines)
239 compared to naïve HCW (black lines) with median titres against IC19 of 2038 and 32 respectively. Post-
240 vaccination sera from pre-exposed HCW also neutralised B.1.1.7 well with a median titre of 968
241 although 3/5 sera showed a greater than 2-fold decrease (2.1-fold to 3.0-fold) in their ability to
242 neutralise B.1.1.7 compared to IC19. This drop in titre against B.1.1.7 was not observed in the PV
243 neutralisation assay with median titres of 1392 against IC19 and 1540 against B.1.1.7 (Figure 4D). For
244 HCW who were naïve on the day of vaccination, titres were low post-vaccination and there was no
245 significant difference in their responses to B.1.1.7 and IC19 (Figure 4C, D). The four sera which were
246 above the threshold of detection of the live virus neutralisation assay against both B.1.1.7 and IC19
247 had median titres of 34 and 29 respectively.



249 **Figure 4 – Neutralisation of live SARS-CoV-2 lineage B.1.1.7 virus by convalescent and post-**
250 **vaccination sera.** (A) Sera raised against early pandemic viruses in May 2020 (n=8) and B.1.1.7 lineage
251 viruses (n=8) were assessed for their ability to neutralise B.1.1.7 isolate 229 and WT IC19 control virus
252 by live neutralisation assay. NT_{50} values were calculated for each serum sample against both viruses.
253 There was no significant difference in the ability of early pandemic sera to neutralise the two viruses
254 but B.1.1.7 sera were significantly better able to neutralise the homologous lineage virus than IC19.
255 Wilcoxon matched-pairs signed rank test was used for comparisons. A 2-fold dilution series of the virus
256 input used for the neutralisation assay was performed and the mean OD of 4 replicate wells is shown
257 indicating that approximately equivalent amounts of each virus were used as challenge. (B) NT_{50} values
258 for May 2020 sera (n=5) and B.1.1.7 sera (n=3) against lentiviral pseudotypes bearing WT or B.1.1.7
259 spike. (C) Sera taken from healthcare workers (HCW) 21-25 days after receiving their first BNT162b2
260 (Pfizer-BioNTech) vaccine dose were assessed for their ability to neutralise B.1.1.7 isolate 246, relative
261 to IC19. HCW sera were divided into those who had been exposed to SARS-CoV-2 prior to receiving the
262 vaccine (red lines, n=5) and those who were naïve when receiving the vaccine (black lines, n=5), as
263 determined by Fortress lateral flow immunoassay test. (D) NT_{50} values for pre-exposed vaccine sera
264 (n=5) and naïve vaccine sera (n=3) against WT and B.1.1.7 pseudotypes.

265 Discussion

266 SARS-CoV-2 lineage B.1.1.7 (Variant of Concern 202012/01) poses a clear threat to ongoing
267 efforts to control the burden of the COVID-19 pandemic due to its increased transmissibility and
268 association with an increased hazard of death. B.1.1.7 has rapidly risen to predominate in the UK and
269 beyond but the virological traits which have allowed it to do this have yet to be described. Here, we
270 show that B.1.1.7 does not demonstrate increased replication in human airway epithelial (HAE) cells.
271 This suggests that increased viral replication is not responsible for the increase of viral transmission
272 seen in the B.1.1.7 lineage. This differs from viruses with the D614G mutation in spike (S), whose global
273 spread was at least partially accounted for by increased viral replication that was evident in HAE
274 cultures (Hou et al., 2021). In infected individuals, a small but significant decrease in Ct values in G614
275 compared to D614 infections in the UK demonstrated increased viral load for variants carrying D614G
276 (Volz, Hill, et al., 2021). For B.1.1.7, early analysis using the number of mapped sequencing reads or Ct
277 values as proxies for viral load suggested an increased viral burden associated with B.1.1.7 infection
278 (Golubchik et al., 2021; Kidd et al., 2020) but more recent reports show no such association at either
279 the population level or longitudinally in individuals (Kissler et al., 2021; Walker et al., 2021). The more
280 recent reports and our data suggest there is no clear replicative advantage for B.1.1.7 *in vitro* or at the
281 population level.

282 In contrast to the equivalent replication we saw in HAE cells, B.1.1.7 showed reduced
283 replication and a corresponding smaller plaque size on Vero cells compared to other tested viral
284 lineages (Figures 1 and 2). Our previous work highlighted that S1/S2 cleavage in the producer cell,
285 conferred by a polybasic stretch at the cleavage site, is advantageous in cells expressing abundant
286 TMPRSS2 but deleterious in cells lacking TMPRSS2 such as Vero cells (Peacock et al., 2020). Analysis
287 here revealed that the P681H substitution directly adjacent to the S1/S2 cleavage site resulted in
288 increased cleavage of S (Figure 3) and could be responsible for the reduced entry into Vero cells.
289 However, increased cleavage as an explanation for the deleterious effect in Vero cells was not

290 accompanied by a corresponding advantage in HAE cells. It is possible that increased cleavage could
291 be beneficial to transmission and entry into the human airway, but that this phenotype is not observed
292 when infecting HAE cultures with a relatively large amount of virus compared to the likely dose during
293 transmission. Further experiments to establish the lowest dose required to initiate infection in vitro
294 and in vivo may clarify this. Additionally, the decreased growth on Vero cells means that care must be
295 taken when growing B.1.1.7 viral stocks since deletion or mutation of the S1/S2 cleavage site might
296 be selected for in these cells. Viral stocks should be sequenced to confirm that the cleavage site
297 remains intact. In addition, viral titres calculated on Vero cells may lead to an underestimation of the
298 number of infectious viruses due to the high pfu:genome ratio.

299 We confirmed that B.1.1.7 does not escape from antibody immunity after natural infection
300 with previously circulating variants, or vaccine expressing the S protein of older variants, suggesting
301 that immune escape does not account for increased transmission (Figure 4). This is unsurprising given
302 that there is no observed increase in reinfection reported with B.1.1.7 nor was there high
303 seroprevalence in the UK during the emergence of this variant. Our finding is supported by many
304 studies using B.1.1.7 viruses and PV which show either no reduction or a modest reduction in
305 polyclonal serum titres (Collier et al., 2021; Diamond et al., 2021; Hu et al., 2021; Muik et al., 2021;
306 Rees-Spear C et al., 2021; Wang et al., 2021; Wu et al., 2021). There is some evidence of heterogeneity
307 of responses and neutralising titres for some individuals with initially low responses against WT virus
308 can drop below limits of detection against the B.1.1.7 variant (Skelly et al., 2021). However, since the
309 correlate of protection is not yet established for SARS-CoV-2, the significance of this drop is still not
310 clear. Whilst the N501Y mutation has been implicated in loss of binding of some RBD targeting
311 monoclonal antibodies, and B.1.1.7 also contains mutations in the N-terminal domain (NTD) of S which
312 may allow for escape against NTD targeting antibodies, the small effect on neutralisation titres by
313 polyclonal sera is reassuring and suggests further antigenic domains on S that contribute to protection.
314 In our study, the reduced ability of sera raised against B.1.1.7 to neutralise the historic IC19 virus

315 relative to the homologous virus (Figure 4A) may suggest that immune responses to B.1.1.7 are more
316 focussed meaning B.1.1.7 S may not be a preferred choice as the basis of future vaccine updates.

317 B.1.1.7 is one of a growing number of variants of concern which are showing increased
318 transmission. These variants share many convergent or parallel mutations of S as well as in NSP6 and
319 ORF8 suggesting that these mutations are likely adaptive and likely multiple mutations are needed for
320 an increase in transmissibility. It is notable that several emerging variants contain mutations which
321 could increase cleavage of S. These include other mutations adjacent to the cleavage site such as
322 P681R, Q677H, and Q677P (Hodcroft et al., 2021) as well as further mutations (H655Y, A701V) which
323 are more distant in primary sequence but proximal to the furin cleavage site in the 3D structure of S.
324 We therefore hypothesise that an increase in the efficiency of furin cleavage is an important
325 contributing factor to the increase in transmission of these variants. The B.1.1.7 lineage continues to
326 evolve and it is notable that several isolates have gained additional S mutations such as E484K which
327 has been shown to cause a 9.6-fold decreased neutralisation by vaccine sera in a B.1.1.7 background
328 (Collier et al., 2021), and which could further increase receptor binding avidity in combination with
329 N501Y. E484K and N501Y together have been associated with other rapidly emerging variants of the
330 B.1.351 and P.1 lineages.

331 It is likely that the increased transmissibility of B.1.1.7 owes to subtle optimisation and balancing of a
332 number of virological traits which include and extend beyond those investigated here. Once the
333 genetic determinants of increased transmissibility of the B.1.1.7 variant are found it will be important
334 to remain vigilant for these hallmarks in other emerging variants, especially if in combination with
335 mutations which confer vaccine escape.

336 **Materials and Methods**

337 **Cells**

338 African green monkey kidney (Vero) cells (Nuvonis Technologies) were maintained in OptiPRO
339 SFM (Life Technologies) containing 2X GlutaMAX (Gibco). Human embryonic kidney cells (293T) were
340 maintained in Dulbecco's modified Eagle's medium (DMEM), 10% fetal calf serum (FCS), 1% non-
341 essential amino acids (NEAA), 1% penicillin-streptomycin (P/S). All primary and continuous cell lines
342 were maintained at 37°C, 5% CO₂. 293T-ACE2 cells were generated as previously described (Peacock
343 et al., 2020; Rebendenne et al., 2021) and were maintained with 293T media supplemented with 1
344 µg/ml of puromycin. Primary nasal human airway epithelial (HAE) cells at air-liquid interface (ALI) were
345 purchased from Epithelix for the experiment in Figure 1B and primary bronchial HAE cells from
346 Epithelix for the experiment in Supplementary 1. The basal MucilAir medium (Epithelix) was changed
347 every 2-3 days for maintenance of HAE cells. For experiment 1A, HAE cells at ALI were differentiated
348 in-house as follows under Health Research Authority study approval (REC ref: 20/SC/0208; IRAS:
349 282739). A nasal brushing of the turbinate was acquired using 3-mm bronchial cytology brush and
350 placing the biopsy into warm PneumaCult-Ex Plus Medium (STEMCELL Technologies, Cambridge, UK).
351 The cells were dissociated from the brush by gentle agitation before seeding into a single well of a
352 collagen (PureCol from Sigma Aldrich) coated plate. Once confluent the cells were passaged and
353 expanded further in a flask before passaging a second time and seeding onto transwell inserts (6.5 mm
354 diameter, 0.4 µm pore size, Corning) at a density of 24,000 cells per insert. Cells were cultured in
355 PneumaCult-Ex Plus (STEMCELL Technologies, Cambridge, UK) until confluent, at which point the
356 media was replaced with PneumaCult-ALI in the basal chamber and apical surface exposed to provide
357 an air liquid interface (ALI). Cilia were observed between 4-6 weeks post transition to ALI.

358 **Serum samples**

359 Sera were collected under ethical approval as stated in the Ethical Approval and heat-
360 inactivated at 56°C for 30 minutes before use in assays.

361 **Viruses**

362 SARS-CoV-2 infectious swabs were collected as approved in Ethical Approval. Viruses were
363 isolated by inoculating 100ul of neat swab material onto 24-well plates of Vero cells, incubating at
364 37°C, 5% CO₂ for 1 hour before adding 1 ml OptiPRO SFM supplemented with 2X Glutamax, 1% P/S
365 and 1% amphotericin and incubating again for 5-7 days until cytopathic effect was observed. Isolates
366 were passaged twice in Vero cells and used for subsequent experiments. For western blot analysis,
367 virus supernatants were concentrated by spinning through an Amicon® Ultra-15 Centrifugal Filter Unit
368 followed by an Amicon® Ultra-0.5 Centrifugal Filter Unit with 50 kDa exclusion size.

Name used in text	Virus name	GISAID Accession ID	PANGO lineage
B.1.1.7 111	hCoV-19/England/205090256/2020	EPI_ISL_747517	B.1.1.7
B.1.1.7 162	hCoV-19/England/205080329/2020	EPI_ISL_722999	B.1.1.7
B.1.1.7 212	hCoV-19/England/204690005/2020	EPI_ISL_693401	B.1.1.7
B.1.1.7 229	hCoV-19/England/204661721/2020	EPI_ISL_693400	B.1.1.7
B.1.1.7 246	hCoV-19/England/205080610/2020	EPI_ISL_723001	B.1.1.7
B.1.117.19	hCoV-19/England/204501194/2020	EPI_ISL_660788	B.1.117.19
B.1.258	hCoV-19/England/204501206/2020	EPI_ISL_660791	B.1.258
B.1.351	hCoV-19/England/205280030/2020	EPI_ISL_770441	B.1.351
IC19	hCoV-19/England/IC19/2020	EPI_ISL_475572	B.1.13
IC32	hCoV-19/England/IC32/2020	n/a	B.1.238

369

370 **Determination of plaque size**

371 Virus inoculum were serially diluted in serum-free DMEM (1:10) supplemented with 1% NEAA
372 and 1% P/S, and added to Vero cell monolayers at 37°C for 1 h. Inoculum was then removed and plates
373 were overlaid with DMEM containing 0.2% w/v bovine serum albumin, 0.16% w/v NaHCO₃, 10 mM
374 HEPES, 2mM L-Glutamine, 1x P/S, and 0.6% w/v agarose at 37°C for 3 d. The overlay was then
375 removed, and monolayers were stained with crystal violet solution. Plates were then washed with tap
376 water, dried and scanned on a flatbed office scanner at 600 dots per inch (dpi). Images were analysed
377 using the Analyze Particles module of Fiji (ImageJ). Areas of ≥ 300 plaques were measured for each
378 virus variant and expressed in pixels². Virus plaque sizes were compared using the Kolmogorov–
379 Smirnov test with Bonferroni correction (Prism 9.0; GraphPad). $P \leq 0.05$ significant difference from WT.

380 **Virus growth kinetics**

381 All dilution of viruses, wash steps and harvests were carried out with OptiPRO SFM (Life
382 Technologies) containing 2X GlutaMAX (Gibco). For HAE cells, all wash and harvest steps were
383 performed by addition of 200ul SFM and incubation for 10 mins at 37°C before removing SFM. To
384 infect, basal medium was replaced, cells were washed once with SFM to remove mucus before
385 addition of inoculum and incubation for 1 h at 37°C. Inoculum was removed, cell washed twice and
386 the second wash taken as harvest for 0 hpi. For infection of Vero cells, overnight growth medium was
387 removed, inoculum added and incubated for 1 h at 37°C before removal, two washes and replacement
388 with 3ml SFM from which harvests were taken at timepoints.

389 **Conventional transmission electron microscopy (TEM)**

390 HAE cells were fixed by placing them in 2.5% glutaraldehyde in 0.05M sodium cacodylate
391 buffer at a pH 7.4 and left for 2 days at room temperature. Subsequently, the samples were incubated
392 in 1% aqueous osmium tetroxide for 1 h at RT before en bloc staining by placing them in undiluted UA-
393 Zero (Agar Scientific) for 30 minutes at RT. The samples were dehydrated using increasing
394 concentrations of ethanol (50%, 70%, 90%, 100%), followed by propylene oxide and a mixture of
395 propylene oxide and araldite resin (1:1). To embed the samples, they were placing in aradite and left
396 at 60°C for 48 h. Ultrathin sections were cut using a Reichert Ultracut E ultramicrotome and stained
397 using Reynold's lead citrate for 10 minutes at RT. Images were acquired on a JEOL 1400Plus
398 transmission electron microscope fitted with an Advanced Microscopy Technologies (AMT) XR16
399 charge coupled device (CCD) camera.

400 **E gene qPCR**

401 RNA was extracted from virus supernatants using QIASymphony DSP Virus/Pathogen Mini Kit
402 on the QIASymphony instrument (Qiagen). qPCR was then performed using AgPath RT-PCR (Life
403 Technologies) kit on a QuantStudio(TM) 7 Flex System with the primers for E gene used in (Corman et

404 al., 2020). A standard curve was also generated using dilutions viral RNA of known copy number to
405 allow quantification of E gene copies in the samples from Ct values. E gene copies per ml of original
406 virus supernatant were then calculated.

407 **Live virus neutralisation assay**

408 The ability of sera to neutralise SARS-CoV-2 virus was assessed by neutralisation assay on Vero
409 cells. Sera were serially diluted in OptiPRO SFM (Life Technologies) and incubated for 1 h at RT with
410 100 TCID₅₀/well of SARS-CoV-2/England/IC19/2020 and transferred to 96-well plates pre-seeded with
411 Vero-E6 cells. Serum dilutions were performed in duplicate. Plates were incubated at 37°C, 5% CO₂ for
412 42 h before fixing cells in 4% PFA. Cells were treated with methanol 0.6% H₂O₂ and stained for 1 h
413 with a 1:3000 dilution of 40143-R019 rabbit mAb to SARS-CoV-2 nucleocapsid protein (Sino Biological).
414 A 1:3000 dilution of sheep anti-rabbit HRP conjugate (Sigma) was then added for 1 h. TMB substrate
415 (Europa Bioproducts) was added and developed for 20 mins before stopping the reaction with 1M HCl.
416 Plates were read at 450nm and 620nm and the concentration of serum needed to reduce virus signal
417 by 50% was calculated to give NT₅₀ values.

418 **Pseudovirus assays**

419 SARS-CoV-2 spike-bearing lentiviral pseudotypes (PV) were generated as previously described
420 (Peacock et al., 2020). PV for western blot analysis were concentrated by ultracentrifugation at
421 100,000 x g for 2 hours over a 20% (w/v) sucrose cushion. PV neutralisation assays were performed
422 by serially diluting sera in 293T growth media and incubating for 1 h at 37°C with equal concentrations
423 of PV. The PV/antisera mix was then added onto 293T-ACE2 cells. Serum dilutions were performed in
424 duplicate. 293T-ACE2 were transduced for 48 hours before lysis with reporter lysis buffer (Promega).
425 Luciferase luminescence was read on a FLUOstar Omega plate reader (BMF Labtech) using the
426 Luciferase Assay System (Promega).

427 **Western blot analysis**

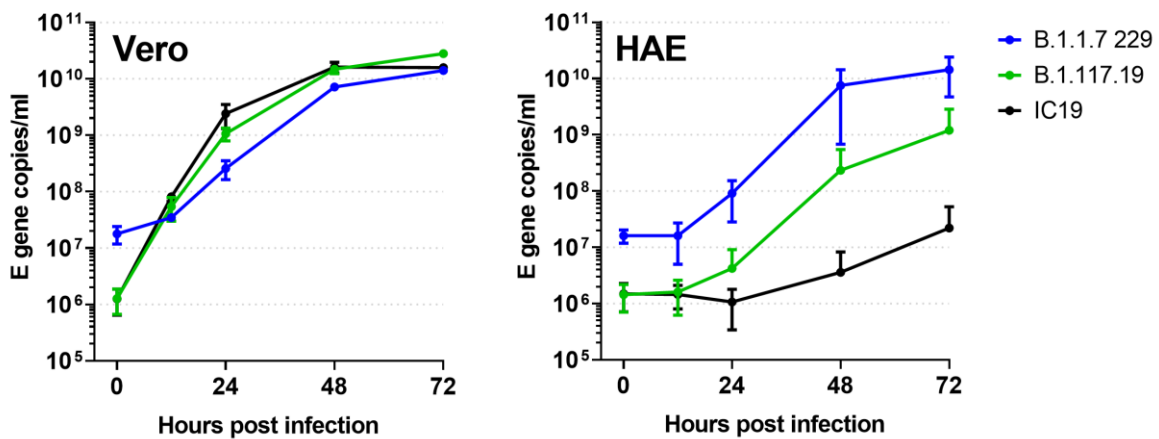
428 Concentrated PV or virus was mixed with 4x Laemmli sample buffer (Bio-Rad) with 10% β -
429 mercaptoethanol and run on SDS-PAGE gels. After semi-dry transfer onto nitrocellulose membrane,
430 membranes were probed with mouse anti-p24 (abcam; ab9071), rabbit anti-SARS spike protein
431 (NOVUS; NB100-56578) or rabbit anti-SARS-CoV-2 nucleocapsid (SinoBiological; 40143-R019). Near
432 infra-red (NIR) secondary antibodies, IRDye[®] 680RD Goat anti-mouse (abcam; ab216776) and IRDye[®]
433 800CW Goat anti-rabbit (abcam; ab216773) were subsequently used to probe membranes. Western
434 blots were visualised using an Odyssey Imaging System (LI-COR Biosciences).

435 **Ethical approval**

436 Convalescent sera from healthcare workers at St. Mary's Hospital at least 21 days since PCR-
437 confirmed SARS-CoV-2 infection were collected in May 2020 as part of the REACT2 study with ethical
438 approval from South Central Berkshire B Research Ethics Committee (REC ref: 20/SC/0206; IRAS
439 283805). Patient swabs for virus isolation and sera raised against B.1.1.7 were collected by the PHE
440 Virology Consortium. The investigation protocol was reviewed and approved by the PHE Research
441 Ethics and Governance Group and Incident Management team. PHE has legal permission, provided by
442 Regulation 3 of the Health Service (Control of Patient Information) Regulation 2002, to process patient
443 confidential information for national surveillance of communicable diseases. Further infectious swabs
444 and sera were collected as part of the Assessment of Transmission and Contagiousness of COVID-19
445 in Contacts (ATACCC). Ethical approval for ATACCC was granted under the Integrated Network for
446 Surveillance, Trials and Investigation of COVID-19 Transmission (INSTINCT; Ethics Ref: 20/NW/0231;
447 IRAS Project ID: 282820) Sera collected from HCW 21-25 days after their first BNT162b2 vaccine dose
448 were collected as part of a study approved by the Health Research Authority (REC ref: 20/WA/0123).

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463

464 **Supplementary 1 – Replication kinetics of B.1.1.7 and non-B.1.1.7 isolates with input normalised by**
465 **infectivity on Vero cells.** Virus isolates were plaqued on Vero cells to determine infectious titre and
466 then used to infect triplicate wells Vero or human airway epithelial (HAE) cells at a multiplicity of 0.01
467 pfu/cell. Replication was measured by E gene qPCR of Vero supernatants and HAE apical harvests.
468 B.1.1.7 had higher genome input despite having an equivalent infectious dose as measured on Vero
469 cells.

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