

1 **A robust, highly multiplexed mass spectrometry assay to identify SARS-CoV-2 variants**

2

3 Matthew M. Hernandez^{1,#}, Radhika Banu¹, Paras Shrestha¹, Ana S. Gonzalez-Reiche², Adriana van de
4 Guchte², Keith Farrugia², Robert Sebra^{2,3,4,5}, Mount Sinai PSP Study Group^{1,2,6,7}, Melissa R. Gitman¹,
5 Michael D. Nowak¹, Carlos Cordon-Cardo¹, Viviana Simon^{1,6,7,8,9}, Harm van Bakel^{2,3}, Emilia Mia
6 Sordillo¹, Nicolas Luna¹⁰, Angie Ramirez¹⁰, Sergio Andres Castañeda¹⁰, Luz Helena Patiño¹⁰, Nathalia
7 Ballesteros¹⁰, Marina Muñoz¹⁰, Juan David Ramírez^{1,10}, Alberto E. Paniz-Mondolfi^{1,#}

8

9 **Affiliations:**

10 ¹ Department of Pathology, Molecular, and Cell-Based Medicine, Icahn School of Medicine at Mount
11 Sinai, New York, NY 10029, USA

12 ² Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York,
13 NY 10029, USA

14 ³ Icahn Genomics Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

15 ⁴ Black Family Stem Cell Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029,
16 USA

17 ⁵ Sema4, a Mount Sinai venture, Stamford, CT 06902, USA

18 ⁶ Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

19 ⁷ Center for Vaccine Research and Pandemic Preparedness (C-VARPP), Icahn School of Medicine at
20 Mount Sinai, New York, NY, USA

21 ⁸ Division of Infectious Diseases, Department of Medicine, Icahn School of Medicine at Mount Sinai,
22 New York, NY 10029, USA

23 ⁹ The Global Health and Emerging Pathogens Institute, Icahn School of Medicine at Mount Sinai, New
24 York, NY 10029, USA

25 ¹⁰ Centro de Investigaciones en Microbiología y Biotecnología-UR (CIMBIUR), Facultad de Ciencias
26 Naturales, Universidad del Rosario, Bogotá, Colombia.

27

28 # Corresponding authors: matthew.hernandez@mssm.edu, alberto.paniz-mondolfi@mountsinai.org;
29 1428 Madison Avenue, Suite B2-15, New York, NY, 10029

30

31 Mount Sinai PSP Study Group (in alphabetical order): Hala Alshammary, Angela A. Amoako,
32 Mahmoud H. Awawda, Christian Cognigni, Aria Rooker, Jose Polanco, Levy A. Sominsky, Komal
33 Srivastava, Huanzhi Shi, Liyong Cao, Feng Chen, Jian Zhang, Zain Khalil

34

35 **Running head:** RT-PCR/MALDI-TOF SARS-CoV-2 variant panel

36 **Keywords:** RT-PCR, MALDI-TOF, SARS-CoV-2, variant panel, multiplex, Omicron

37

38 **Abstract**

39 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants are characterized by
40 differences in transmissibility and response to therapeutics. Therefore, discriminating among them is
41 vital for surveillance, infection prevention, and patient care. While whole viral genome sequencing
42 (WGS) is the “gold standard” for variant identification, molecular variant panels have become
43 increasingly available. Most, however, are based on limited targets and have not undergone
44 comprehensive evaluation. We assessed the diagnostic performance of the highly multiplexed Agena
45 MassARRAY[®] SARS-CoV-2 Variant Panel v3 to identify variants in a diverse set of 391 SARS-CoV-2
46 clinical RNA specimens collected across our health systems in New York City, USA as well as in
47 Bogotá, Colombia (September 2, 2020 – March 2, 2022). We demonstrate almost perfect levels of
48 interrater agreement between this assay and WGS for 9 of 11 variant calls ($\kappa \geq 0.856$) and 25 of 30
49 targets ($\kappa \geq 0.820$) tested on the panel. The assay had a high diagnostic sensitivity ($\geq 93.67\%$) for
50 contemporary variants (e.g., Iota, Alpha, Delta, Omicron [BA.1 sublineage]) and a high diagnostic
51 specificity for all 11 variants ($\geq 96.15\%$) and all 30 targets ($\geq 94.34\%$) tested. Moreover, we highlight
52 distinct target patterns that can be utilized to identify variants not yet defined on the panel including the
53 Omicron BA.2 and other sublineages. These findings exemplify the power of highly multiplexed
54 diagnostic panels to accurately call variants and the potential for target result signatures to elucidate new
55 ones.

56

57

58 **Importance**

59 The continued circulation of SARS-CoV-2 amidst limited surveillance efforts and inconsistent
60 vaccination of populations has resulted in emergence of variants that uniquely impact public health
61 systems. Thus, in conjunction with functional and clinical studies, continuous detection and
62 identification are quintessential to inform diagnostic and public health measures. Furthermore, until
63 WGS becomes more accessible in the clinical microbiology laboratory, the ideal assay for identifying
64 variants must be robust, provide high resolution, and be adaptable to the evolving nature of viruses like
65 SARS-CoV-2. Here, we highlight the diagnostic capabilities of a highly multiplexed commercial assay
66 to identify diverse SARS-CoV-2 lineages that circulated at over September 2, 2020 – March 2, 2022
67 among patients seeking care at our health systems. This assay demonstrates variant-specific signatures
68 of nucleotide/amino acid polymorphisms and underscores its utility for detection of contemporary and
69 emerging SARS-CoV-2 variants of concern.

70

71 **Introduction**

72 Since the onset of the coronavirus disease 2019 (COVID-19) pandemic, suboptimal surveillance
73 and diagnostic efforts have not been able to prevent the rapid, unchecked spread of severe acute
74 respiratory syndrome coronavirus 2 (SARS-CoV-2) (1–4). In conjunction with various factors (e.g.,
75 variable healthcare access, limitations to effective infection prevention efforts), continued spread has led
76 to the emergence of viral variants characterized by increased genomic diversity including the most
77 recent Omicron (B.1.1.529) variant and its sublineages (5–8). This poses a unique challenge to
78 healthcare systems and diagnostic laboratories, alike, as genomic variation has the potential to impact
79 viral fitness (5, 9), disease pathogenesis (10–12), response to therapeutics (e.g., antibodies) (5, 13, 14),
80 and molecular target detection (15–17).

81 Ideally, SARS-CoV-2 diagnostic assays should be scalable to test increased clinical specimens
82 and should be robust enough to accommodate genomic variation in viruses over time. Although
83 improved technologies have made high-throughput platforms more available, most are limited in the
84 level of multiplexing and, thus, risk target dropout and failing to capture infected individuals. Indeed,
85 current nucleic acid amplification tests (e.g., reverse-transcription polymerase chain reaction (RT-PCR))
86 largely utilize 1-3 targets to detect (e.g., presence/absence) SARS-CoV-2 nucleic acids. Moreover, as
87 new variants have emerged, diagnostic panels are based on targets designed for detection of nucleotide
88 changes that yield specific amino acid substitutions and call variants based on distinct target result
89 combinations (18–20). However, most of these assays distinguish viral variants through result patterns
90 of 3-9 molecular targets across multiple reaction wells (20–28), which are constrained to distinguishing
91 current circulating variants but may not be sufficient to distinguish nascent, increasingly divergent
92 variants.

93 Whole viral genome sequencing (WGS) has, therefore, largely served as the “gold standard” for
94 pathogen genomic surveillance. Still, this methodology is not realistic for most diagnostic laboratories as
95 it requires staff with bioinformatic expertise, bioinformatics infrastructure, is relatively expensive and
96 restricted in lower income countries (LICs) and lower middle income countries (LMCs) (29, 30).
97 Therefore, there is great potential for highly multiplexed assays that target an expansive repertoire of
98 polymorphisms. Currently, these platforms are rare in number (31, 32) and most have not yet been
99 evaluated for their diagnostic capabilities in the clinical setting.

100 Here, we recovered 391 SARS-CoV-2 viral RNA from clinical specimens collected from
101 infected individuals who presented for testing at the Mount Sinai Health System (MSHS) in New York
102 City (NYC) and at the Universidad del Rosario in Bogotá, Colombia from September 2, 2020 – March
103 2, 2022. These specimens had previously undergone WGS for epidemiologic surveillance, and we used
104 this data as a benchmark to evaluate the diagnostic performance of the Agena MassARRAY[®] SARS-
105 CoV-2 Variant Panel v3 (research use only, RUO). We tested this highly diverse set of viral variants to
106 interrogate the level of agreement and diagnostic sensitivity and specificity across 12 distinct variants on
107 the panel and 30 distinct polymorphic targets in the *Spike (S)* gene region. We demonstrate a high level
108 of assay agreement and high levels of diagnostic sensitivity and specificity across most variant and
109 individual targets tested. Furthermore, we highlight the utility of the variant panel to elucidate undefined
110 or emergent variants based on unique target result signatures.

111

112 **Materials and Methods**

113 *Ethics statement*

114 For specimens obtained through routine testing at MSHS, the Mount Sinai Pathogen Surveillance
115 Program was reviewed and approved by the Human Research Protection Program at the Icahn School of
116 Medicine at Mount Sinai (ISMMS) (HS#13-00981). For specimens from Colombia, the study was
117 reviewed and approved by the ethics Committee from Universidad del Rosario in Bogotá, Colombia
118 (Act number DVO005 1550-CV1499). This study was performed following the Declaration of Helsinki
119 and its later amendments, and all patient data was anonymized to minimize risk to participants.

120

121 *SARS-CoV-2 specimen collection and testing*

122 Residual viral RNA from a total of 391 specimens that were previously collected from
123 September 2, 2020 – March 2, 2022 for routine diagnostic testing were utilized for this study.

124 Specifically, 349 upper respiratory tract (e.g., nasopharyngeal, anterior nares) and saliva
125 (September 2, 2020 – March 2, 2022) specimens were originally collected for SARS-CoV-2 diagnostic
126 testing in the Molecular Microbiology Laboratory of the MSHS Clinical Laboratory, which is certified
127 under Clinical Laboratory Improvement Amendments of 1988, 42 U.S.C. §263a and meets requirements
128 to perform high-complexity tests were eligible for inclusion in this study. Viral RNA was extracted from
129 300µL of each specimen using the Viral DNA/RNA 300 Kit H96 (PerkinElmer, CMG□1033□S) on the
130 automated chemagic™ 360 instrument (PerkinElmer, 2024□0020) per manufacturer’s protocol as
131 previously described (33, 34). After routine testing and extraction, viral RNA was stored at -80°C prior
132 to recovery for testing in this study.

133 Forty-two nasopharyngeal specimens were collected from patients from the Valle del Cauca
134 department for SARS-CoV-2 testing at Universidad del Rosario from March 29, 2021 – July 28, 2021.

135 Details of processing and SARS-CoV-2 testing of upper respiratory specimens have been described
136 previously (35). After diagnostic testing, residual viral RNA was also stored at -80°C prior to recovery
137 for testing in this study.

138

139 *SARS-CoV-2 sequencing, assembly, and phylogenetics*

140 As part of the ongoing Mount Sinai Pathogen Surveillance Program, SARS-CoV-2 viral RNA
141 from MSHS underwent RT-PCR and next-generation sequencing followed by genome assembly and
142 lineage assignment using a phylogenetic-based nomenclature as described by Rambaut et al. (36) using
143 the Pangolin v4.0.6 tool and PANGO-v1.2.81 nomenclature scheme ([https://github.com/cov-](https://github.com/cov-lineages/pangolin)
144 [lineages/pangolin](https://github.com/cov-lineages/pangolin)) as previously described (4, 37).

145 Sequence libraries were prepared from RNA from Colombian specimens using the ARTIC
146 Network protocol (<https://artic.network/ncov-2019> accessed on 1 February 2021) as previously
147 described (38). Briefly, long-read Oxford Nanopore MinION sequencing was conducted by the
148 MinKNOW application (v1.5.5). Raw Fast5 files were base called and demultiplexed using Guppy.
149 Reads were filtered to remove possible chimeric reads, and genome assemblies were obtained following
150 the MinION pipeline described in the ARTIC bioinformatics pipeline ([https://artic.network/ncov-](https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html)
151 [2019/ncov2019-bioinformatics-sop.html](https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html) accessed on 1 February 2021).

152 Of 391 specimens, 381 single variant consensus genome sequences were identified, and the
153 remaining 10 yielded mixed assemblies and, thus, a putative (inconclusive) consensus genome sequence
154 was generated. All FASTA consensus genome sequences underwent mutation calling and phylogenetic
155 lineage assignment by the Nextclade Web Interface (<https://clades.nextstrain.org/>, last accessed 4/18/22)
156 and the Pangolin COVID-19 Lineage Assigner (<https://pangolin.cog-uk.io/>, last accessed 4/18/22).

157

158 *SARS-CoV-2 Variant Panel Testing*

159 We recovered residual viral RNA from all 391 specimens from -80°C storage to undergo testing
160 on the Agena MassARRAY[®] SARS-CoV-2 Variant Panel v3
161 ([https://www.agenabio.com/products/panel/coronavirus-sars-cov-2-variant-detection-research-](https://www.agenabio.com/products/panel/coronavirus-sars-cov-2-variant-detection-research-panel/#:~:text=The%20MassARRAY%C2%AE%20SARS%2DCoV,SARS%2DCoV%2D2%20variants)
162 [panel/#:~:text=The%20MassARRAY%C2%AE%20SARS%2DCoV,SARS%2DCoV%2D2%20variants](https://www.agenabio.com/products/panel/coronavirus-sars-cov-2-variant-detection-research-panel/#:~:text=The%20MassARRAY%C2%AE%20SARS%2DCoV,SARS%2DCoV%2D2%20variants)
163 ., last accessed 4/25/22). The panel combines RT-PCR and matrix-assisted laser desorption/ionization
164 time-of-flight (MALDI-TOF) to detect targeted viral polymorphisms in the Spike (S) gene (**Fig. 1A**). It
165 consists of a two-well multiplex qualitative assay that utilizes primer mixes that target a total of 36
166 polymorphisms which – in various signature combinations – reflect 16 distinct SARS-CoV-2 variants
167 (**Fig. 1B**).

168

169 RT-PCR and generation of analytes

170 Per manufacturer's protocol, for each specimen, viral RNA underwent RT-PCR by combining
171 0.355µL nuclease-free water, 1µL RT-PCR Mastermix, 0.125µL RNase Inhibitor, and 0.020µL of
172 MMLV Enzyme in each of two wells in a 384-well format. To one well, 0.500µL SARS-CoV-2 Variant
173 v3 PCR Primer P01 was added, and to the second, 0.500µL SARS-CoV-2 Variant v3 PCR Primer P02
174 was added. Three microliters of sample RNA were added to each of the two wells for a final RT-PCR
175 reaction volume of 5µL. Four positive controls of synthetic SARS-CoV-2 RNA (Twist Synthetic SARS-
176 CoV-2 RNA Controls 1 (MT007544.1, #102019), 14 (B.1.1.7_710528, #103907), 16 (EPI_ISL_678597,
177 #104043), and 17 (EPI_ISL_792683, #104044)) were diluted in a mixture of nuclease-free water
178 (Ambion #AM9916) and human liver total RNA (Takara Bio #636531) and included in each RT-PCR
179 run. This resulted in a total of 8 wells with 1,500 SARS-CoV-2 genome copies/well and 10ng human

180 liver total RNA/well for all positive controls. A negative control of nuclease-free water was included in
181 each RT-PCR run. RT-PCR thermocycler conditions are depicted in **Table S1**.

182 RT-PCR products underwent reaction with shrimp alkaline phosphatase (SAP). Directly to each
183 RT-PCR well, a mastermix of 1.53 μ L nuclease-free water, 0.17 μ L SAP Buffer, and 0.30 μ L SAP was
184 added for a total volume of 7 μ L including 2 μ L of SAP mastermix. SAP reaction thermocycler
185 conditions are described in **Table S2**.

186 Extension products were generated with SARS-CoV-2 Variant v3 Extend Primers using the
187 iPLEX[®] Pro Reagent Set. Mastermixes were created as follows: 1.06 μ L nuclease-free water, 0.20 μ L
188 iPLEX Buffer Plus, GPR; 0.20 μ L iPLEX Termination Mix, 0.04 μ L iPLEX Pro Enzyme, and 0.50 μ L
189 MassARRAY[®] SARS-CoV-2 Variant v3 Extend Primers (E01 or E02. Two microliters of E01
190 mastermix were added to each well amplified by P01 primers, and 2 μ L E02 mastermix was added to
191 each well amplified by P02 primers for the total extension reaction volume of 9 μ L. Extension
192 thermocycler conditions are detailed in **Table S3**.

193

194 Analyte dispensing, data acquisition, and data analyses

195 Twenty microliters of nuclease-free water were added to each well prior to desalting and
196 dispensing in the MassARRAY[®] System for data acquisition. Analytes were desalted using suspended
197 clean resin (Agena #08060) and dispensed onto SpectroCHIP[®] Arrays (CPM-384) for data acquisition
198 with the MassARRAY[®] Analyzer with Chip Prep Module 384 as per manufacturer's protocol.
199 Instrument settings for iPLEX Pro genotyping panels were used with Genotype+Area selected as the
200 Process Method. After data acquisition, MassARRAY[®] Typer Analyzer was used to analyze data and
201 generate variant report output results for each specimen. All variant target results and individual target
202 results for each specimen are depicted in **Table S4**.

203 *Diagnostic performance analyses*

204 To compare performance of the panel to the WGS “gold standard”, we generated 2x2
205 contingency tables for detected and not detected results of each variant call or target call. To measure the
206 level of agreement between WGS and the variant panel, we performed agreement analyses with kappa
207 (κ) results and 95% confidence intervals (95% CI) using the publicly-available GraphPad Prism web
208 calculator (<https://graphpad.com/quickcalcs/kappa2/>, last accessed April 20, 2022). Level of agreement
209 was interpreted from kappa values as previously described (39). Interpretations included no ($\kappa < 0$),
210 slight ($0 \leq \kappa \leq 0.20$), fair ($0.21 \leq \kappa \leq 0.40$), moderate ($0.41 \leq \kappa \leq 0.60$), substantial ($0.61 \leq \kappa \leq 0.80$), and
211 almost perfect agreement ($0.80 \leq \kappa \leq 1.00$). In addition, we measured diagnostic sensitivity and
212 specificity and negative (NPV) and positive predictive values (PPV) for each variant and individual
213 target polymorphism tested (GraphPad Prism v.9.3.1). Ninety-five percent confidence intervals were
214 calculated by the hybrid Wilson/Brown method. Fisher’s exact tests were performed for each
215 contingency table for each variant and/or target tested.

216 Statistical analyses were performed for variant call results of 12 of the possible 16 variants on the
217 panel. We also performed these analyses for 30 of the 36 possible targets on the panel as clinical
218 specimens that encoded 6 specific amino acid polymorphisms (D80G, Y453F, E484Q, Q493K, N501T,
219 I692V) were not recovered for this study. In addition, we did not recover any specimens that harbored
220 the native D614 amino acid (A23403 nucleotide), and, therefore, we were not able to compute level of
221 agreement or diagnostic specificity for the D614G variant or D614G target calls. In addition, for
222 performance analyses of targets H69_V70del, N439K, and E484K, we excluded specimens that resulted
223 in target dropout as we could not infer nucleotide polymorphisms that caused dropout given that
224 primer/probe sequences are proprietary and not known.

225 To assess prevalence of variant panel targets across Omicron sublineages, we interrogated
226 publicly-available SARS-CoV-2 genomes on the GISAID database (last accessed May 6, 2022). Using
227 the online graphical user interface, we counted the number of genomes that harbored each of the 36
228 possible substitutions for each of four Omicron sublineages: BA.2.12.1 (n = 12324 genomes), BA.3 (n =
229 184), BA.4 (n = 857), BA.5 (n = 437). Prevalence of each substitution was determined by dividing the
230 number of genomes with the substitution by the total number of genomes analyzed for the sublineage in
231 question.

232

233 *Display Items*

234 All figures are original and were generated using the GraphPad Prism software, Microsoft Excel
235 v16.60, and finished in Adobe Illustrator (v.26.1). **Fig. 1A** was created in BioRender.com and finished
236 in Adobe Illustrator.

237

238 *Data availability*

239 All single variant consensus genome sequences were deposited in the publicly-available Global
240 Initiative on Sharing Avian Influenza Data (GISAID) database (www.gisaid.org) [accession identifiers
241 indicated in **Table S4**]. The remaining 10 genomes with mixed patterns of mutations were not deposited
242 into GISAID as single variant consensus genomes could not be resolved (data available upon request).

243 **Results**

244 We analyzed a diverse set of 391 SARS-CoV-2 viral RNA specimens that were collected from
245 infected patients over 18 months of the COVID-19 pandemic in the NYC metropolitan area and
246 Colombia. These RNA all underwent WGS that resulted in consensus genomes that comprise 56 distinct
247 phylogenetic Pango lineages and corresponded to 12 of the 16 possible variant calls on the panel (**Fig.**
248 **1C**). These included 39 Iota (B.1.526), 40 Alpha (B.1.1.7), 110 Delta (B.1.617.2 (n = 3) + AY.x (n =
249 107)), and 79 Omicron (B.1.1.529 [BA.1 sublineage]) specimens. We also included 45 specimens that
250 corresponded to 3 variants that are not defined by the panel (e.g., Lambda (C.37), Mu (B.1.621),
251 Omicron (BA.2 sublineage)) to interrogate the assay's ability to distinguish these based on target result
252 patterns.

253

254 *Diagnostic performance of variant calling*

255 To evaluate the diagnostic performance of the Agena MassARRAY[®] Variant Panel, phylogenetic
256 results of consensus sequences based on WGS data served as the “gold standard” for comparison. Of
257 391 specimens tested on the variant panel, there were 62 with variant calls that were discordant from
258 WGS data; however, 45 of these consisted of specimens whose sequenced variant was without an
259 appropriately defined variant algorithm on the panel. Therefore, only 17 (4.91%) of clinical RNA tested
260 yielded discordant results between WGS and the expected results on the variant panel.

261 We measured the level of agreement between the overall variant calls from WGS and the variant
262 panel (**Table 1**). Of the 12 panel variants in our study set, we performed agreement analyses on 11. We
263 could not measure level of agreement – or diagnostic sensitivity/specificity – for the isolated D614G
264 result because specimens which concurrently encoded the native D614 amino acid and did not yield any
265 other variant result were not recovered for this study.

266 Overall, we observed a high level of agreement (e.g., $\kappa \geq 0.856$) for 9/11 variants including the
267 contemporary Delta (B.1.617.2+AY.x) and Omicron (BA.1) variants. The Zeta (P.2) and Eta (B.1.525)
268 variant calls demonstrated the lowest level of agreement. The single Zeta variant confirmed by WGS
269 that was tested (PV26936) resulted as the Florida variant on the panel (**Table S4**). Interestingly,
270 although the E484K and the four native amino acids – L18, K417, A701, Q677H – were correctly
271 detected as part of the Zeta target algorithm, detection of K1191N and Q493K targets met the Florida
272 variant target result criteria. The low level of agreement for the Zeta variant call is also impacted by the
273 fact that all 15 Mu (B.1.621) specimens tested on the panel were incorrectly identified as Zeta; however,
274 it is important to note that the Mu variant is not yet defined on this panel. Of the 7 Eta (B.1.525) variants
275 tested on the panel, only 1 correctly identified as Eta while the remaining 6 resulted as detected D614G.
276 These did not meet the minimum number of detectable targets to yield the Eta result and only resulted in
277 2-3 of the 4 minimum required targets. These results may be the consequences of RNA degradation over
278 long-term storage. For example, the six discordant specimens encode the H69_V70del by WGS, but all
279 yielded dropout of that target on the panel which further supports this scenario.

280 We also measured the diagnostic sensitivity and specificity of the panel (**Fig. 2A-B**) for the
281 variants tested. Diagnostic sensitivity ranged from 0% (95% CI: 0-94.87%) [Zeta] to 100% (95% CI:
282 91.24-100%) [Alpha]. Unsurprisingly, the specimens for which we had limited (e.g., <10) specimens
283 available for testing had the lowest measured sensitivities and broadest CIs including Zeta and Eta
284 variants. Among variants for which we recovered >10 specimens for testing, diagnostic sensitivity was
285 $\geq 93.67\%$ [Omicron (BA.1)]. In addition, the variant panel demonstrated a high level of diagnostic
286 specificity across all 11 variants tested that ranged from 96.15% (95% CI: 93.75-97.66%) for the Zeta
287 variant to >99% for all other variants (**Fig. 2B**). Furthermore, excluding the Zeta variant, the panel
288 results showed high PPVs (≥ 0.933) and high NPVs (≥ 0.984) for all variant calls (**Table S5**).

289 *Diagnostic performance of distinct target calls*

290 To evaluate the diagnostic capabilities of each of the 36 targeted polymorphisms that comprise
291 the variant panel, we performed interrater agreement analyses and measured the diagnostic sensitivity
292 and specificity of each assay target. Across all 391 viral RNA specimens, each of 30 of the possible 36
293 polymorphisms were present in at least one specimen.

294 When we performed agreement analyses on each of these 30 targets (**Table 2**), 25 demonstrated
295 almost perfect levels of agreement ($\kappa \geq 0.820$). The targets with suboptimal levels of agreement for our
296 dataset included D215G ($\kappa = 0$; no agreement), L242_244del ($\kappa = 0.568$; moderate agreement), N501Y
297 ($\kappa = 0.528$; moderate agreement), and K1191N ($\kappa = 0.799$; substantial agreement). This low level of
298 agreement may be impacted by small sample sizes tested. Indeed, we only recovered 2-4 specimens that
299 encoded each of the D215G, L242_244del, and K1191N targets, Therefore, small frequencies (e.g., 2-4)
300 of inaccurate calls may explain this result. It is important to note that our study set did not include
301 specimens with the native D614 amino acid (A24303 nucleotide), and level of agreement could not be
302 calculated for the D614G target.

303 Interestingly, for N501Y target, we found that of 158 specimens with the polymorphism by
304 WGS, 79 yielded a false-negative result on the variant panel. All 79 belong to the Omicron (BA.1)
305 variant lineage, and when reanalyzed excluding these BA.1 specimens, interrater agreement was almost
306 perfect ($\kappa = 0.975$) (**Table 2**). This suggests genomic variation outside original assay design may impact
307 primer/probe binding and yield distinct target results for novel variants.

308 Across the 30 targets tested, the average diagnostic sensitivity measured was 90.2% (**Fig. 2C**).
309 The targets with the lowest sensitivities included D80A (75.00%, 95% CI: 30.06-98.72%), D215G (0%,
310 95% CI: 0.00-56.00%), L242_244del (50.00%, 95% CI: 9.00-91.00%), and N501Y (50.00%, 95% CI:

311 42.00-58.00%). Notably, when all BA.1 specimens were excluded from the analyses, the sensitivity of
312 the N501Y target improved to 100.00% (95% CI: 95.00-100.00%).

313 The variant panel assay demonstrated a high diagnostic specificity across nearly all 30 targets
314 tested in this study (**Fig. 2D**). On average, the diagnostic specificity was >99.00% across all of the tested
315 diagnostic targets. Specificity was not calculated for the D614G target because no clinical specimens
316 with the native D614 amino acid were recovered in this study. In addition, across the 30 targets, the
317 PPVs and NPVs were 0.900 and 0.959, respectively (**Table S6**).

318

319 *Diagnostic target signatures of undefined variants*

320 Given that the variant panel has a uniquely high level of multiplexing, we also interrogated the
321 capabilities of the assay to reveal unique signatures of variants not defined by the panel software. To do
322 this, we included 45 clinical specimens that included the older Lambda (C.37) (n = 21) and Mu
323 (B.1.621) (n = 15) variants as well as the contemporary Omicron BA.2 variants (n = 9) recently captured
324 in NYC (**Fig. 3A**). Each of the three were called as D614G, Zeta (P.2), and D614G, respectively on the
325 panel.

326 Based on the current design of the panel, most of the Lambda specimens tested (18/21) only have
327 detectable D614G polymorphisms among the 36 targets. The remaining 3 additionally yield a T95I
328 (C21846U) polymorphism; however, this substitution is not found in any of the 3 consensus sequences,
329 and, therefore, may represent a nonspecific reaction or a minority intra-host variant. Notably, this
330 polymorphism is rare and found in only 24/10186 (0.23%) Lambda genomes deposited in GISAID (last
331 accessed May 6, 2022). The current target design does not target common Lambda substitutions
332 including G75V, T76I, D253N, L452Q, T859N, or deletions (e.g., amino acids 246-252).

333 All 15 Mu specimens were appropriately called as Zeta (P.2) based on the presence of the native
334 L18, K417, Q677, and A701 amino acids as well as the E484K which meets the threshold for the Zeta
335 variant call. All Mu specimens display a signature of six detectable targets that is unique among all other
336 variant patterns on this assay: T95I, Y144del, E484K, N501Y, D614G, and P681H. Interestingly, none
337 of the specimens' consensus genome sequences encode the Y144del which suggests other sequence
338 variation may alter primer/probe binding to this target. In fact, 12 of the Mu sequences harbor
339 substitutions at positions 144-145 (e.g., Y144S, Y145N) which may impact target detection. The five
340 other amino acid substitutions are encoded in the consensus genomes of all 15 specimens. Of note,
341 although specimen K42 features an adenosine insertion at genome position 21995, the downstream
342 nucleotide sequence encodes these amino acid polymorphisms. In addition to these 5 substitutions, there
343 are 3 Mu specimens which yielded a detectable Q677H target. However, these genomes harbor the
344 G23593 nucleotide which encodes the native Q677 amino acid and suggests this is a nonspecific result
345 or detection of a minor intra-host variant.

346 We also found that the 9 Omicron BA.2 specimens generated a distinct target result signature on
347 the variant panel assay. All 9 resulted in detection of S477N, T478K, N501Y, D614G, and P681H
348 targets as well as dropout of the N439K target. The five detected targets each were confirmed by the
349 presence of the amino acid substitutions in WGS data. We cannot delineate the cause of the N439K
350 target dropout because we do not know primer/probe sequences at the site of the targeted nucleotide
351 substitution at position 22879. However, one can speculate that sequence variation around this region
352 may interfere with primer/probe binding. Indeed, all 9 of the BA.2 specimens harbor the T22882G
353 polymorphism which results in the N440K substitution. Furthermore, the K417N substitution is found in
354 all BA.2 consensus genomes but is only detected in 7 specimens. This may be the result of different
355 nucleic acid quantities across specimens and reflect a limit in analytic sensitivity for the target.

356 Together, these are important findings to gauge the capabilities of this assay to highlight unique target
357 signatures of variants that may be captured by this platform.

358 Finally, to assess the capabilities of the assay to detect other Omicron sublineages (e.g.,
359 BA.2.12.1, BA.3, BA.4, BA.5), we independently interrogated the prevalence of each variant panel
360 target substitution among publicly-available genomes (GISAID) (**Fig. 3B**). We found that 94.9-99.7% of
361 BA.2.12.1 genomes harbor each of the K417N, S477N, T478K, N501Y, D614G, and P681H
362 substitutions. With the exception of the N439K target dropout in BA.2 genomes tested in this study, the
363 BA.2.12.1 target signature harbors the same detectable polymorphisms. While 70.7-99.5% of BA.3
364 genomes also encode the S477N, T478, N501Y, D614G, and P681H, 63.0-66.3% harbor the
365 H69_V70del, T95I, and Y144del substitution which help to distinguish this sublineage from BA.2 and
366 BA.2.12.1. Furthermore, 88.1% of BA.4 and 99.3% of BA.5 genomes encode the L452R substitution
367 which may help to differentiate these genomes from other Omicron sublineages.

368

369 **Discussion**

370 Monoclonal antibody treatments are effective in limiting severe COVID-19 but emerging
371 variants of concern often carry mutations that render the virus partially or completely resistant to
372 antibody neutralization (13, 14, 40–44). Rapid SARS-CoV-2 variant calling is therefore essential for
373 personalized COVID-19 treatment interventions. With the advent of commercial and lab-developed
374 variant panels, however, accurate variant requires robust, high-resolution platforms which are limited in
375 number and have not undergone evaluation prior to implementation. Indeed, a multi-laboratory external
376 quality assessment in late 2021 revealed gaps in calling of contemporary variants that stemmed from
377 inadequate selection of diagnostic targets to discern between variants (27). Given this, highly
378 multiplexed, efficient platforms are invaluable but are limited in number and warrant comprehensive
379 evaluation before implementation in the molecular microbiology laboratory.

380 Here, we report a comprehensive diagnostic evaluation of one of the highest multiplexed variant
381 panel assays on the market. Based on a diverse cohort of clinical specimens across two continents and a
382 wide timeline of the pandemic, we highlight almost perfect levels of interrater agreement between this
383 assay and the “gold standard” WGS for 9 of 11 variants and 25 of 30 distinct targets tested. The assay
384 has a high diagnostic specificity across all variants ($\geq 96.15\%$) and all targets ($\geq 94.34\%$) tested.
385 Furthermore, the panel shows a high diagnostic specificity and sensitivity for contemporary variants in
386 global circulation (e.g., Delta, Omicron (BA.1)).

387 Our study does present some limitations particularly with respect to limited sampling. While the
388 panel has defined target signatures for 16 different variants, we were only able to recover clinical
389 specimens that corresponded to 11 of these variants for testing. Indeed, variants with the lowest level of
390 agreement and diagnostic performance metrics were those with some of the fewest specimens recovered
391 and tested (e.g., Zeta ($n = 1$), Beta ($n = 4$), Eta ($n = 7$)). We also did not include specimens from the early

392 phase of the pandemic including D614 viruses (45, 46) which limited diagnostic analyses of the D614G
393 variant and individual target. It is important to note, however, that the D614G polymorphism has
394 undergone positive selection to eventuate emergent variants (47), and these older viruses have largely
395 been replaced by the emergent Omicron lineage(s) (6, 48). We also recognize that we did not conduct
396 this study at the extraction step of clinical specimens given limited availability of remnant upper
397 respiratory or saliva specimens.

398 A unique benefit of a highly multiplexed molecular assay is its adaptability to the natural
399 evolution of the pathogen at hand which confers the ability to identify changes in circulating viruses that
400 manifest as distinct target result signatures. To assess this potential, we included undefined variants to
401 determine if the discrete assay target result patterns could elucidate a variant's identity without
402 necessarily providing a defined result as the current software stands. Testing of Mu specimens resulted
403 in a distinguishable combination of 5 detectable substitutions, but each result was interpreted as a Zeta
404 (P.2) variant. This scenario highlights the utility of distinct target results to point to new viruses that
405 rapidly arise the circulating milieu of variants. Moreover, this underscores the importance of adaptable
406 target result interpretation software to address acute changes detected in patient populations. We also
407 tested clinical specimens that corresponded with the most current variant in circulation – the Omicron
408 sublineage BA.2 – which has largely replaced BA.1 globally over January through April 2022
409 (<https://covariants.org/>, last accessed 4/26/22). From our results, we report a BA.2-specific pattern of
410 target results on this panel that can be used to readily discriminate the BA.2 from the BA.1 subtype. The
411 following weeks/months will be key to monitoring to understand the utility of this platform for capturing
412 other emerging Omicron sublineages (e.g., BA.2.12.1, BA.3, BA.4, BA.5).

413 Accurate identification of currently circulating and emerging SARS-CoV-2 variants is key to
414 effective pathogen surveillance and providing optimal care to patients. Although WGS is the mainstay

415 for surveillance, it is important to consider in the global context of a pandemic, this may not be a
416 realistic technology for many LICs and LMCs (29). Therefore, there is a great need for rapid, cost-
417 effective, conventional technologies (e.g., RT-PCR) in the clinical laboratory. However, these require
418 increased diagnostic resolution to adequately capture viral evolution and meet the needs of pathogen
419 surveillance. Thus, highly multiplexed molecular assays such as the one presented benefit from high
420 discriminatory power and are a vital tool to shed light on changing viral dynamics.

421

422 **References**

- 423 1. Kalia K, Saberwal G, Sharma G. 2021. The lag in SARS-CoV-2 genome submissions to GISAID.
424 Nat Biotechnol 39:1058–1060.
- 425 2. Litwin T, Timmer J, Berger M, Wahl-Kordon A, Müller MJ, Kreutz C. 2022. Preventing COVID-
426 19 outbreaks through surveillance testing in healthcare facilities: a modelling study. BMC Infect
427 Dis 22:105.
- 428 3. Deng X, Gu W, Federman S, du Plessis L, Pybus OG, Faria N, Wang C, Yu G, Bushnell B, Pan C-
429 Y, Guevara H, Sotomayor-Gonzalez A, Zorn K, Gopez A, Servellita V, Hsu E, Miller S, Bedford
430 T, Greninger AL, Roychoudhury P, Starita LM, Famulare M, Chu HY, Shendure J, Jerome KR,
431 Anderson C, Gangavarapu K, Zeller M, Spencer E, Andersen KG, MacCannell D, Paden CR, Li Y,
432 Zhang J, Tong S, Armstrong G, Morrow S, Willis M, Matyas BT, Mase S, Kasirye O, Park M,
433 Masinde G, Chan C, Yu AT, Chai SJ, Villarino E, Bonin B, Wadford DA, Chiu CY. 2020.
434 Genomic surveillance reveals multiple introductions of SARS-CoV-2 into Northern California.
435 Science <https://doi.org/10.1126/science.abb9263>.
- 436 4. Hernandez MM, Gonzalez-Reiche AS, Alshammary H, Fabre S, Khan Z, van De Guchte A, Obla
437 A, Ellis E, Sullivan MJ, Tan J, Albuquerque B, Soto J, Wang C-Y, Sridhar SH, Wang Y-C, Smith
438 M, Sebra R, Paniz-Mondolfi AE, Gitman MR, Nowak MD, Cordon-Cardo C, Luksza M, Krammer
439 F, van Bakel H, Simon V, Sordillo EM. 2021. Molecular evidence of SARS-CoV-2 in New York
440 before the first pandemic wave. Nat Commun 12:3463.
- 441 5. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, Fera D, Shafer RW.
442 2021. The biological and clinical significance of emerging SARS-CoV-2 variants. Nat Rev Genet
443 22:757–773.

- 444 6. 2021. Classification of Omicron (B.1.1.529): SARS-CoV-2 Variant of Concern. World Health
445 Organization. [https://www.who.int/news/item/26-11-2021-classification-of-omicron-\(b.1.1.529\)-](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern)
446 [sars-cov-2-variant-of-concern](https://www.who.int/news/item/26-11-2021-classification-of-omicron-(b.1.1.529)-sars-cov-2-variant-of-concern). Retrieved 3 March 2022.
- 447 7. Dhar MS, Marwal R, Vs R, Ponnusamy K, Jolly B, Bhojar RC, Sardana V, Naushin S, Rophina M,
448 Mellan TA, Mishra S, Whittaker C, Fatihi S, Datta M, Singh P, Sharma U, Ujjainiya R, Bhatheja N,
449 Divakar MK, Singh MK, Imran M, Senthivel V, Maurya R, Jha N, Mehta P, A V, Sharma P, Vr A,
450 Chaudhary U, Soni N, Thukral L, Flaxman S, Bhatt S, Pandey R, Dash D, Faruq M, Lall H, Gogia
451 H, Madan P, Kulkarni S, Chauhan H, Sengupta S, Kabra S, Indian SARS-CoV-2 Genomics
452 Consortium (INSACOG)[‡], Gupta RK, Singh SK, Agrawal A, Rakshit P, Nandicoori V, Tallapaka
453 KB, Sowpati DT, Thangaraj K, Bashyam MD, Dalal A, Sivasubbu S, Scaria V, Parida A, Raghav
454 SK, Prasad P, Sarin A, Mayor S, Ramakrishnan U, Palakodeti D, Seshasayee ASN, Bhat M,
455 Shouche Y, Pillai A, Dikid T, Das S, Maitra A, Chinnaswamy S, Biswas NK, Desai AS,
456 Pattabiraman C, Manjunatha MV, Mani RS, Arunachal Udipi G, Abraham P, Atul PV, Cherian SS.
457 2021. Genomic characterization and epidemiology of an emerging SARS-CoV-2 variant in Delhi,
458 India. *Science* 374:995–999.
- 459 8. Arora P, Zhang L, Rocha C, Sidarovich A, Kempf A, Schulz S, Cossmann A, Manger B, Baier E,
460 Tampe B, Moerer O, Dickel S, Dopfer-Jablonka A, Jäck H-M, Behrens GMN, Winkler MS,
461 Pöhlmann S, Hoffmann M. 2022. Comparable neutralisation evasion of SARS-CoV-2 omicron
462 subvariants BA.1, BA.2, and BA.3. *Lancet Infect Dis* [https://doi.org/10.1016/S1473-](https://doi.org/10.1016/S1473-3099(22)00224-9)
463 [3099\(22\)00224-9](https://doi.org/10.1016/S1473-3099(22)00224-9).
- 464 9. Plante JA, Liu Y, Liu J, Xia H, Johnson BA, Lokugamage KG, Zhang X, Muruato AE, Zou J,
465 Fontes-Garfias CR, Mirchandani D, Scharton D, Bilello JP, Ku Z, An Z, Kalveram B, Freiberg AN,

- 466 Menachery VD, Xie X, Plante KS, Weaver SC, Shi P-Y. 2021. Spike mutation D614G alters
467 SARS-CoV-2 fitness. *Nature* 592:116–121.
- 468 10. Tang P, Hasan MR, Chemaitelly H, Yassine HM, Benslimane FM, Al Khatib HA, AlMukdad S,
469 Coyle P, Ayoub HH, Al Kanaani Z, Al Kuwari E, Jeremijenko A, Kaleeckal AH, Latif AN, Shaik
470 RM, Abdul Rahim HF, Nasrallah GK, Al Kuwari MG, Al Romaihi HE, Butt AA, Al-Thani MH, Al
471 Khal A, Bertollini R, Abu-Raddad LJ. 2021. BNT162b2 and mRNA-1273 COVID-19 vaccine
472 effectiveness against the SARS-CoV-2 Delta variant in Qatar. *Nat Med*
473 <https://doi.org/10.1038/s41591-021-01583-4>.
- 474 11. Malden DE, Bruxvoort KJ, Tseng HF, Ackerson B, Choi SK, Florea A, Tubert J, Takhar H,
475 Aragonés M, Hong V, Talarico CA, McLaughlin JM, Qian L, Tartof SY. 2021. Distribution of
476 SARS-CoV-2 Variants in a Large Integrated Health Care System - California, March-July 2021.
477 *MMWR Morb Mortal Wkly Rep* 70:1415–1419.
- 478 12. Nyberg T, Ferguson NM, Nash SG, Webster HH, Flaxman S, Andrews N, Hinsley W, Bernal JL,
479 Kall M, Bhatt S, Blomquist P, Zaidi A, Volz E, Aziz NA, Harman K, Funk S, Abbott S, COVID-19
480 Genomics UK (COG-UK) consortium, Hope R, Charlett A, Chand M, Ghani AC, Seaman SR,
481 Dabrera G, De Angelis D, Presanis AM, Thelwall S. 2022. Comparative analysis of the risks of
482 hospitalisation and death associated with SARS-CoV-2 omicron (B.1.1.529) and delta (B.1.617.2)
483 variants in England: a cohort study. *Lancet* 399:1303–1312.
- 484 13. Bruel T, Hadjadj J, Maes P, Planas D, Seve A, Staropoli I, Guivel-Benhassine F, Porrot F, Bolland
485 W-H, Nguyen Y, Casadevall M, Charre C, Péré H, Veyer D, Prot M, Baidaliuk A, Cuypers L,
486 Planchais C, Mouquet H, Baele G, Mouthon L, Hocqueloux L, Simon-Loriere E, André E, Terrier
487 B, Prazuck T, Schwartz O. 2022. Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1

- 488 and BA.2 in patients receiving monoclonal antibodies. *Nat Med* [https://doi.org/10.1038/s41591-](https://doi.org/10.1038/s41591-022-01792-5)
489 022-01792-5.
- 490 14. Tada T, Zhou H, Dcosta BM, Samanovic MI, Chivukula V, Herati RS, Hubbard SR, Mulligan MJ,
491 Landau NR. 2022. Increased resistance of SARS-CoV-2 Omicron variant to neutralization by
492 vaccine-elicited and therapeutic antibodies. *eBioMedicine* 78.
- 493 15. Hernandez MM, Banu R, Gonzalez-Reiche AS, Gray B, Shrestha P, Cao L, Chen F, Shi H, Hanna
494 A, Ramírez JD, van de Guchte A, Sebra R, Gitman MR, Nowak MD, Cordon-Cardo C, Schutzbank
495 TE, Simon V, van Bakel H, Sordillo EM, Paniz-Mondolfi AE, Mount Sinai PSP Study Group.
496 2021. RT-PCR/MALDI-TOF diagnostic target performance reflects circulating SARS-CoV-2
497 variant diversity in New York City. *bioRxiv*.
- 498 16. Bal A, Destras G, Gaymard A, Stefic K, Marlet J, Eymieux S, Regue H, Semanas Q, d'Aubarede C,
499 Billaud G, Laurent F, Gonzalez C, Mekki Y, Valette M, Bouscambert M, Gaudy-Graffin C, Lina B,
500 Morfin F, Josset L, COVID-Diagnosis HCL Study Group. 2021. Two-step strategy for the
501 identification of SARS-CoV-2 variant of concern 202012/01 and other variants with spike deletion
502 H69-V70, France, August to December 2020. *Euro Surveill* 26.
- 503 17. Sánchez-Calvo JM, Alados Arboledas JC, Ros Vidal L, de Francisco JL, López Prieto MD. 2021.
504 Diagnostic pre-screening method based on N-gene dropout or delay to increase feasibility of
505 SARS-CoV-2 VOC B.1.1.7 detection. *Diagn Microbiol Infect Dis* 101:115491.
- 506 18. Erster O, Mendelson E, Levy V, Kabat A, Mannasse B, Asraf H, Azar R, Ali Y, Shirazi R, Bucris
507 E, Bar-Ilan D, Mor O, Mandelboim M, Sofer D, Fleishon S, Zuckerman NS. 2021. Rapid and High-

- 508 Throughput Reverse Transcriptase Quantitative PCR (RT-qPCR) Assay for Identification and
509 Differentiation between SARS-CoV-2 Variants B.1.1.7 and B.1.351. *Microbiol Spectr* 9:e0050621.
- 510 19. Teng I-T, Nazzari AF, Choe M, Liu T, de Souza MO, Petrova Y, Tsybovsky Y, Wang S, Zhang B,
511 Artamonov M, Madan B, Huang A, Lopez Acevedo SN, Pan X, Ruckwardt TJ, DeKosky BJ,
512 Mascola JR, Misasi J, Sullivan NJ, Zhou T, Kwong PD. 2021. Molecular probes of spike
513 ectodomain and its subdomains for SARS-CoV-2 variants, Alpha through Omicron. *bioRxiv*.
- 514 20. Ashford F, Best A, Dunn SJ, Ahmed Z, Siddiqui H, Melville J, Wilkinson S, Mirza J, Cumley N,
515 Stockton J, Ferguson J, Wheatley L, Ratcliffe E, Casey A, Plant T, COVID-19 Genomics UK
516 (COG-UK) Consortium, Quick J, Richter A, Loman N, McNally A. 2022. SARS-CoV-2 Testing in
517 the Community: Testing Positive Samples with the TaqMan SARS-CoV-2 Mutation Panel To Find
518 Variants in Real Time. *J Clin Microbiol* 60:e0240821.
- 519 21. Nörz D, Grunwald M, Tang HT, Weinschenk C, Günther T, Robitaille A, Giersch K, Fischer N,
520 Grundhoff A, Aepfelbacher M, Pfefferle S, Lütgehetmann M. 2022. Clinical Evaluation of a Fully-
521 Automated High-Throughput Multiplex Screening-Assay to Detect and Differentiate the SARS-
522 CoV-2 B.1.1.529 (Omicron) and B.1.617.2 (Delta) Lineage Variants. *Viruses* 14.
- 523 22. Yeung Priscilla S.-W., Wang Hannah, Sibai Mamdouh, Solis Daniel, Yamamoto Fumiko, Iwai
524 Naomi, Jiang Becky, Hammond Nathan, Truong Bernadette, Bihon Selamawit, Santos Suzette, Mar
525 Marilyn, Mai Claire, Mfuh Kenji O., Miller Jacob A., Huang ChunHong, Sahoo Malaya K.,
526 Zehnder James L., Pinsky Benjamin A., Caliendo Angela M. 2022. Evaluation of a Rapid and
527 Accessible Reverse Transcription-Quantitative PCR Approach for SARS-CoV-2 Variant of
528 Concern Identification. *J Clin Microbiol* 0:e00178-22.

- 529 23. Hale R, Crowley P, Dervisevic S, Coupland L, Cliff PR, Ebie S, Snell LB, Paul J, Williams C,
530 Randell P, Pond M, Stanley K. 2021. Development of a Multiplex Tandem PCR (MT-PCR) Assay
531 for the Detection of Emerging SARS-CoV-2 Variants. *Viruses* 13.
- 532 24. De Pace V, Bruzzone B, Orsi A, Ricucci V, Domnich A, Guarona G, Randazzo N, Stefanelli F,
533 Battolla E, Dusi PA, Lillo F, Icardi G. 2022. Comparative Analysis of Five Multiplex RT-PCR
534 Assays in the Screening of SARS-CoV-2 Variants. *Microorganisms* 10.
- 535 25. Nussbaum EZ, Thiriveedhi V, Uddin R, Cho HE, Carroll S, Rosenberg ES, Lemieux JE, Turbett
536 SE. 2022. Implementation of a SARS-CoV-2 Genotyping Panel for Prompt Omicron Variant
537 Identification: A Pragmatic Tool for Clinical Laboratories. *Ann Intern Med*
538 <https://doi.org/10.7326/M22-0023>.
- 539 26. Vogels CBF, Breban MI, Ott IM, Alpert T, Petrone ME, Watkins AE, Kalinich CC, Earnest R,
540 Rothman JE, Goes de Jesus J, Morales Claro I, Magalhães Ferreira G, Crispim MAE, Brazil-UK
541 CADDE Genomic Network, Singh L, Tegally H, Anyaneji UJ, Network for Genomic Surveillance
542 in South Africa, Hodcroft EB, Mason CE, Khullar G, Metti J, Dudley JT, MacKay MJ, Nash M,
543 Wang J, Liu C, Hui P, Murphy S, Neal C, Laszlo E, Landry ML, Muyombwe A, Downing R,
544 Razeq J, de Oliveira T, Faria NR, Sabino EC, Neher RA, Fauver JR, Grubaugh ND. 2021.
545 Multiplex qPCR discriminates variants of concern to enhance global surveillance of SARS-CoV-2.
546 *PLoS Biol* 19:e3001236.
- 547 27. Buchta C, Camp JV, Jovanovic J, Radler U, Benka B, Puchhammer-Stöckl E, Müller MM,
548 Griesmacher A, Aberle SW, Görzer I. 2021. Inadequate design of mutation detection panels
549 prevents interpretation of variants of concern: results of an external quality assessment for SARS-
550 CoV-2 variant detection. *Clin Chem Lab Med* <https://doi.org/10.1515/cclm-2021-0889>.

- 551 28. Dikdan RJ, Marras SAE, Field AP, Brownlee A, Cironi A, Hill DA, Tyagi S. 2022. Multiplex PCR
552 Assays for Identifying all Major Severe Acute Respiratory Syndrome Coronavirus 2 Variants. *J*
553 *Mol Diagn* 24:309–319.
- 554 29. Brito AF, Semenova E, Dudas G, Hassler GW, Kalinich CC, Kraemer MUG, Ho J, Tegally H,
555 Githinji G, Agoti CN, Matkin LE, Whittaker C, Danish Covid-19 Genome Consortium, COVID-19
556 Impact Project, Network for Genomic Surveillance in South Africa (NGS-SA), GISAID core
557 curation team, Howden BP, Sintchenko V, Zuckerman NS, Mor O, Blankenship HM, de Oliveira
558 T, Lin RTP, Siqueira MM, Resende PC, Vasconcelos ATR, Spilki FR, Aguiar RS, Alexiev I,
559 Ivanov IN, Philipova I, Carrington CVF, Sahadeo NSD, Gurry C, Maurer-Stroh S, Naidoo D, von
560 Eije KJ, Perkins MD, van Kerkhove M, Hill SC, Sabino EC, Pybus OG, Dye C, Bhatt S, Flaxman
561 S, Suchard MA, Grubaugh ND, Baele G, Faria NR. 2021. Global disparities in SARS-CoV-2
562 genomic surveillance. *medRxiv* <https://doi.org/10.1101/2021.08.21.21262393>.
- 563 30. Xuan J, Yu Y, Qing T, Guo L, Shi L. 2013. Next-generation sequencing in the clinic: promises and
564 challenges. *Cancer Lett* 340:284–295.
- 565 31. Lai E, Becker D, Brzoska P, Cassens T, Davis-Turak J, Diamond E, Furtado M, Gandhi M, Gort D,
566 Greninger AL, Hajian P, Hayashibara K, Kennedy EB, Laurent M, Lee W, Leonetti NA, Lozach J,
567 Lu J, Nguyen JM, O'Donovan KMC, Peck T, Radcliffe GE, Ramirez JM, Roychoudhury P,
568 Sandoval E, Walsh B, Weinell M, Wesselman C, Wesselman T, White S, Williams S, Wong D, Yu
569 Y, Creager RS. 2022. A method for variant agnostic detection of SARS-CoV-2, rapid monitoring of
570 circulating variants, detection of mutations of biological significance, and early detection of
571 emergent variants such as Omicron. *bioRxiv*.

- 572 32. Welch NL, Zhu M, Hua C, Weller J, Mirhashemi ME, Nguyen TG, Mantena S, Bauer MR, Shaw
573 BM, Ackerman CM, Thakku SG, Tse MW, Kehe J, Uwera M-M, Eversley JS, Bielwaski DA,
574 McGrath G, Braidt J, Johnson J, Cerrato F, Moreno GK, Krasilnikova LA, Petros BA, Gionet GL,
575 King E, Huard RC, Jalbert SK, Cleary ML, Fitzgerald NA, Gabriel SB, Gallagher GR, Smole SC,
576 Madoff LC, Brown CM, Keller MW, Wilson MM, Kirby MK, Barnes JR, Park DJ, Siddle KJ,
577 Happi CT, Hung DT, Springer M, MacInnis BL, Lemieux JE, Rosenberg E, Branda JA, Blainey
578 PC, Sabeti PC, Myhrvold C. 2022. Multiplexed CRISPR-based microfluidic platform for clinical
579 testing of respiratory viruses and identification of SARS-CoV-2 variants. *Nat Med*
580 <https://doi.org/10.1038/s41591-022-01734-1>.
- 581 33. Hernandez MM, Banu R, Shrestha P, Patel A, Chen F, Cao L, Fabre S, Tan J, Lopez H, Chiu N,
582 Shifrin B, Zapolskaya I, Flores V, Lee PY, Castañeda S, Ramírez JD, Jhang J, Osorio G, Gitman
583 MR, Nowak MD, Reich DL, Cordon-Cardo C, Sordillo EM, Paniz-Mondolfi AE. 2021. RT-
584 PCR/MALDI-TOF mass spectrometry-based detection of SARS-CoV-2 in saliva specimens. *J Med*
585 *Virol* <https://doi.org/10.1002/jmv.27069>.
- 586 34. Hernandez MM, Banu R, Gonzalez-Reiche AS, van de Guchte A, Khan Z, Shrestha P, Cao L, Chen
587 F, Shi H, Hanna A, Alshammary H, Fabre S, Amoako A, Obla A, Albuquerque B, Patiño LH,
588 Ramírez JD, Sebra R, Gitman MR, Nowak MD, Cordon-Cardo C, Schutzbank TE, Simon V, van
589 Bakel H, Sordillo EM, Paniz-Mondolfi AE. 2022. Robust clinical detection of SARS-CoV-2
590 variants by RT-PCR/MALDI-TOF multitarget approach. *J Med Virol* 94:1606–1616.
- 591 35. Patiño LH, Castañeda S, Muñoz M, Ballesteros N, Ramirez AL, Luna N, Guerrero-Araya E, Pérez
592 J, Correa-Cárdenas CA, Duque MC, Méndez C, Oliveros C, Shaban MV, Paniz-Mondolfi AE,

- 593 Ramírez JD. 2022. Epidemiological Dynamics of SARS-CoV-2 Variants During Social Protests in
594 Cali, Colombia. *Front Med* 9:863911.
- 595 36. Rambaut A, Holmes EC, O’Toole Á, Hill V, McCrone JT, Ruis C, du Plessis L, Pybus OG. 2020.
596 A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology. *Nat*
597 *Microbiol* 5:1403–1407.
- 598 37. Gonzalez-Reiche AS, Hernandez MM, Sullivan MJ, Ciferri B, Alshammary H, Obla A, Fabre S,
599 Kleiner G, Polanco J, Khan Z, Albuquerque B, van de Guchte A, Dutta J, Francoeur N, Melo BS,
600 Oussenko I, Deikus G, Soto J, Sridhar SH, Wang Y-C, Twyman K, Kasarskis A, Altman DR,
601 Smith M, Sebra R, Aberg J, Krammer F, García-Sastre A, Luksza M, Patel G, Paniz-Mondolfi A,
602 Gitman M, Sordillo EM, Simon V, van Bakel H. 2020. Introductions and early spread of SARS-
603 CoV-2 in the New York City area. *Science* 369:297–301.
- 604 38. Ramírez JD, Muñoz M, Ballesteros N, Patiño LH, Castañeda S, Rincón CA, Mendez C, Oliveros C,
605 Perez J, Márquez EK, Ortiz F de LS, Correa-Cárdenas CA, Duque MC, Paniz-Mondolfi A. 2021.
606 Phylogenomic Evidence of Reinfection and Persistence of SARS-CoV-2: First Report from
607 Colombia. *Vaccines (Basel)* 9.
- 608 39. Landis JR, Koch GG. 1977. The measurement of observer agreement for categorical data.
609 *Biometrics* 33:159–174.
- 610 40. Planas D, Veyer D, Baidaliuk A, Staropoli I, Guivel-Benhassine F, Rajah MM, Planchais C, Porrot
611 F, Robillard N, Puech J, Prot M, Gallais F, Gantner P, Velay A, Le Guen J, Kassis-Chikhani N,
612 Edriss D, Belec L, Seve A, Courtellemont L, Péré H, Hocqueloux L, Fafi-Kremer S, Prazuck T,

- 613 Mouquet H, Bruel T, Simon-Lorière E, Rey FA, Schwartz O. 2021. Reduced sensitivity of SARS-
614 CoV-2 variant Delta to antibody neutralization. *Nature* 596:276–280.
- 615 41. Liu C, Ginn HM, Dejnirattisai W, Supasa P, Wang B, Tuekprakhon A, Nutalai R, Zhou D, Mentzer
616 AJ, Zhao Y, Duyvesteyn HME, López-Camacho C, Slon-Campos J, Walter TS, Skelly D, Johnson
617 SA, Ritter TG, Mason C, Costa Clemens SA, Gomes Naveca F, Nascimento V, Nascimento F,
618 Fernandes da Costa C, Resende PC, Pauvolid-Correa A, Siqueira MM, Dold C, Temperton N,
619 Dong T, Pollard AJ, Knight JC, Crook D, Lambe T, Clutterbuck E, Bibi S, Flaxman A, Bittaye M,
620 Belij-Rammerstorfer S, Gilbert SC, Malik T, Carroll MW, Klenerman P, Barnes E, Dunachie SJ,
621 Baillie V, Serafin N, Ditse Z, Da Silva K, Paterson NG, Williams MA, Hall DR, Madhi S, Nunes
622 MC, Goulder P, Fry EE, Mongkolsapaya J, Ren J, Stuart DI, Screaton GR. 2021. Reduced
623 neutralization of SARS-CoV-2 B.1.617 by vaccine and convalescent serum. *Cell* 184:4220-
624 4236.e13.
- 625 42. Lusvarghi S, Pollett SD, Neerukonda SN, Wang W, Wang R, Vassell R, Epsi NJ, Fries AC, Agan
626 BK, Lindholm DA, Colombo CJ, Mody R, Ewers EC, Lalani T, Ganesan A, Goguet E, Hollis-Perry
627 M, Coggins SA, Simons MP, Katzelnick LC, Wang G, Tribble DR, Bentley L, Eakin AE, Broder
628 CC, Erlandson KJ, Laing ED, Burgess TH, Mitre E, Weiss CD. 2022. SARS-CoV-2 BA.1 variant
629 is neutralized by vaccine booster-elicited serum, but evades most convalescent serum and
630 therapeutic antibodies. *Sci Transl Med* eabn8543.
- 631 43. Yu J, Collier A-RY, Rowe M, Mardas F, Ventura JD, Wan H, Miller J, Powers O, Chung B,
632 Siamatu M, Hachmann NP, Surve N, Nampanya F, Chandrashekar A, Barouch DH. 2022.
633 Neutralization of the SARS-CoV-2 Omicron BA.1 and BA.2 Variants. *N Engl J Med* 386:1579–
634 1580.

- 635 44. Guo D, Duan H, Cheng Y, Wang Y, Hu J, Shi H. 2022. Omicron-included mutation-induced
636 changes in epitopes of SARS-CoV-2 spike protein and effectiveness assessments of current
637 antibodies. *Molecular Biomedicine* 3:12.
- 638 45. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, Hengartner N, Giorgi EE,
639 Bhattacharya T, Foley B, Hastie KM, Parker MD, Partridge DG, Evans CM, Freeman TM, de Silva
640 TI, Sheffield COVID-19 Genomics Group, McDanal C, Perez LG, Tang H, Moon-Walker A,
641 Whelan SP, LaBranche CC, Saphire EO, Montefiori DC. 2020. Tracking Changes in SARS-CoV-2
642 Spike: Evidence that D614G Increases Infectivity of the COVID-19 Virus. *Cell* 182:812-827.e19.
- 643 46. Isabel S, Graña-Miraglia L, Gutierrez JM, Bundalovic-Torma C, Groves HE, Isabel MR, Eshaghi
644 A, Patel SN, Gubbay JB, Poutanen T, Guttman DS, Poutanen SM. 2020. Evolutionary and
645 structural analyses of SARS-CoV-2 D614G spike protein mutation now documented worldwide.
646 *Sci Rep* 10:14031.
- 647 47. Chakraborty C, Saha A, Sharma AR, Bhattacharya M, Lee S-S, Agoramoorthy G. 2021. D614G
648 mutation eventuates in all VOI and VOC in SARS-CoV-2: Is it part of the positive selection
649 pioneered by Darwin? *Mol Ther Nucleic Acids* 26:237–241.
- 650 48. Mallapaty S. 2022. Where did Omicron come from? Three key theories. Nature Publishing Group
651 UK. <https://doi.org/10.1038/d41586-022-00215-2>. Retrieved 25 April 2022.

652

653 **Acknowledgments**

654 We thank the members of MSHS MML, Simon, and van Bakel laboratories. We are grateful for
655 the continuous expert guidance provided by the ISMMS Program for the Protection of Human Subjects
656 (PPHS).

657 We are thankful for the efforts of the Mount Sinai PSP Study Group members (in alphabetical
658 order): Hala Alshammary, Angela A. Amoako, Mahmoud H. Awawda, Christian Cognigni, Aria
659 Rooker, Jose Polanco, Levy A. Sominsky, Komal Srivastava, Paras Shrestha, Steve Shi, Jian Zhang,
660 Zain Khalil.

661

662 **Funding**

663 This research was supported by the Center for Research on Influenza Pathogenesis, a National
664 Institutes of Health (NIH) funded Center of Excellence for Influenza Research and Surveillance (CEIRS,
665 contract number HHSN272201400008C; CEIRR contract number 75N93021C00014) [Icahn School of
666 Medicine at Mount Sinai (ISMMS)], the NIH Office of Research Infrastructure under award numbers
667 S10OD018522 and S10OD026880 [ISMMS Scientific Computing and Data, Patricia Kovatch],
668 institutional and philanthropic funds (Open Philanthropy Project, #2020-215611 [ISMMS Department of
669 Microbiology, Dr. Adolfo Garcia-Sastre, V.S. and H.v.B.], the Pershing Square Foundation [Mount
670 Sinai Health System, Dr. David L. Reich and A.E.PM.], as well as a Robin Chemers Neustein
671 Postdoctoral Fellowship Award [A.S.GR.]. We thank the Universidad del Rosario in the framework of
672 its strategic plan RUTA2025. Thanks to President and the University Council for leading the strategic
673 projects (JR).

674

675 **Author contributions**

676 M.M.H., R.B., P.S., M.S.PSP.S.G., A.E.PM., M.R.G., M.D.N., M.M., N.L., A.R., S.C., N.B.,
677 J.D.R. and E.M.S. provided clinical samples for the study. M.M.H., R.B., P.S., M.S.PSP.S.G., and
678 A.E.PM. accessioned clinical samples. A.S.GR., A.v.d.G, K.F., M.S.PSP.S.G., M.M., N.L., A.R.,
679 S.A.C., N.B., L.H.P., and H.v.B. performed viral genome sequencing experiments. R.S. provided NGS
680 services. A.S.GR. and M.S.PSP.S.G. performed genome assembly and data curation. M.M.H., R.B.,
681 P.S., J.D.R., M.R.G., M.D.N., C.C.C., V.S., H.v.B., E.M.S., and A.E.PM analyzed, interpreted, or
682 discussed data. M.M.H. wrote the manuscript. M.M.H., J.D.R., and A.E.PM. conceived the study.
683 E.M.S. and A.E.PM. supervised the study. H.v.B., V.S., A.E.PM., and E.M.S. raised financial support.

684 M.M.H. and A.E.PM are the guarantors of this work and, as such, had full access to all of the
685 data in the study and take responsibility for the integrity of the data and the accuracy of the data
686 analysis.

687 **Competing Interests**

688 Robert Sebra is VP of Technology Development and a stockholder at Sema4, a Mount Sinai
689 Venture. This work, however, was conducted solely at Icahn School of Medicine at Mount Sinai.
690 Otherwise, the authors declare no competing interests.

691

692 **Table and Figure Legends**

693 **TABLE 1. Diagnostic agreement between WGS and panel variant calls**

694

Variant	Number of Specimens^a	Agreement (%)	Kappa (95% CI)	Interpretation^b
Omicron (BA.1)	79	98.72 %	0.959 (0.924-0.995)	Almost perfect
Delta	110	98.72 %	0.968 (0.940-0.996)	Almost perfect
Alpha	40	100.00 %	1.000 (1.000-1.000)	Almost perfect
Beta	4	99.74 %	0.856 (0.577-1.000)	Almost perfect
Gamma	14	99.74 %	0.964 (0.894-1.000)	Almost perfect
Zeta	1	95.91 %	-0.005 (-0.014-0.004)	None
Eta	7	98.47 %	0.247 (0.147-0.640)	Fair
Iota	39	99.74 %	0.986 (0.957-1.000)	Almost perfect
Epsilon	19	100.00 %	1.000 (1.000-1.000)	Almost perfect
B.1.258	1	100.00 %	1.000 (1.000-1.000)	Almost perfect
D614G ^c	27	NA	NA	NA
Broad USA	5	100.00 %	1.000 (1.000-1.000)	Almost perfect

695

696 ^a Number of specimens confirmed by WGS as the indicated variant.

697 ^b Interpretation of level of agreement is based on reference (39).

698 ^c Agreement analyses were not performed as specimens that harbor the native D614 amino acid were not recovered for
699 testing. NA, not available.

700

701

702

703 **TABLE 2. Diagnostic agreement between WGS and panel target calls**
704

Target	Number of Specimens ^{a, b}	Agreement (%)	Kappa (95% CI)	Interpretation ^c
L5F	42	99.49 %	0.973 (0.935-1.000)	Almost perfect
S13I	19	99.74 %	0.972 (0.916-1.000)	Almost perfect
L18F	15	98.99 %	0.877 (0.758-0.996)	Almost perfect
T19R	110	98.72 %	0.968 (0.940-0.996)	Almost perfect
H69_V70del	51	97.35 %	0.906 (0.841-0.970)	Almost perfect
D80A	4	99.74 %	0.856 (0.577-1.000)	Almost perfect
D80G	0	NA	NA	NA
T95I	159	96.42 %	0.927 (0.889-0.964)	Almost perfect
Y144del	126	92.33 %	0.824 (0.764-0.885)	Almost perfect
W152C	19	100.00 %	1.000 (1.000-1.000)	Almost perfect
D215G	3	99.23 %	0	None
L242_244del	4	99.23 %	0.568 (0.127-1.000)	Moderate
D253G	39	98.98 %	0.944 (0.890-0.999)	Almost perfect
K417N	92	97.95 %	0.942 (0.902-0.982)	Almost perfect
K417T	14	99.23 %	0.899 (0.786-1.000)	Almost perfect
N439K	1	100.00 %	1.000 (1.000-1.000)	Almost perfect
L452R	124	98.47 %	0.965 (0.937-0.933)	Almost perfect
Y453F	0	NA	NA	NA
S477N	90	100.00 %	1.000 (1.000-1.000)	Almost perfect
T478K	194	98.72 %	0.974 (0.952-0.997)	Almost perfect
E484Q	0	NA	NA	NA
E484K	65	95.79 %	0.860 (0.791-0.929)	Almost perfect
Q493K	0	NA	NA	NA
N501Y	158	79.03 %	0.528 (0.447-0.609)	Moderate
N501Y (exclude BA.1)	79	99.04 %	0.975 (0.947-1.000)	Almost perfect
N501T	0	NA	NA	NA
A570D	40	100.00 %	1.000 (1.000-1.000)	Almost perfect
D614G ^d	388	99.49 %	NA	NA
Q677H	7	99.23 %	0.820 (0.620-1.000)	Almost perfect
Q677P	5	100.00 %	1.000 (1.000-1.000)	Almost perfect

P681H	141	98.47 %	0.967 (0.940-0.993)	Almost perfect
P681R	110	98.47 %	0.961 (0.931-0.992)	Almost perfect
I692V	0	NA	NA	NA
A701V	49	98.88 %	0.955 (0.911-0.999)	Almost perfect
T716I	40	100.00 %	1.000 (1.000-1.000)	Almost perfect
S982A	40	99.74 %	0.986 (0.958-1.000)	Almost perfect
K1191N	2	99.74 %	0.799 (0.413-1.000)	Substantial

705

706

707

708

709

710

711

712

^a Number of specimens that harbor the given target polymorphism by WGS.

^b Analyses were not performed if specimens with the given target polymorphism by WGS were not recovered for testing. NA, not available.

^c Interpretation of level of agreement is based on reference (39).

^d Kappa could not be determined as specimens that harbor the native D614 amino acid were not recovered for testing.

713 **FIG 1. Detection of viral variants by the Agena MassARRAY[®] SARS-CoV-2 Variant Panel.** (A)
714 SARS-CoV-2 genome with nucleotide positions from 5'-to-3' direction depicted above. *S* gene
715 polymorphisms targeted by the variant panel (lollipops) and corresponding amino acids are depicted
716 below. (B) A color map depicts algorithms of target combinations that define 16 distinct SARS-CoV-2
717 variants on the panel. Variant results are depicted (left) which include the WHO designation (e.g.,
718 Omicron, Delta, etc.) and corresponding PANGO lineage assignments. Note that the B.1.526.1 variant
719 was re-designated as B.1.637 to distinguish it from the Iota variant lineage (<https://cov->
720 [lineages.org/lineage_list.html](https://cov-lineages.org/lineage_list.html), last accessed 4/26/22). The minimum number of targets required to
721 support the corresponding variant result are indicated (right). Target results are depicted as colored cells
722 indicating detectable native (e.g., unchanged from Wuhan-Hu-1 reference) amino acids which do not
723 contribute to the variant target algorithm (grey), detectable native amino acids which do contribute to the
724 algorithm (yellow), detectable amino acid polymorphisms (red), and dropout of the given target
725 polymorphism. (C) Phylogenetic composition of 391 clinical specimen viral RNA recovered for
726 diagnostic evaluation of the variant panel. Numbers of each lineage tested are depicted in brackets.
727

728 **FIG 2. Diagnostic sensitivity and specificity of the Agena MassARRAY[®] SARS-CoV-2 Variant**
729 **Panel.** (A) Diagnostic sensitivity and (B) diagnostic specificity of eleven variant calls on the panel are
730 depicted. The number of specimens that correspond with each variant according to WGS are annotated
731 in brackets. Depiction of (C) diagnostic sensitivity and (D) diagnostic specificity of each of thirty
732 distinct panel targets. The number of specimens that correspond with each amino acid polymorphism
733 according to WGS are annotated in brackets for each target. Asterisks (*) indicate targets for which
734 dropout results were excluded from analyses (see Methods). For target N501Y, a separate diagnostic
735 analysis was conducted excluding BA.1 specimens (“N501Y_Excl-BA.1”). Error bars reflect 95% CI in
736 all four panels. ND, not determined.
737

738 **FIG 3. Target result patterns of undefined variants on the Agena MassARRAY[®] SARS-CoV-2**
739 **Variant Panel. (A)** A color map depicts the observed target results for three undefined SARS-CoV-2
740 variants tested on the panel: Lambda (C.37), Mu (B.1.621), and Omicron (BA.2). Distinct target patterns
741 observed among each of the variant types are depicted. Cells indicate the distinct target results including
742 detectable native amino acid (grey), detection of target polymorphism (red), and target dropout (green).
743 The number of specimens that yielded each of the distinct target result patterns are indicated on the right
744 as well as the output variant ID result generated by the variant panel software. (B) A heatmap depicts the
745 measured prevalence of each variant panel target substitution among publicly-available Omicron
746 sublineage genomes as of May 6, 2022.
747

Figure 1

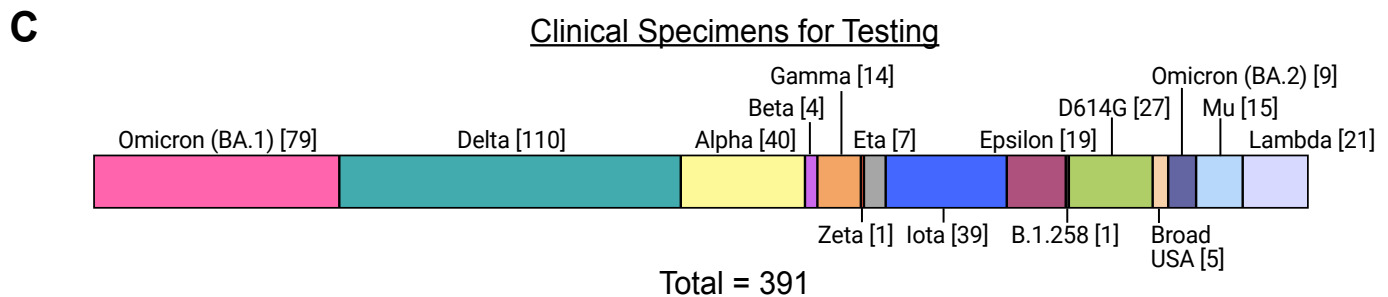
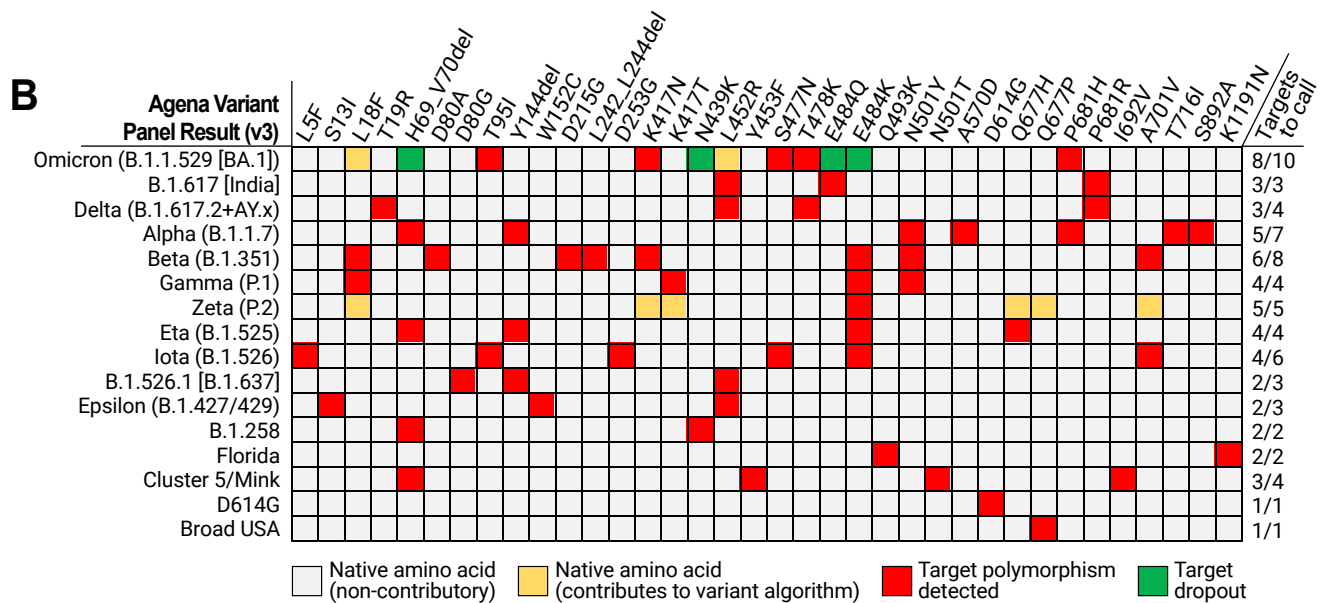
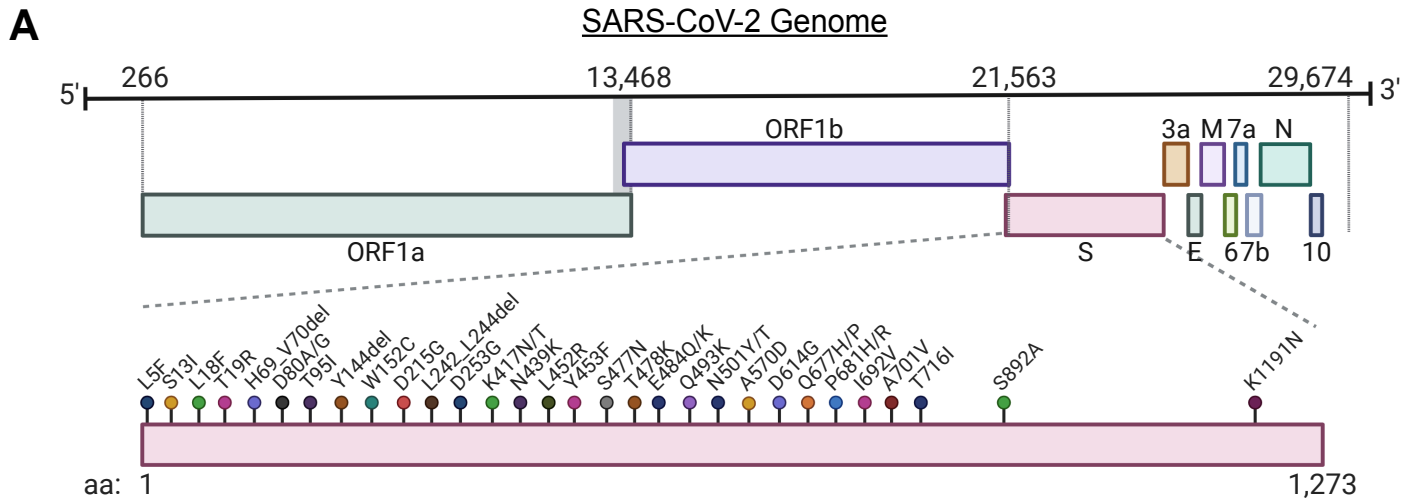
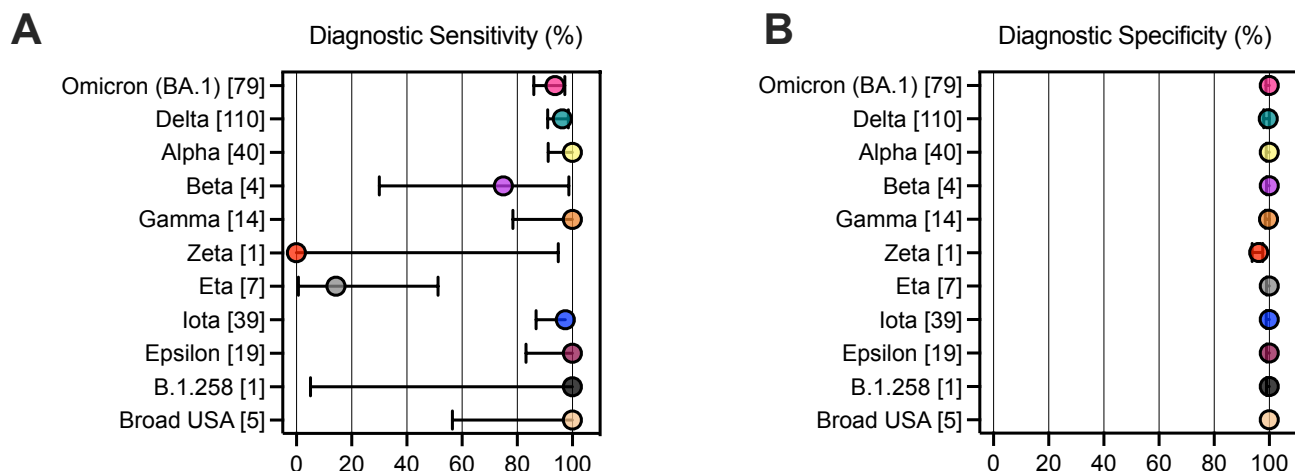


Figure 2

Variant Performance



Target Performance

