1 **SARS-CoV-2 testing in the community: Testing positive samples with the TaqMan SARS-CoV-2**
2 **Mutation Panel to find variants in real-time**

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17

18 **ABSTRACT**

19 Genome sequencing is a powerful tool for identifying SARS-CoV-2 variant lineages, however there can
20 be limitations due to sequence drop-out when used to identify specific key mutations. Recently,
21 Thermo Fisher Scientific have developed genotyping assays to help bridge the gap between testing
22 capacity and sequencing capability to generate real-time genotyping results based on specific variants.
23 Over a 6-week period during the months of April and May 2021, we set out to assess the Thermo
24 Fisher TaqMan Mutation Panel Genotyping Assay, initially for three mutations of concern and then an
25 additional two mutations of concern, against SARS-CoV-2 positive clinical samples and the
26 corresponding COG-UK sequencing data. We demonstrate that genotyping is a powerful in-depth
27 technique for identifying specific mutations, an excellent complement to genome sequencing and has
28 real clinical health value potential allowing laboratories to report and action variants of concern much
29 quicker.

30 **Keywords:** SARS-CoV-2, variants of concern, genotyping, genome sequencing, real-time, SNPs

31

INTRODUCTION

Viruses mutate and SARS-CoV-2 is no exception. As the COVID-19 pandemic continues around the world, mutations are naturally occurring resulting in the emergence of divergent clusters / variants containing sets of mutations. These clusters/variants have differing prevalence in different geographical regions, likely in response to changing immune profiles of the human population **(1)**. Movement of people enabled by global air travel, allow these variants to spread and mutate further under differing selection pressures. In the United Kingdom, the Alpha variant (B.1.1.7), first identified in Kent, rapidly swept to dominance by December 2020 **(2)**. In April 2021, the Delta variant (B.1.617.2), first identified in India, rapidly outcompeted the Alpha variant to become dominant in a matter of weeks **(3)**. The geographical location where these variants emerged is not relevant **(4)**, rather it is the specific mutations present which greatly impacts virus characteristics including transmissibility and antigenicity, where mutations of significance have been identified in the SARS-CoV-2 Spike Protein of these variants of concern (VOC), that contribute to enhanced transmission and/or immune invasion **(5)**. Other VOC have been identified and characterised, including both Beta (B.1.351 – first identified in South Africa) and Gamma (P.1 – first identified in Brazil) **(5).**

Previously at the University of Birmingham, we set up a SARS-CoV-2 modular testing facility at the request of the United Kingdom Department of Health and Social Care **(6)**. Our PCR assay of choice was the 3-target design (ORF1ab, S and N genes) TaqPath COVID-19 CE-IVD RT PCR Kit, where target areas are unique to SARS- CoV-2 to reduce detection of other coronaviruses and compensate for virus mutations. Initially, we detected all three genes in SARS-CoV-2 samples, however, during November 2020, we along with other laboratories using the same PCR assay, started to notice a drop-off in the detection of the S-gene and then a rapid rise in this S-gene target failure (SGTF) in early December 2020 **(7)**. From discussions with the University of Birmingham genome sequencing laboratory (as part of the COG-UK Consortium) and as confirmed by other laboratories, it was demonstrated that S-gene did amplify, therefore confirming this gene was still present, but was not being detected using the

57 TaqPath COVID-19 assay. This was attributed to a 6-bp deletion (Δ69/70) in the middle of the S-gene
58 where the fluorescent specific probe binds, thus negating the probe's ability to fluoresce (**8**).
59 Simultaneously, these findings of a new VOC were reported by our laboratory and multiple testing
60 facilities across the UK, where this finding identified the VOC B.1.1.7 (Alpha variant). Although SGTF
61 identification with the Thermo Fisher TaqPath RT-qPCR assay was not intentional, this observation
62 with this PCR assay allowed us and other COVD-19 testing facilities to use this assay as an accurate
63 epidemiological tool to track the rise, spread and dominance of this VOC in the UK. By April 2021,
64 COVID-19 TaqPath PCR testing facilities, including our laboratory, noted an increasing number of
65 samples without SGTF, where the 3 target genes were amplifying and upon genome sequencing
66 analysis these samples were identified as the Delta (B.1.617.2) variant (**9**).

67 To better understand viral transmission and evolution and to inform public health responses and
68 vaccine development, genomic sequencing is essential. In March 2020, the COVID-19 Genomics UK
69 Consortium (COG-UK) was created for this purpose (**10**). To date, COG-UK have sequenced over
70 1,100,000 SARS-CoV-2 samples, providing a vast amount of data to the global COVID-19 response. This
71 yields crucial information about the number of variants circulating in the population and possible lines
72 of transmission, however, sequencing can be timely, costly and in some cases full coverage of the virus
73 is not possible.

74 Recently, Thermo Fisher have developed genotyping assays to help bridge the gap between testing
75 capacity and sequencing capability to receive results in real-time, so in addition to our modular system
76 for COVID-19 testing, we decided to build the Thermo Fisher TaqMan™ SARS-CoV-2 mutation panel
77 into our workflow (Figure 1). Here we present our data showing that the TaqMan genotyping assays
78 identify variants in all samples tested with zero failure rate, and that often the assay confirms
79 mutations in a viral isolate that cannot be definitively identified from genome sequence data alone.
80 We conclude that the genotyping assay is an excellent complement to genome sequencing efforts and

81 allows rapid, point-of-testing determination of the presence of any genetic variant for SARS-CoV-2 for
82 which an assay can be designed.

83 METHODS

84 Patient samples

85 500 Nasopharyngeal Pillar 1 swab samples in Virus Transfer Medium (VTM) samples were sent to the
86 University of Birmingham during April and May 2021 from Birmingham Health Trust Hospital. SARS-
87 CoV-2 positive samples from the University of Birmingham Lateral Flow testing site were also sent and
88 archived. Five pillar 2 SARS-CoV-2 samples previously identified in February 2021 at the University of
89 Birmingham Turnkey laboratory were also utilised. The use of anonymised samples in this study was
90 allowed under ethics gained to aid assay development (NRES Committee West Midlands - South
91 Birmingham 2002/201 Amendment Number 4, 24 April 2013).

92 RNA extraction

93 RNA was extracted from 200 µl of patient sample using the Thermo Fisher MagMax Viral/Pathogen II
94 Nucleic Acid isolation kits with MagMax magnetic beads and MS-2 phage internal control, using the
95 automated Thermo Fisher Kingfisher Flex Magnetic Particle Processor (11).

96 TaqPath™ COVID-19 assay

97 Reactions were prepared using the Thermo Fisher TaqPath™ COVID-19 CE-IVD RT-PCR Kit protocol
98 (**12**). RT-PCR of reactions were performed using the Applied Biosystems™ QuantStudio™ 5 Real-Time
99 PCR Instrument. Subsequent EDT files were transferred to a computer with QuantStudio™ Design and
100 Analysis Desktop Software v2.5.1 for analysis of exponential curves. The TaqPath™ COVID-19 assay
101 co-amplifies three target genes: ORF1ab, N-gene and S-gene. Results were classified as positive with
102 respect to at least 2 single-target genes (Orf1ab, N-and S-) provided the raw RT-Ct values were below
103 37 for single gene target signals. Bacteriophage MS2 RNA was added to each sample as an internal
104 positive control for each sample and to monitor potential sample inhibition. A negative control (dH2O)

105 is included on every plate. The SGTF of the TaqPath™ COVID-19 CE-IVD RT-PCR Kit was considered a
106 proxy for the presence of Δ69/70 in the S gene of SARS-CoV-2.

107 **TaqMan™ SARS-CoV-2 Mutation Panel Workflow**

108 Sample inclusion for the mutation assay required RNA extracts from positive samples (Ct ≤ 30, as
109 defined in the manufacturer's protocol, available from Thermo Fisher) for the TaqMan™ SARS-CoV-2
110 Mutation Panel workflow. From our pool of positive samples 185 were randomly selected for the
111 mutation panel assay following the assay workflow (Figure 1). Samples containing S-gene single-target
112 failure (SGTF) were included in the assay as long as ORF1a and N-gene Ct values were within range as
113 there was no compromise of assay accuracy.

114 **Designing a Genotyping Panel for the Mutation Assay**

115 TaqMan probes specific to SNPs found in variants known to be circulating widely within the United
116 Kingdom around March-May 2021 were used in this study. S-gene mutations chosen were N501Y,
117 E484K, K417N, P618R and L452R (Figure 2). Probes detect both the reference and mutation sequences
118 of SARS-CoV-2. Reporter dye information for the TaqMan™ SARS-CoV-2 Mutation Panel is represented
119 in the assay context sequence, which is the nucleotide sequence surrounding the mutation site in the
120 SARS-CoV-2 reference genome (hCoV-19/Wuhan/2019; GISAID EPI_ISL_402124). The variant allele is
121 detected by FAM™ dye and the reference allele is detected by VIC™ dye.

122 Presence of SGTF and N501Y was indicative of the Alpha variant, Beta variant was defined with the
123 presence of K417N, E484K and N501Y, Gamma as E484K and N501Y without the presence of K417N,
124 while the presence of L452R and P681R was indicative of the Delta variant.

125 Positive controls for both the Original SARS-CoV-2 sequence and chosen SNP mutations were used in
126 the assay. The AcroMetrix™ coronavirus 2019 (COVID-19) RNA control (Low Positive Control),
127 prepared using full length genomic RNA from SARS-CoV-2, was used as a positive control for SARS-
128 CoV-2. A plasmid control containing mutation sequences for N501Y, E484K and K417N was used as a

positive control for SNP mutations. However, at the time of running these experiments a plasmid control for mutations P681R and L452R was unavailable.

RT-PCR reaction mix was prepared as per the assay protocol (**12**). For samples with Ct values < 30, 5 µl of RNA was added to the reaction, for samples with Ct < 16, 2.5 µl of RNA was added to the reaction. Reactions and real- time PCR program were set up according to the mutation panel assay protocol (**13**).

Library preparation and sequencing

Library preparation of positive SARS-CoV-2 samples (cycle threshold <30) was performed using the nCoV-2019 LoCost Sequencing Protocol version 3 (**14**), using normalised primers (New England Biolabs) for the V3 ARTIC primer scheme (ARTIC network) (**15**). Sequencing was performed on a MinION flow cell (R9.4.1) run on a GridION sequencing device (Oxford Nanopore Technologies).

The ARTIC network "nCoV-2019 novel coronavirus bioinformatics protocol" (**16**) was used to process the raw sequencing data including genome assembly and variant calling using nanopolish 0.11.3 (**17**). The genotyping was then performed using a nextflow pipeline (https://github.com/BioWilko/genotyping-pipeline). Briefly; genotypes were called using aln2type (https://github.com/connor-lab/aln2type) utilising custom variant definition files for each mutation (included in repository), and lineages were called using Pangolin (**18**).

Data Analysis

Results were plotted as Allelic Discrimination Plots using the QuantStudio Design & Analysis v2.5 with the Genotyping Analysis Module.

RESULTS

Allelic discrimination plots show clear discrimination between Wild-type samples and Mutation samples using QuantStudio Design & Analysis

152 The Design & Analysis software genotype calling algorithm was initially designed for diploid organism
153 genotype calling. This is leveraged for the TaqPath assay by amplification and detection of both wild
154 type and variant alleles (labelled allele 1 and 2 respectively). This allows the software to identify
155 samples with a clear amplification curve matching either allele 1 (reference/wildtype) or allele 2
156 (variant) for each specific mutation respectively (Figure 3). In some instances, the software will identify
157 heterozygosity (i.e. presence of both wild type and variant alleles), which indicates the need for
158 further inspection of that sample. Heterozygosity may indicate a mutation at the underlying assay
159 binding site, or a truly heterologous sample (e.g. multiple strains present in sample).

160 Each probe is labelled with VIC dye to detect the reference (WT) sequence and FAM dye to detect the
161 mutation sequence, which has one nucleotide difference. This allows clear discrimination on a cluster
162 plot between WT and mutation samples, as seen with the AcroMetrix control (reference sequence)
163 and plasmid control containing the mutation sequence, Figure 3B.

164 Where input samples have similar Ct values they appear as clusters on the discrimination plot, as seen
165 in the mut/mut samples (Figure 3B) or in the case of a range of Ct values, samples are dispersed
166 along/up the axis as seen in ref/ref samples (Figure 3B and C). We were able to detect samples with
167 Ct's varying from 12 to 29, in respect to ORF1ab, N-gene and S-gene. In one case we could detect
168 mutations with Ct value 33, albeit with slightly reduced sensitivity. Samples with high viral load cluster
169 in the upper x/y-axis and low viral load in the lower x/y-axis.

170 Independent mutations that are located next to one another in SARS-CoV-2 virus genome, such as
171 P681R and P681H, can complicate genotype analysis, as probes of an assay for one mutation will fail
172 to bind to viral sequences that contain other adjacent mutations. Mutations under the probe can
173 appear as ref/mut and slope away from ref/ref or mut/mut samples, cluster along the X-axis, near to
174 the NTCs, thus exhibiting weak amplification due to the probes non-specific activity, Figure 3C.
175 Genotyping calls can manually be adjusted to 'no amp', or two separate assays run such as P681R and

176 P681H to compare and facilitate accurate genotype analysis. If it is not possible to make a call, then
177 further characterisation by genome sequencing would be necessary.

178

179 **The Mutation Panel Assay is extremely effective at identifying mutations in laboratory samples**

180 All samples run through the mutation panel assay produced a result, with either mutation present,
181 absent or reference/mutation, indicating another mutation within that SNP was present. Input
182 samples had varying Ct values (Ct 12-29) with regards to each of the three single-target genes Orf1ab,
183 N gene and S gene (Supplementary data 1). Using the Orf1ab gene as marker for Ct distribution across
184 a Ct value group, good distribution of different Ct values was observed (Figure 4). RNA quality was not
185 measured, and in some cases, samples had been stored at -80°C for up to 2 months and through no
186 more than 2 freeze/thaw cycles. However, no effect on the performance of the mutation assay was
187 observed. Furthermore, we also noted that for positive samples Ct ≤16 RNA was added at 2.5ul instead
188 of 5ul into the Mutation Panel PCR reaction, therefore, allowing more RNA to be archived.

189

190 All samples run through this assay were sent for sequencing onsite at the University of Birmingham as
191 part of COG-UK. This provided us with the ability to compare the mutation panel assay results with
192 that of genome sequencing. By cross referencing the genome sequencing results for each SNP we
193 could identify whether the mutation assay correctly calls each SNP mutation. Our data shows that for
194 all samples, where sequencing data was available, the mutation panel is in 100% agreement (Figure
195 5A - Also refer to Supplementary Data 1 for complete data set for all samples run). While Nanopore
196 sequencing may miss a SNP mutation (Figure 5B), the mutation assay can identify this. This can be due
197 to the RNA quality, sequencing platform used, or issues with primers required for genome sequencing,
198 but highlights the importance of the genotyping assay for rapid identification and subsequent action.

199 The mutation assay cannot distinguish what the substitution is in 'ref/mut' results, which highlights
200 the continued importance of sequencing and updating SNP mutations which can be added when
201 designing an assay panel. The mutation assay cannot be used to identify variant lineages, however it
202 can, due to the detection of specific mutations, give an indication as to which variant the sample may
203 be and can also exclude the presence of a VOC in a sample based on the absence of key characterising
204 mutations of significance. This is of particular importance as case numbers rise, and sequencing
205 capacity and turnaround time may not be matched. Specifically for VOC Alpha (B.1.1.7) ref/mut
206 further analysis of genome sequencing data revealed that this variant, although negative for P681R,
207 was positive for P681H. Importantly, it was noted that one ref/mut was also positive for E484K and
208 clarification from the University of Birmingham arm of the COG UK consortium confirmed that this
209 was a small cluster of B1.1.7 variants that was being monitored and actioned in the Birmingham area.

210 **The Mutation Panel Assay is highly adaptable to newly emerging variants and mutations**

211 The mutation panel designed for this assay was to identify samples containing SNPs associated with
212 variants of SARS-CoV-2 widely circulating within the United Kingdom March 2021- May 2021, this
213 included mutations found in the table 2. Between April and May B.1.617 variant numbers were
214 increasing rapidly and beginning to overtake the B.1.1.7 variant within the UK population. Therefore,
215 as experiments were being conducted in real-time the addition of P618R and L452R assays were
216 essential for rapid identification of B.1.617 variants. Samples that had previously been run for the
217 original assays and sent for sequencing meant that RNA availability was limited. However, freshly
218 isolated samples from May provided the opportunity to run all assays at once.

219 186 samples were assayed with 182 run for N501Y, 183 for E484K and 178 for K417N, and a total of
220 42 samples were assayed for all 5 mutations; 68.7% of samples assayed were positive for N501Y (WT
221 ref/ref = 31.3%); 2% of samples were positive for E484K (WT ref/ref = 97.2%); 2.8% of samples assayed
222 were positive for K417N (WT ref/ref = 97.2%) ; 57.1% of samples were positive for L425R (WT ref/ref

223 = 42.9%) and 61% were positive for P681R (WT ref/ref = 19%). A ref/mut 'call' was also noted for E484K
224 (1 sample, 0.6%) and P681R (8 samples, 19%).

225 Lineage was identifiable in 80.1% (Figure 6) of samples sent for sequencing, which as mentioned
226 previously may be due to the sequencing protocol used and/or RNA quality. From our pool of samples,
227 654.4% of samples were the B.1.1.7 variant, 18.3% B.1.617.2, 3.2% B.1.177, 2.2% B.1.137, 1.1%
228 B.1.351 and B.52 with B.1.177.16, B.1.1.372, B.1.1 and B.1 variants making up 4%. Cases of B.1.617.2
229 were first identified at the end of April 2021.

230 **Discussion**

231 Genomic epidemiology is a powerful tool for tracking transmission and importation of SARS-CoV-2 as
232 well as assessing the effectiveness of public health measures (**1, 2, 3**). Tracking transmission within
233 the population in real-time enables laboratories to report and governments to action. Recently,
234 Thermo Fisher have developed genotyping assays to help bridge the gap between testing capacity and
235 sequencing capability to receive results in real-time. A previous study from our laboratory
236 demonstrated that B.1.1.7 was associated with significantly higher viral loads (**17**) and had a
237 genotyping assay been available at the time, this would have helped to identify the Alpha variant much
238 quicker and identify speed and spread of infection for quicker action and containment.

239 In our laboratory, an opportunity arose to genotype RNA extracted in real-time from Birmingham Trust
240 Hospital, Pillar 2 and University of Birmingham Lateral Flow, positive SARS-CoV-2 samples. Initially
241 using three verified SNP assays from Thermo Fisher's Applied Biosystems™ TaqMan™ SARS-CoV-2
242 Mutation Panel and then expanding to five to include SNPS for the delta variant, we matched
243 genotypes for specific mutations in variants Alpha (B.1.1.7), Beta (B.1.351) and Delta (B.1.617.2).

244 Here we demonstrate that these mutation panels provide robust detection of VOC even if RNA is of
245 low quality and after more than one freeze/thaw step. Importantly, a specific mutation can be

246 identified on the same day that a nasopharyngeal swab tests positive by RT-PCR. Where sequencing
247 data was available, the genotyping assay always matched 100% to the correct lineage.

248 The ref/mut function is a key to the genotyping assay as when detected this will imply an amino acid
249 change for the specific mutation at the genomic site where the primers amplify. This is crucial in
250 alerting to the potential rise of a new VOI (variants of interest) so it can be monitored to see its
251 potential to become a VOC.

252 Our study confirms that same day rapid real-time detection of variants present in the population is
253 very achievable - from a swab entering the lab, processing through the TaqPath COVID-19 RT-PCR and
254 mutation assay, where results were then available in approximately 5 hours. Confirmation of
255 mutations present and lineage identification following genomic sequencing on-site was provided in
256 approximately 72hours, however, when on-site sequencing is not available this may increase
257 turnaround time significantly. The mutation panel is also significantly cheaper than sequencing, at
258 ~£0.45p per reaction, compared to ~£35 per genome when operating at scale. One limitation of the
259 TaqPath assay is the limit of detection, which may impact laboratories wishing to test extremely low
260 yield samples. This is also an issue for genome sequencing of samples, which in the UK is only possible
261 on samples with Ct values < 30 on the TaqPath assay.

262 Rapid identification of VOCs enables test-and-trace to identify regional clusters and perform targeted
263 testing to prevent spread of more transmissible variants. Having both PCR set-ups in our modular
264 testing system and on-site sequencing removes the logistics, costs and time of moving samples
265 between testing labs and sequencing labs and reporting the results.

266 Importantly, the genotyping panel can be updated readily as new SNP mutations are identified via
267 genome sequencing data from COG-UK. Delta variants emerged quickly over a few weeks and during
268 our study we were able to source very quickly, two mutation panels for the Delta variant (L452R and
269 P681R) and on arrival were able to action on the same day.

270 Genome sequencing can be inconclusive for identifying key mutations found by the mutation panels -
271 we observed several instances where the TaqPath assay identified specific alleles that were not
272 detected via genome sequencing (Supplementary Data 1) - but nonetheless, it is a powerful tool for
273 identifying SARS-CoV-2 variant lineages through phylogenetic trees. However, previous studies have
274 discussed there are limitations due to sequence drop-out when used to identify specific key mutations
275 **(18, 19, 20, 21)**. The presence of SNPs in the forward and / or reverse primer binding sites may lead
276 to complete or partial lack of amplification. These allelic drop-outs specifically affect PCR-based (tile
277 amplicon) targeted sequencing, thus resulting in incomplete genome coverage, especially at lower
278 amounts, resulting in the loss of both 5' and 3' regions that fall outside primer binding positions. This
279 can be mitigated through adjustments to the specific primer scheme used, something that happens at
280 regular intervals for the ARTIC protocol employed world-wide for Nanopore based SARS-CoV-2
281 sequencing.

282 For the study presented here, we demonstrated that genotyping has two major functions. 1)
283 Genotyping is a powerful additional, more in-depth assay for identifying specific mutations and has
284 real clinical health value allowing laboratories to report and action VOC much quicker than genome
285 sequencing. 2) Genotyping is an excellent additional complement to the already powerful tool of
286 genome sequencing already proven for assigning lineages via phylogenetic trees.

287 Our data confirms that SARS-CoV-2 genotyping is essential for real-time identification of VOC here
288 now and tracking those that emerge for informing public health strategy.

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297 **Footnotes**

298 All authors except AM confirm that they do not have any commercial or other potential conflicts of
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300 Thermo Fisher on the use of the original TaqMan assay in the UoB laboratory.

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352

353 **Figure Legends**

354 ***Figure 1.*** *Workflow for Thermo Fisher TaqMan™ SARS-CoV-2 Mutation Panel Assay. Blue workflow: Protocol used*
355 *to detect SARS-CoV-2. Orange workflow: Protocol using SNP assays to confirm mutations associated with SARS*
356 *CoV-2 emerging variants.*

357 ***Figure 2.*** *Thermo Fisher Mutation Panel Assay targets with associated SARS-CoV-2 variants and phenotype.*

358 ***Figure 3****. Mutation Assay Results viewed using QuantStudio Design & Analysis software with the*
359 *Genotyping Analysis Module.* ***(A)*** *Assay results are 'called' in 4 colours according to their outcome. Red*
360 *indicates allele1/allele1 (ref/ref) for WT, blue allele2/allele2 (mut/mut) for mutation present, green*
361 *refers to allele1/allele2 (ref/mut) and orange showing no amplification.* ***(B)*** *Allelic discrimination plot*
362 *showing clear discrimination between wild type (WT) samples (red dots along the x-axis) and the*
363 *mutation samples (blue dots along the y-axis) with high and low viral loads.* ***(C)*** *Allelic discrimination*
364 *plot showing an example of a ref/mut sample (green dots). Abbreviations: Ct (Cycle threshold). Cut offs*
365 *are determined by the QuantStudio Design & Analysis software.*

366 ***Figure 4****. Distribution of TaqPath COVID-19 PCR Ct values for the Orf1ab gene. The Y-axis indicates the number*
367 *of samples SARS-CoV-2 positive for the Orf1ab gene. The X-axis is grouped into ranges for Ct values up to and*
368 *including 30. Abbreviations: Ct (Cycle threshold), Orf1ab (Open reading frame 1ab).*

369 ***Figure 5. (A)*** *Example of mutation assay results compared with Nanopore sequencing results from a selection of*
370 *samples run through the assay. All results for all samples run are included in Supplementary Data 1. Orange*
371 *Square 'N' = mutation not present; Green Square 'Y' = mutation present; White 'ref/mut' = mutation present on*
372 *one allele only. The Red square 'X' indicates that there was not sufficient coverage of that SNP after sequencing.*
373 ***(B)*** *Percentage comparison results of all mutation assay results and corresponding Nanopore sequencing data****.***
374 *Green Bars (Mutation Assay) = Percentage (%) SNP agreement when compared to the corresponding sequencing*
375 *data. Blue Bars (Sequencing) = Percentage SNP identified when sequencing data for each sample was assigned a*
376 *lineage by Pangolin.*

377 ***Figure 6.*** *Lineage of sequenced samples identified by Pangolin.* ***(A)*** *Number of samples assigned a SARS CoV-2*

378 *lineage.* ***(B)*** *Percentage of samples assigned a lineage as compared to the total number of samples assigned a*

379 *linage.*