

Equipment-free detection of SARS-CoV-2 and Variants of Concern using Cas13

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Abstract

The COVID-19 pandemic, and the recent rise and widespread transmission of SARS-CoV-2 Variants of Concern (VOCs), have demonstrated the need for ubiquitous nucleic acid testing outside of centralized clinical laboratories. Here, we develop SHINEv2, a Cas13-based nucleic acid diagnostic that combines quick and ambient temperature sample processing and lyophilized reagents to greatly simplify the test procedure and assay distribution. We benchmarked a SHINEv2 assay for SARS-CoV-2 detection against state-of-the-art antigen-capture tests using 96 patient samples, demonstrating 50-fold greater sensitivity and 100% specificity. We designed SHINEv2 assays for discriminating the Alpha, Beta, Gamma and Delta VOCs, which can be read out visually using lateral flow technology. We further demonstrate that our assays can be performed without any equipment in less than 90 minutes. SHINEv2 represents an important advance towards rapid nucleic acid tests that can be performed in any location.

1 Introduction

2
3 Frequent and widespread testing is critical to prevent and respond to infectious disease
4 outbreaks. For example, large-scale testing to track the prevalence and transmission of severe
5 acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been essential to manage the
6 ongoing coronavirus disease 2019 (COVID-19) pandemic¹⁻³. Ubiquitous and frequent diagnostic
7 testing leads to the rapid identification of new cases and permits the swift treatment or isolation
8 of infected individuals, thereby preventing further viral spread⁴. However, the gold standard for
9 COVID-19 diagnosis, reverse transcription quantitative polymerase chain reaction (RT-qPCR),
10 remains suboptimal for orchestrating this response. RT-qPCR requires specialized equipment
11 and expertise rarely found outside of centralized laboratories. In addition, an insufficient testing
12 infrastructure coupled with reagent shortages and high testing demand have led to slow sample-
13 to-answer times (often of several days) for RT-qPCR^{5,6}. Alternative diagnostic technologies that
14 enable rapid and decentralized testing are vital to respond to the current and future pandemics.

15
16 Lateral flow antigen-capture tests and isothermal nucleic acid diagnostics with visual readouts
17 represent promising alternatives for SARS-CoV-2 testing outside of centralized laboratories. The
18 effectiveness of such tests in curbing the spread of SARS-CoV-2 has been demonstrated in
19 multiple settings, ranging from nursing homes to whole countries^{3,7}. Antigen-capture tests, such
20 as Abbott's BinaxNow COVID - 19 Antigen Self Tests, are quick and user-friendly but their
21 moderate sensitivity means they can miss potentially infectious individuals⁸⁻¹⁰. Isothermal nucleic
22 acid amplification methods, such as loop-mediated isothermal amplification (LAMP), are more
23 sensitive than antigen-capture tests and operate at a single temperature^{11,12}. The deployment of
24 LAMP-based tests has been facilitated by the use of auxiliary devices to eliminate the need for
25 intensive nucleic acid purification and maintain a stable temperature during amplification¹³.
26 However, these devices are often too costly for single-use and difficult to manufacture at the scale
27 required for population-wide distribution, which can limit their utility for frequent and widespread
28 testing¹⁴. Hence, the continued development and deployment of user-friendly, sensitive and
29 equipment-free diagnostics is key to enhance the public health response to COVID-19.

30
31 CRISPR-based diagnostics (CRISPR-Dx) are promising technologies for SARS-CoV-2 testing
32 with minimal equipment requirements. CRISPR-Dx usually combine isothermal nucleic acid
33 amplification methods (often LAMP or recombinase polymerase amplification (RPA)) with a RNA-
34 guided CRISPR-Cas nuclease (usually Cas12 or Cas13) for pathogen detection¹⁵⁻¹⁸. Detection of
35 an amplified target nucleic acid triggers cleavage of a reporter molecule by the CRISPR-Cas
36 enzyme. This reporter cleavage can be detected by the appearance of visual bands on a lateral
37 flow strip, among other methods^{18,19}. Pairing isothermal nucleic acid amplification methods with
38 Cas nuclease detection considerably enhances specificity and sensitivity, but at the expense of
39 increasing assay complexity. Some groups have simplified the workflow by eliminating the need
40 for amplification using more active Cas enzymes, although they rely on custom-built equipment
41 and nucleic acid extraction^{20,21}. Several groups have combined multiple enzymatic steps into
42 individual reactions to streamline CRISPR-Dx^{22,23}. We previously developed SHINE (Sstreamlined
43 Highlighting of Infections to Navigate Epidemics), henceforth SHINEv1, a diagnostic assay that
44 does not require nucleic acid extractions or custom equipment²³. However, SHINEv1 involves

45 multiple heating steps, and uses reagent mixes that require cold temperature storage and manual
46 preparation by trained personnel. Further simplifications and technical improvements to CRISPR-
47 Dx would greatly facilitate test distribution and expand their use cases.

48
49 Frequent and widespread testing is even more important with the rise of highly transmissible
50 SARS-CoV-2 variants. In recent months, the evolution of SARS-CoV-2 has led to the emergence
51 and sustained transmission of viral variants with sets of mutations that can increase infectivity or
52 reduce neutralization by antibodies, making the virus harder to contain²⁴. The World Health
53 Organization (WHO) has designated some of the SARS-CoV-2 variants with increased
54 transmissibility, virulence and/or antigenicity as Variants of Concern (VOCs). Notably, the Alpha,
55 Beta, Gamma and Delta VOCs (designated B.1.1.7, B.1.351, P.1 and B.1.617.2 with the PANGO
56 nomenclature system, respectively) are widely circulating in many countries around the world and
57 have placed unprecedented strain on several healthcare systems^{25,26}. Surveillance of SARS-CoV-
58 2 VOCs is vital to inform public health and clinical decisions, such as for prioritizing testing or
59 vaccination in the populations most heavily impacted by the pandemic. Currently, the identification
60 of viral mutations and tracking of circulating SARS-CoV-2 variants is performed by viral genomic
61 sequencing and a small number of mutation-specific RT-qPCR tests²⁷⁻²⁹. However, the lack of
62 infrastructure and expertise have made routine genomic surveillance difficult to perform outside
63 of specialized genomic centers³⁰.

64
65 CRISPR-Dx are uniquely suited to detect VOCs for routine surveillance applications. In contrast
66 to available point-of-need tests, CRISPR-Dx can easily detect individual SARS-CoV-2 mutations.
67 They do so by measuring relative signal intensities using a set of two crRNAs designed against
68 the variant region^{15,19,31}. To date, only a few CRISPR-Dx have been developed for the
69 identification of single or multiple nucleotide substitutions present in various SARS-CoV-2
70 VOCs³²⁻³⁵. However, these tests consist of multiple liquid handling steps or rely on custom-built
71 equipment^{32,35}. The development of equipment-free and user-friendly CRISPR-Dx assays to
72 distinguish SARS-CoV-2 VOCs would fill a major unmet need in COVID-19 diagnostics.

73
74 Here, we develop SHINEv2, a fast, user-friendly and widely deployable technology for the
75 detection of SARS-CoV-2 and its variants in clinical samples. We improve upon SHINEv1 by
76 incorporating a fast and ambient-temperature sample lysis procedure and enabling the
77 lyophilization of the assay reagents. These improvements considerably reduce assay complexity
78 and facilitate its distribution. We demonstrate that SHINEv2 can detect SARS-CoV-2 RNA in
79 unextracted patient samples with 100% specificity and 50-fold higher sensitivity than cutting-edge
80 antigen-capture tests. Moreover, we designed and validated SHINEv2 assays to discriminate the
81 Alpha, Beta, Gamma and Delta VOCs in clinical samples. Finally, we introduced a Cas12-based
82 human RNase P control, further simplified the lateral flow assay and examined the performance
83 of SHINEv2 at ambient-temperature. These additional advances could expand the use cases of
84 SHINEv2 to virtually any location, including at-home settings.

85

86 Results

87

88 *Design of SHINE assay for SARS-CoV-2 detection - S gene assay*

89 We previously developed a SHINEv1 assay to detect SARS-CoV-2 open reading frame 1a
90 (ORF1a) directly from clinical samples²³. We wondered if the performance of SHINE could be
91 further enhanced through the selection of target sites with more active RPA primers and crRNAs.
92 Using ADAPT³⁶, a genomics-informed computational design tool for nucleic acid based
93 diagnostics, on 308,315 SARS-CoV-2 genomes collected before February 2021, we identified
94 RPA primers and 3 crRNAs with high predicted activity in detecting the S gene of SARS-CoV-2.
95 We selected the most active crRNA by measuring Cas13 cleavage activity on synthetic RNA
96 targets without prior amplification (Supplementary Fig. 1) and we optimized the RPA primer
97 concentration to create the S gene assay. We performed bioinformatic analysis to ensure that the
98 crRNA and RPA primer sequences were highly conserved across SARS-CoV-2 genomes. The
99 crRNA sequence was fully conserved in 99.79% of the 308,315 SARS-CoV-2 genomes analysed
100 and the RPA primers were predicted to amplify 99.77% of the genomes. When compared to the
101 ORF1a assay, the S gene assay was found to be 1-2x faster and 10x more sensitive
102 (Supplementary Fig. 2). Therefore, given its increased sensitivity and favorable kinetics, we
103 selected the S gene assay for further development.

104

105 *Improving the accessibility of SHINEv1*

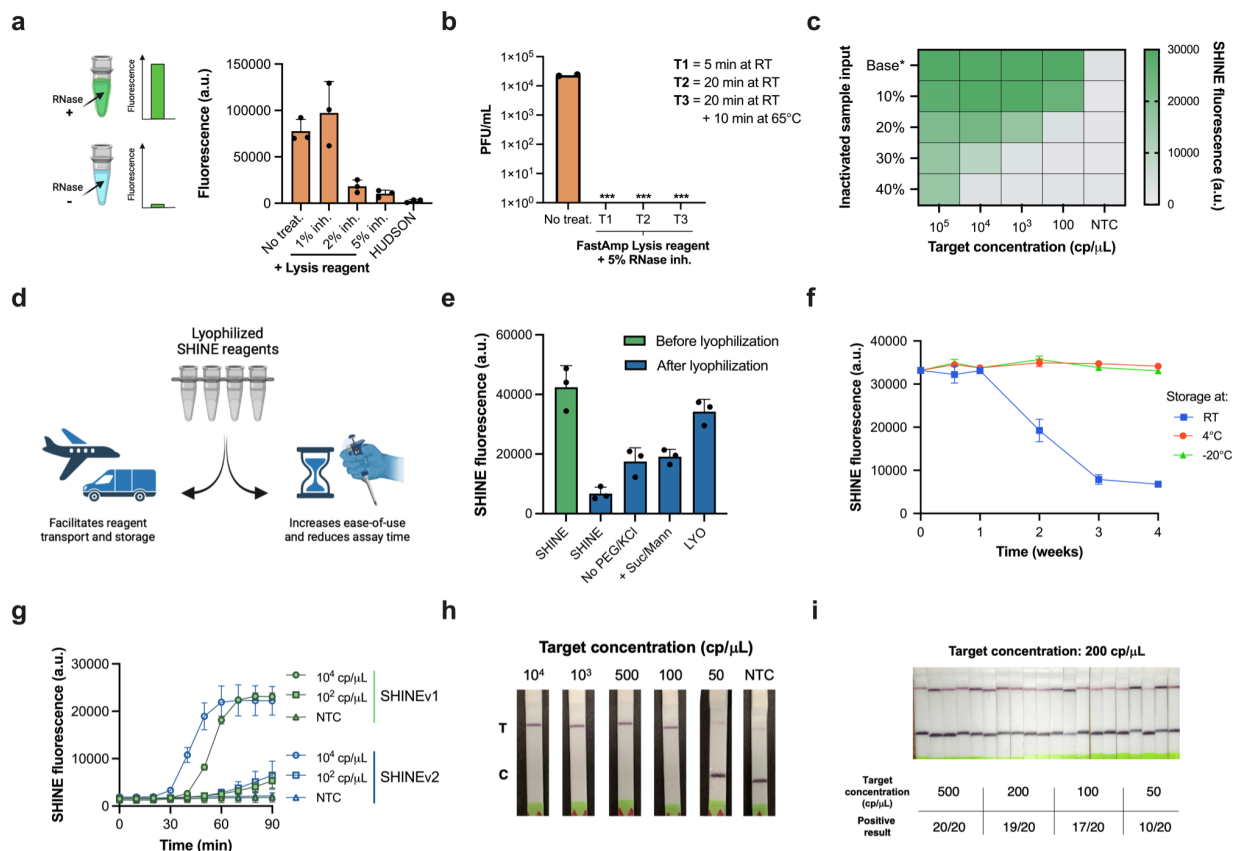
106 For SARS-CoV-2 diagnostic testing to occur in virtually any location, improvements were needed
107 to increase user-friendliness and facilitate test distribution. Towards this goal, we developed
108 SHINEv2, which incorporates ambient-temperature sample processing and lyophilized testing
109 reagents, thereby greatly simplifying reagent transport and storage and decreasing the overall
110 complexity of the assay.

111

112 To be an effective approach, extraction-free sample processing must inactivate nucleases and
113 infectious viral particles, and must be compatible with the assay, allowing for good diagnostic
114 performance. Previously, we circumvented the need for clinical sample extraction using
115 HUDSON, a nuclease inactivation and viral lysis method that relies on heat and chemical
116 reduction^{19,23}. To eliminate HUDSON's need for heating elements and incubation at two
117 temperatures, we sought to find an ambient temperature alternative for sample processing.
118 FastAmp Viral and Cell Solution for COVID-19 testing (Intact Genomics) functions at ambient
119 temperature and has been reported to be compatible with isothermal nucleic acid amplification
120 methods³⁷. Therefore, we investigated whether this lysis reagent was also compatible with Cas13-
121 based detection methods.

122

123 We assessed the ability of the FastAmp lysis reagent to inactivate RNases in contrived nasal
124 samples. Our contrived samples consisted of 10% (v/v) healthy nasal fluid in universal transport
125 medium (UTM). After treatment, we added RNaseAlert, a reporter that fluoresces in the presence
126 of RNase activity (Fig. 1a). Without treatment, contrived nasal samples contained high levels of
127 RNases, as indicated by the high fluorescence.



128
 129 **Fig. 1 | Increasing the ease-of-use and deployability of SHINE.** **a**, RNase activity in nasal fluid
 130 mixed with universal viral transport medium (UTM) untreated or treated with FastAmp Lysis reagent
 131 supplemented with RNase inhibitor (inh.) or treated with HUDSON (a heat- and chemical- treatment).
 132 Activity measured using RNaseAlert at room temperature (RT) for 30 minutes. **b**, SARS-CoV-2
 133 seedstock titer without treatment or after being incubated with lysis reagent (+5% RNase inh.) at RT
 134 for 5 minutes, 20 minutes or 20 minutes plus 10 minutes at 65°C. ***, infection not detected; PFU,
 135 plaque forming units. **c**, SHINE fluorescence with different proportions of inactivated sample input (i.e.
 136 FastAmp lysis reagent, RNase inh. and UTM) after a 90 minute incubation. Base*, baseline (i.e. no
 137 inactivated sample added). **d**, Schematic of the advantages of lyophilizing SHINE. **e**, SHINE
 138 fluorescence on synthetic RNA target (10^4 copies/ μL) before and after lyophilization using different
 139 buffers. Fluorescence measured after 90 minutes. For buffer composition, see *Methods*. **f**, SHINE
 140 fluorescence after a 90 minute incubation using lyophilized (LYO) reagents stored at RT, 4°C or -20°C
 141 over time. Target concentration: 10^4 copies/ μL . **g**, Fluorescence kinetics for SHINEv1 and SHINEv2
 142 using synthetic RNA targets; NTC, no target control. **h**, Lateral flow detection of SARS-CoV-2 RNA in
 143 lysis buffer treated viral seedstocks using SHINEv2, after a 90 minute incubation. C = control band; T
 144 = test band. **i**, Determination of analytical limit of detection with 20 replicates of SHINEv2 at different
 145 concentrations of SARS-CoV-2 RNA from lysis reagent treated contrived samples. Incubated for 90
 146 minutes. For **(a,e,g)**, center = mean and error bars = standard deviation (s.d.) for 3 technical replicates.
 147 In **c**, the heatmap values represent the mean for 3 technical replicates. For **f**, center = mean and error
 148 bars = s.d. for 3 biological replicates with 3 technical replicates each.

149

150 Treatment with FastAmp lysis reagent supplemented with 5% RNase inhibitors decreased the
151 RNase activity by over 85% in contrived nasal samples (Fig. 1a). In addition, the ratio of nasal
152 fluid to UTM in clinical samples derived from nasal swabs is likely to be significantly lower than
153 10% and therefore, the residual RNase activity in lysed patient samples is expected to be even
154 lower.

155
156 Having determined the nuclease inactivation capacity of this lysis solution, we tested its ability to
157 inactivate SARS-CoV-2 viral seedstocks. For each treatment, we quantified the amount of viable
158 virus after two passages using a plaque-based assay (Fig. 1b). Incubation of SARS-CoV-2 with
159 the lysis solution for as little as 5 minutes at room temperature (RT) yielded no discernible
160 plaques, thereby confirming the ability of our lysis solution to swiftly inactivate the virus.

161
162 We then examined the compatibility of this lysis solution with good SHINE assay performance.
163 Solution-based sample processing methods often introduce an upper bound of inactivated sample
164 input that can be added, which is dictated by the balance between increased sample volume and
165 decreased assay performance caused by the lysis reagents. Therefore, we supplemented these
166 SHINE reactions with different proportions of inactivated sample inputs (i.e. contrived nasal
167 samples treated with lysis solution) (Fig. 1c). An input of 10% (v/v) inactivated sample into SHINE
168 was able to retain full activity while still doubling the amount of sample added with respect to the
169 previous instantiation of SHINE, which used 5% (v/v) inactivated sample²³ (Supplementary Fig.
170 3). Larger input volumes, which would increase the volume of clinical sample added, led to a
171 marked decrease in the sensitivity of SHINE. (Fig. 1C and Supplementary Fig. 3). Thus, our lysis
172 procedure - FastAmp lysis reagent with 5% RNase inhibitor for 5 min - is compatible with SHINE
173 and can effectively inactivate nucleases and SARS-CoV-2 virions, making it ideal for rapid and
174 equipment-free sample processing at ambient temperature.

175
176 Lyophilization can facilitate the transport and storage of point-of-care CRISPR-Dx by eliminating
177 the need for a cold chain and increasing the shelf life (Fig. 1d). Lyophilization also simplifies
178 reaction setup, by allowing the full reaction to be easily reconstituted by the end user. Initially,
179 lyophilization with the original SHINE buffer led to a profound drop in activity post-lyophilization
180 (Fig. 1e). We sought to improve the lyophilization process through the addition of non-reducing
181 disaccharides (e.g. sucrose), which can act as stabilizers; the addition of bulking agents (e.g.
182 mannitol) and the removal of potentially destabilizing components, such as polyethylene glycol
183 (PEG) and potassium chloride (KCl). Specifically, the removal of 3.5% PEG-8000 and 60 mM KCl
184 from the original SHINE buffer or the addition of 5% (w/v) sucrose and 150 mM mannitol each
185 moderately increased SHINE's activity post-lyophilization. With these modifications combined (i.e.
186 LYO buffer), SHINE retained its full activity after lyophilization (Fig. 1e and Supplementary Fig.
187 4).

188
189 Having optimized the lyophilization excipients and procedure, we conducted a month-long
190 experiment to determine the stability of the lyophilized SHINE reagents at different temperatures
191 over time. After being stored at RT, 4°C or -20°C for 4, 7, 14, 21 or 28 days, SHINE pellets were
192 rehydrated and tested on full genome synthetic RNA standards (Twist Bioscience). SHINE pellets
193 retained full activity for >1 week at RT and at least a month at 4°C/-20°C (Fig. 1f). By enabling

194 transport and storage at above-freezing temperatures, lyophilized SHINE greatly simplifies
195 distribution logistics.

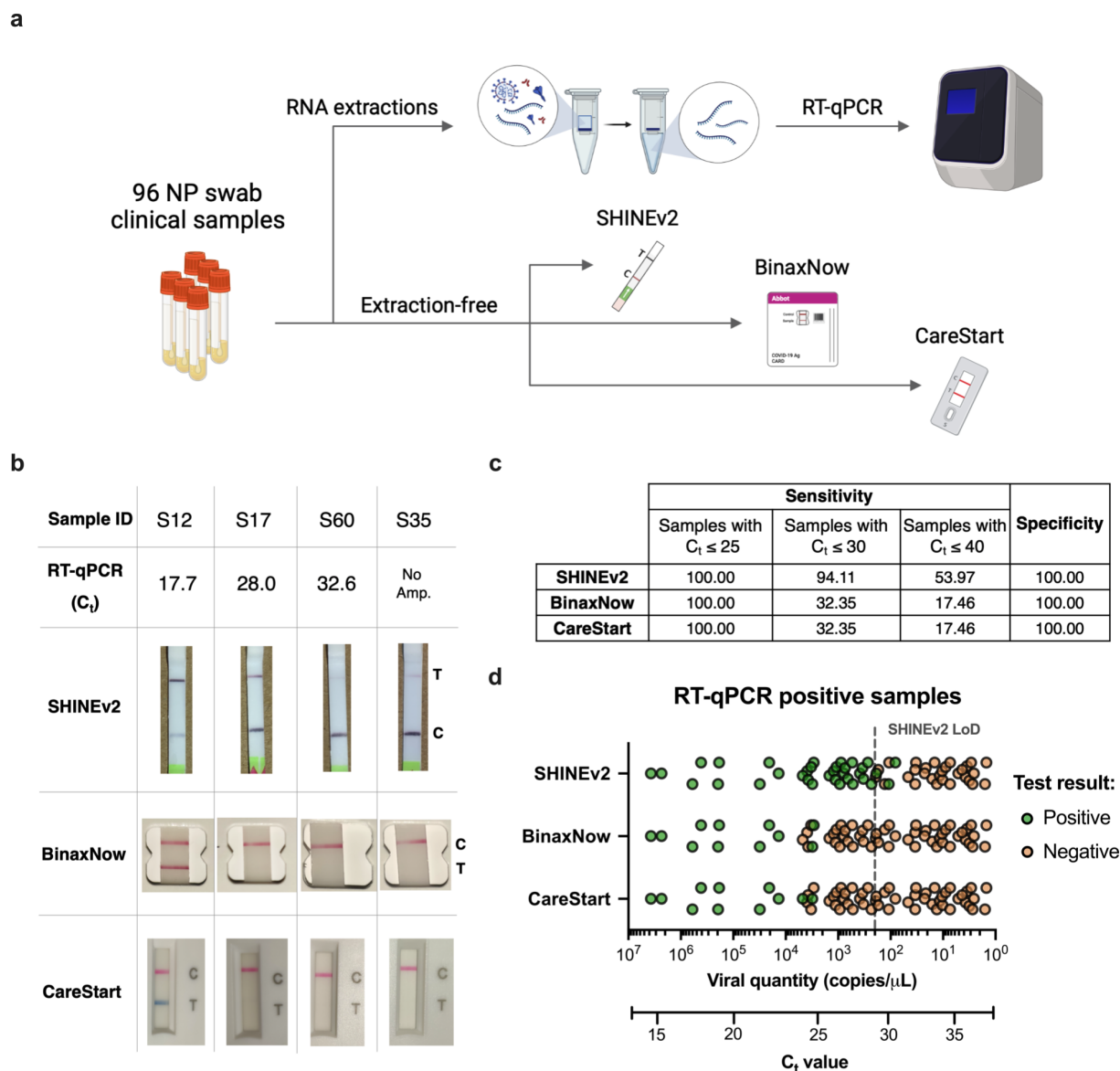
196
197 Our SHINEv2 technology, which incorporates a new sample lysis procedure and lyophilization
198 into SHINEv1, can sensitively detect nucleic acids with a simple workflow. SHINEv2 substantially
199 reduced the hands-on time and liquid-handling steps needed from the user, from over 45 minutes
200 and 20 pipetting steps in SHINEv1 to less than 10 minutes and 5 user-operations in SHINEv2
201 (Supplementary Fig. 5). When tested side-by-side with SHINEv1, we found the sensitivity and
202 reaction kinetics of the two methods to be equivalent at multiple target concentrations (Fig. 1g).
203 SHINEv2 detected down to 100 copies per microliter (cp/ μ L) within 90 minutes, using either a
204 plate-based fluorescence readout or a colorimetric lateral flow based readout (Fig. 1g and 1h).
205 Moreover, we evaluated the impact of transportation and storage on SHINEv2 by comparing its
206 performance over time in a second laboratory in Nigeria. Six weeks after production, and after
207 transportation to a different continent, SHINEv2 was shown to detect down to 100 cp/ μ L of full
208 genome synthetic RNA standards using a colorimetric lateral flow based readout (Supplementary
209 Fig. 6).

210
211 We sought to determine the analytical limit-of-detection (LoD) of SHINEv2 following the guidance
212 released by the Food and Drug Administration (FDA)³⁸. Under this guidance, the LoD is defined
213 as the lowest concentration at which at least 19/20 technical replicates are detected. Using
214 SHINEv2 in lateral flow format, we tested 20 replicates of a dilution series of heat-inactivated
215 SARS-CoV-2 viral seedstock in UTM. We determined the analytical LoD of SHINEv2 to be 200
216 cp/ μ L (Fig. 1i and Supplementary Fig. 7), which is over an order of magnitude lower than that of
217 state-of-the-art antigen-capture tests and comparable to that of some equipment-based
218 isothermal nucleic acid tests^{39,40}. Given the optimal performance of SHINEv2 on contrived SARS-
219 CoV-2 samples, we sought to determine its performance on clinical samples.

220
221 *Assessing the clinical performance of SHINEv2 on NP swab samples.*

222 We assessed the clinical performance of SHINEv2 on 96 unextracted nasopharyngeal (NP) swab
223 samples from SARS-CoV-2 positive and negative patients, compared to three widely used FDA
224 EUA-authorized diagnostic tests. We first compared the performance of SHINEv2 to the CDC-
225 recommended RT-qPCR protocol for SARS-CoV-2, as the gold-standard reference test⁴¹. We
226 also compared SHINEv2 to two state-of-the-art antigen-capture tests: Abbott's BinaxNow COVID-
227 19 antigen self-test (BinaxNow) and Access Bio's CareStart COVID-19 antigen test
228 (CareStart)^{42,43}. The BinaxNow test, the most widely used rapid test in the United States,
229 represents the cutting-edge in antigen-capture tests. As a second point of comparison, we
230 selected the CareStart test, which has the most similar workflow to SHINEv2 and has comparable
231 performance to BinaxNow.

232
233 We performed SHINEv2 testing side-by-side with the RT-qPCR, BinaxNow and CareStart tests
234 (Fig. 2a,b). RT-qPCR detected SARS-CoV-2 RNA in 63 samples out of a total of 96



235
 236 **Fig. 2 | Performance of SHINEv2 on clinical samples.** **a**, Schematic of side-by-side clinical
 237 sample testing using SHINEv2, BinaxNow, CareStart and RT-qPCR. NP, nasopharyngeal swab.
 238 **b**, SHINEv2, BinaxNow and CareStart test results for a subset of clinical NP swab samples with
 239 different C_t values (CDC EUA N1 RT-qPCR). C = control band; T = test band. No Amp., no
 240 amplification detected. For all test results, see *Supplementary Fig. 8-10*. **c**, Side-by-side clinical
 241 performance of SHINEv2, BinaxNow and CareStart versus RT-qPCR. **d**, Positive and negative
 242 test results for SHINEv2, BinaxNow and CareStart tests for RT-qPCR-positive clinical samples
 243 relative to viral RNA concentration and C_t value.
 244

245 samples tested, with cycle threshold (C_t) values ranging from 14.7 to 37.2 (Supplementary Table
246 1). SHINEv2 detected SARS-CoV-2 RNA in 9/9 RT-qPCR positive samples with $C_t < 25$ (100%
247 sensitivity), 32/34 samples with $C_t < 30$ (94.1% sensitivity), and 34/63 samples with $C_t < 40$ (54.0%
248 sensitivity), (Fig. 2c and Supplementary Fig. 8-10). Notably, our assay correctly identified all
249 positive samples with titers above our analytical LoD of 200 copies/ μ L and several samples below
250 this LoD. This confirmed that the clinical performance of SHINEv2 is equivalent to its analytical
251 performance.

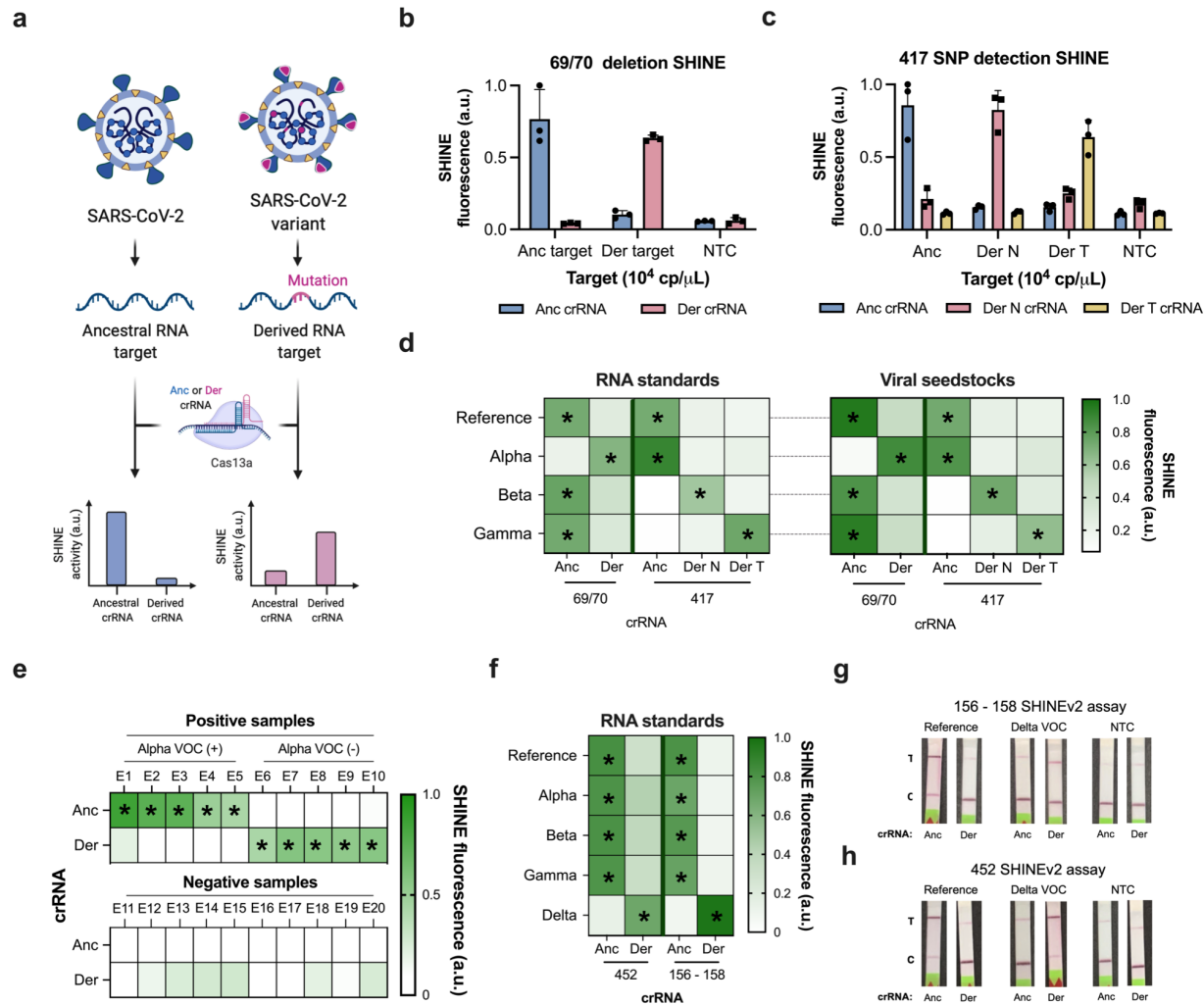
252
253 Moreover, SHINEv2 was 50 times more sensitive than either antigen-capture test⁴²⁻⁴⁵ (Fig. 2d).
254 SHINEv2 excelled at detecting samples with moderate viral loads (25-30 C_t), thereby allowing the
255 identification of potentially infectious individuals that antigen-capture tests would miss⁸⁻¹⁰.
256 Additionally, SHINEv2, BinaxNow and CareStart were shown to be highly specific, each having
257 no false positive results in any of the 33 RT-qPCR-negative samples (100% specificity) (Fig. 2c).
258 This is consistent with previously reported results for these antigen-capture tests^{42,43} and is also
259 expected for CRISPR-Dx, given that the RPA primers during amplification and the crRNA during
260 Cas13-based detection provide two layers of specificity¹⁵. Importantly, the sensitivity and
261 specificity of SHINEv2 on patient samples falls within the recommended range for population-
262 wide screening in settings of reopening^{10,46}.

263
264 *Design and testing of SHINEv2 assays for SARS-CoV-2 VOC identification*

265 The emergence of SARS-CoV-2 variants with increased transmissibility and virulence, and the
266 specificity and flexibility of SHINEv2, prompted us to develop assays to detect and distinguish
267 four widely circulating VOCs. Initially, we focused on detecting the 69/70 deletion in the S gene,
268 a hallmark of the Alpha VOC^{24,47}. Then, we expanded to the K417N and K417T mutations found
269 in the Beta and Gamma VOCs, respectively^{24,47}. Finally, in light of the rampant spread of the Delta
270 VOC in many countries around the world, we sought to rapidly develop new SHINEv2 assays to
271 detect the L452R mutation and 156/157 deletion + R158G mutation present in this variant^{47,48}.
272 We designed sets of crRNAs with differential activities on the reference RNA genome (ancestral
273 target) with respect to the RNA genome containing a given mutation (derived target), or vice versa
274 (Fig. 3a).

275
276 For the Alpha VOC, we manually designed a set of 10 ancestral and derived crRNAs to detect
277 the 69/70 deletion. We selected an ancestral crRNA and a derived crRNA based on Cas13
278 cleavage activity and their ability to discriminate between ancestral and derived RNA targets
279 (Supplementary Fig. 11). The 69/70 deletion falls within the region amplified by the RPA primers
280 from the S gene assay we previously developed (Supplementary Fig. 12). Using these primers,
281 the 69/70 assay successfully detected the 69/70 deletion in synthetic RNA targets
282 (Supplementary Fig. 13). For instance, on the ancestral RNA target, using the ancestral crRNA
283 led to 7x greater fluorescence than the derived crRNA (Fig. 3b). Conversely, on derived RNA
284 target, we observed 5x higher fluorescence with the derived crRNA than with the ancestral crRNA.
285

286 Next, we sought to design additional assays for the identification of the Beta and Gamma VOCs.
287 These variants contain distinct single nucleotide polymorphisms (SNPs) at amino acid



288
 289 **Fig. 3 | Development of SHINEv2 assays for the detection of SARS-CoV-2 VOCs.** **a**,
 290 Schematic of Cas13a-based detection of mutations in SARS-CoV-2 using a fluorescent readout;
 291 anc, ancestral; der, derived. **(b,c)**, SHINE fluorescence of the ancestral and derived crRNAs for
 292 the **(b)** 69/70 deletion assay and **(c)** on synthetic RNA targets after 90 minutes; NTC, no target
 293 control. **d**, Identification of SARS-CoV-2 VOCs using SHINE fluorescence on full-genome
 294 synthetic RNA standards and RNA extracted from viral seedstocks; target RNA concentration:
 295 10⁴ copies/μL. **e**, Mean fluorescence of 69/70 SHINEv2 assay on SARS-CoV-2 RNA extracted
 296 from clinical samples, after 90 minutes. **f**, Discrimination of SARS-CoV-2 VOCs using SHINE
 297 fluorescence of Delta assays on full-genome synthetic RNA standards, after 90 minutes. Target
 298 RNA concentration: 10⁴ genomes/μL. **(g,h)**, Colorimetric lateral flow based detection of full-
 299 genome synthetic RNA standards using the **(g)** 156 - 158 and **(h)** SHINEv2 assays. SHINEv2
 300 incubation time: 90 minutes. NTC, no target control. T, test line; C, control line. For **b** and **c**, center
 301 = mean and error bars = s.d. for 3 technical replicates. In **d,e** and **f**, the heatmap values represent
 302 the mean for 3 technical replicates.
 303

304 position 417 in the S gene (i.e. K417N for Beta and K417T for Gamma), none of which are present
305 on the reference genome or the Alpha VOC^{24,47}. However, discriminating between 3 different
306 codons in the same amino acid position using point-of-care diagnostics is remarkably difficult and,
307 to our knowledge, has not been reported to date. We used a computational design technique (see
308 Methods; manuscript *in prep.*) to generate crRNAs that maximize predicted SNP discrimination
309 at position 417, and used ADAPT³⁶ to help in designing RPA primers for these crRNAs. After
310 assay optimization, we demonstrated that our 417 assays can differentiate between the three
311 genotypes with high accuracy (Fig. 3c and Supplementary Fig. 14). When tested on synthetic
312 RNA targets, each 417 assay was shown to be at least 3x more active (i.e. higher fluorescence)
313 on its preferred target than on the non-preferred targets (Fig. 3c).

314
315 Having demonstrated the sensitivity and specificity of our 69/70 and 417 assays on short synthetic
316 RNA targets, we next tested their performance on full genome synthetic RNA standards and on
317 RNA extracted from SARS-CoV-2 viral seedstocks. In each case, these assays correctly identified
318 the presence or absence of the mutations (Fig. 3d). For the Alpha VOC, the fluorescence signal
319 of the derived crRNA was over 3x higher than that of the ancestral crRNA, indicating the presence
320 of the 69/70 deletion. Conversely, the fluorescence signal for the ancestral crRNA was 1.25-3.5x
321 higher for the reference strain as well as the Beta and Gamma VOCs, which confirmed the
322 absence of the S gene deletion in these cases. Similarly, the genotype of each variant was
323 correctly identified with the 417 assays, based on which crRNA showed the highest activity (Fig
324 3d). For example, for the Gamma VOC, the derived T crRNA led to the highest fluorescence level,
325 thereby correctly identifying the presence of the K417T SNP in this variant.

326
327 Given the promising performance of these assays, we sought to assess their capacity to detect
328 SARS-CoV-2 VOCs in clinical samples. We focused on validating the Alpha VOC assay, the only
329 assay for which we had access to clinical samples, and reasoned that the results obtained could
330 be extended to the other assays. We assessed the clinical performance of the 69/70 assay on
331 RNA extracted from 20 NP swab samples. SHINEv2 correctly identified the presence of the 69/70
332 deletion in the Alpha VOC-positive samples (Ct values 23 - 29), as indicated by the higher
333 fluorescence with the derived crRNA in samples E6 to E10 (Fig. 3e). In addition, the 69/70 deletion
334 assay showed excellent specificity (100%), detecting all RT-qPCR positive samples and none of
335 the RT-qPCR negative samples (Fig. 3e).

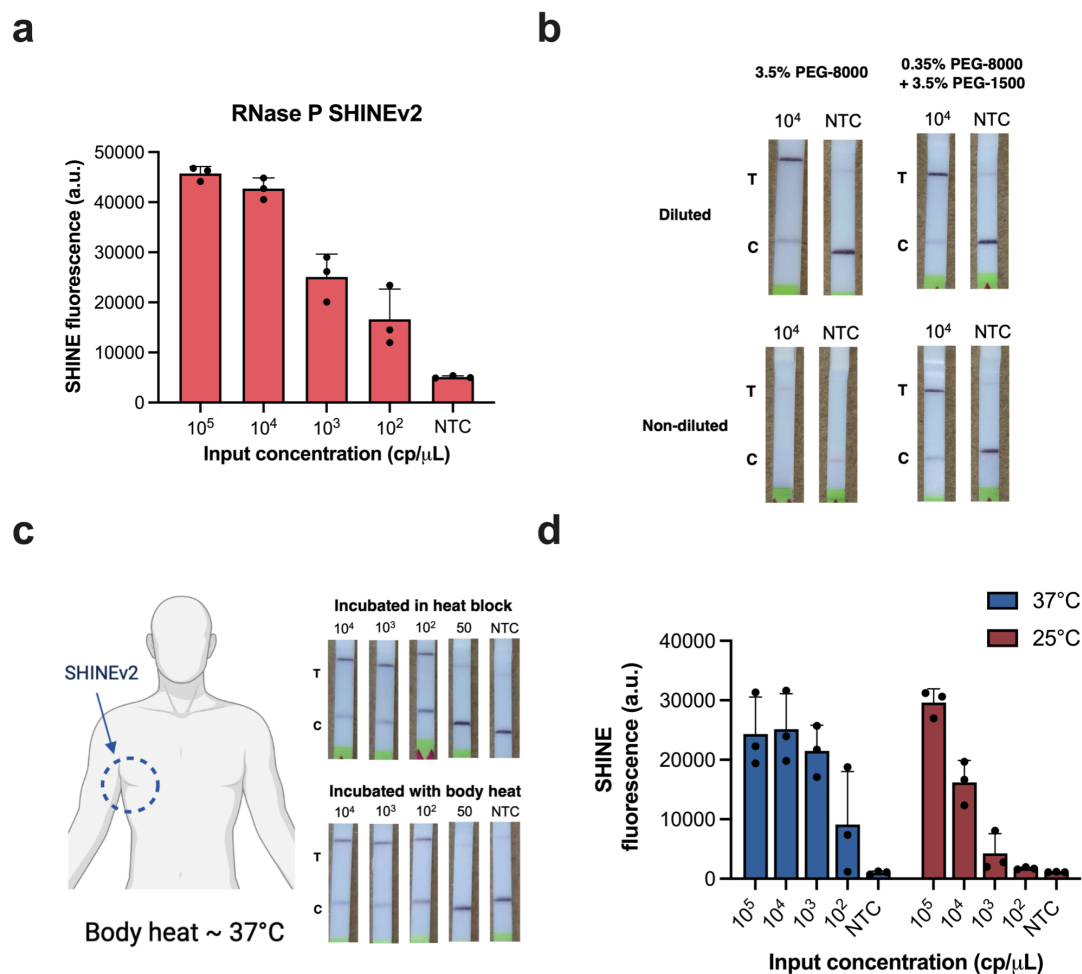
336
337 We further assessed the performance of the 69/70 assay on contrived unextracted samples. To
338 emulate unextracted NP swab samples, we spiked in heat-inactivated viral seedstocks (reference
339 strain or Alpha VOC) into SARS-CoV-2 negative clinical samples. Using a colorimetric lateral flow
340 based readout, SHINEv2 correctly identified the presence or absence of the alpha VOC in the
341 range of viral RNA concentrations (10^5 - 10^3 cp/ μ L) tested (Supplementary Fig. 15). The presence
342 of a test band for the ancestral crRNA and lack of it for the derived crRNA confirmed the presence
343 of the 69/70 deletion in Alpha VOC positive contrived samples with lower viral RNA concentrations
344 (10^4 and 10^3 cp/ μ L). At higher viral RNA concentrations (10^5 cp/ μ L), the presence or absence of
345 the deletion was determined by the difference in test band intensity between the ancestral and
346 derived crRNAs. Altogether, these data indicate that SHINEv2 can identify SARS-CoV-2 VOCs in
347 clinical samples.

348
349 SHINEv2 is a flexible technology that can be rapidly adapted to detect new viral variants. This
350 allowed us to develop new SHINEv2 assays for the 452 and 156 - 158 mutations present in the
351 Delta VOC, a VOC that arose as we were preparing this manuscript for publication. We designed
352 a set of crRNAs and RPA primers for each assay as described above, and evaluated their
353 performance on full genome synthetic RNA standards. Both assays successfully detected the
354 mutations in synthetic RNA targets with high sensitivity and specificity (Supplementary Fig. 16).
355 The 156-158 assay, in particular, displayed excellent specificity. Both the ancestral and derived
356 crRNAs detected their respective targets with high accuracy while showing no detection for the
357 opposite target (Supplementary Fig. 16b). Next, we examined the compatibility of these assays
358 with lyophilization and ambient-temperature sample processing. These SHINEv2 assays showed
359 excellent specificity on full genome synthetic RNA standards, clearly distinguishing the Delta VOC
360 from the reference strain and the other VOCs (Fig 3f). The 452 and 156-158 assays correctly
361 identified the genotype of each variant tested, based on the differential fluorescence activity of
362 each crRNA pair. Additionally, both SHINEv2 assays correctly identified the presence or absence
363 of each mutation using a colorimetric lateral flow based readout (Fig. 3g and 3h). All in all, we
364 have developed mutation-specific SHINEv2 assays which are widely deployable and, in
365 combination, can effectively discriminate between four widely circulating SARS-CoV-2 VOCs.

366
367 *Towards at home SHINEv2 testing*
368 SHINEv2 increases the accessibility of CRISPR-based SARS-CoV-2 diagnostics, and enables
369 VOC identification outside of laboratory settings. However, further improvements are necessary
370 for SHINEv2 to be used in virtually any environment. For example, the incorporation of internal
371 controls or further simplifying the assay readout could greatly expand the use cases of SHINEv2,
372 and enable its use in at home settings.

373
374 Nucleic acid diagnostic tests often require an internal control as part of the regulatory approval
375 process. Therefore, we designed and developed a Cas12-based assay for human RNase P, to
376 serve as an internal control for SHINEv2. After optimizing the concentration of RPA primers, our
377 RNase P assay was able to detect synthetic DNA down to 100 copies/ μ L (Supplementary Fig.
378 17a and 17b). Next, we confirmed that this Cas12-based assay was compatible with the FastAmp
379 lysis solution and with lyophilization. In this format, the Cas12-based RNase P assay detected
380 synthetic DNA target down to 100 copies/ μ L using a fluorescence-based readout, which supports
381 its potential use as an internal control for SHINEv2 (Fig. 4a). Therefore, we sought to determine
382 the compatibility of the Cas12-based RNase P assay with the Cas13-based S gene assay and
383 evaluate the performance of this duplex assay on synthetic nucleic acid targets. The duplex
384 SHINEv2 assay successfully detected both targets, with an LOD of 10^3 copies/ μ L for each target
385 (Supplementary Fig. 17c). Although additional improvements will be required to achieve clinically-
386 relevant sensitivity with the duplex SHINEv2 assay, we have taken steps towards incorporating
387 an internal control for SHINEv2. Moreover, our results confirmed that the improvements made to
388 SHINEv2 are applicable to other systems, including in Cas12-based assays and potentially in
389 other CRISPR-Dx.

390



391
 392 **Fig. 4 | Enhancing the accessibility of SHINEv2.** a, SHINE fluorescence of the RNase P
 393 SHINEv2 assay on synthetic DNA target after 90 minutes; NTC, no target control. b, Lateral flow
 394 based detection of SARS-CoV-2 RNA using SHINEv2 with different polyethylene glycol (i.e. PEG)
 395 compositions; with or without dilution after a 90 minute incubation. NTC, no target control. c,
 396 Lateral flow based SHINEv2 detection of SARS-CoV-2 RNA after a 90 minute incubation in a heat
 397 block or using body heat (underarm incubation). NTC, no target control. d, SHINE fluorescence
 398 on SARS-CoV-2 RNA after 90 minutes at 37°C or 25°C; NTC, no target control. For d, center =
 399 mean and error bars = s.d. for 3 technical replicates.
 400

401 Moreover, the accessibility of SHINEv2 could also be enhanced by further simplifying the assay.
402 The lateral flow based readout for CRISPR-Dx, the preferred option for point-of-need
403 implementation, requires a buffer to be added after the detection reaction is performed, increasing
404 the risk of sample contamination and adding a liquid-handling step for the user. Directly inserting
405 the paper strip into the SHINEv2 reaction would significantly reduce this risk. However, undiluted
406 SHINEv2 reactions block the flow of nanoparticles through the paper strip, resulting in a failed
407 test (Fig. 4b). We reasoned that high molecular weight PEG – an indispensable crowding agent
408 for RPA – could be responsible for the lack of flow. We sought to identify a combination of PEGs
409 with different molecular weights that enables flow while retaining SHINEv2's activity. In the
410 absence of RNA targets, lowering the concentration and molecular weight of PEG in SHINEv2
411 reactions facilitated the flow of nanoparticles through the paper strip (Supplementary Fig. 18a).
412 However, the sensitivity of SHINEv2 was markedly reduced when lowering the concentration of
413 PEG-8000 in the reaction (Supplementary Fig. 19). Using a combination of 0.35% PEG-8000 and
414 3.5% PEG-1500, undiluted SHINEv2 was able to detect SARS-CoV-2 RNA without a drop in
415 performance while retaining flow (Figure 4b and Supplementary Fig. 18b). Thus, we managed to
416 eliminate a liquid handling step in SHINEv2 and simplify the test readout.

417
418 In addition, SHINEv2 can be run without equipment due to its minimal heating requirements.
419 SHINE's single 90-minute incubation step at 37°C can be easily maintained with low-cost and
420 energy-efficient heat blocks. However, the removal of all heating elements would enable the
421 unrestricted deployment of SHINEv2. Using body temperature, which is at approximately 37°C,
422 could provide an alternative for fully equipment-free SHINEv2 testing⁴⁹. Indeed, the underarm
423 incubation of SHINEv2 was successful at detecting down to 100 cp/μL of SARS-CoV-2 RNA, and
424 with a similar efficiency to SHINEv2 incubated in a heat block (Fig. 4c). An ideal test would perform
425 with similar efficiency at ambient temperature as well. We examined the performance of SHINEv2
426 at 25°C using both a plate-based fluorescence readout and a colorimetric paper-based readout.
427 SHINEv2 was able to detect SARS-CoV-2 RNA at 25°C with the plate reader, albeit with lower
428 speed and sensitivity than at 37°C (Fig. 4d). We observed a larger drop in sensitivity with the
429 lateral flow based readout (Supplementary Fig. 20). We believe the drop in sensitivity was caused
430 by a decline in the performance of RPA at lower temperatures⁵⁰, since Cas13 detection alone
431 performed well at 25°C and 37°C (Supplementary Fig. 21). Collectively, these improvements
432 increase the accessibility of SHINEv2 and facilitate its use in almost any situation.

433 434 **Discussion**

435 Here we present SHINEv2, a widely deployable CRISPR-Dx for SARS-CoV-2 RNA detection and
436 VOC identification from unextracted samples with a straightforward workflow. Previously
437 developed CRISPR-Dx are not well poised for widespread deployment, as they are often too
438 complex and/or require a cold chain and auxiliary equipment^{15,20,32}. SHINEv2 addresses these
439 limitations. Lyophilization considerably simplifies the assay and facilitates its transportation and
440 storage. SHINEv2 can be distributed overseas without a loss in performance. Moreover, the use
441 of an equipment-free and ambient-temperature sample lysis method further increases the user-
442 friendliness of the assay. SHINEv2 involves as few steps from the user as antigen-capture tests,
443 while providing a 50-fold boost in sensitivity^{7,42,43}. Importantly, SHINEv2 demonstrates perfect

444 (100%) concordance with RT-qPCR, the gold standard for SARS-CoV-2 diagnosis, in samples
445 with RNA levels above our analytical LoD of 200 copies/ μ L. This level of sensitivity could enable
446 the detection of every potentially infectious individual, including those missed by antigen-capture
447 tests^{10,46}.

448
449 SHINEv2 can accurately identify several clade-specific mutations in the Alpha, Beta, Gamma and
450 Delta SARS-CoV-2 VOCs, and it can be rapidly adapted to respond to emerging viral variants as
451 well as other viruses in current and future outbreaks. Thus, SHINEv2 can provide critical
452 information to inform public health responses, and it fills a major gap in point-of-need diagnostics.
453 At the population level, SHINEv2 could be used to prioritize testing and vaccine rollout in highly-
454 affected communities or to select subsets of samples for further viral sequencing. SHINEv2 could
455 also assist clinicians in selecting the right treatment (e.g. monoclonal antibody cocktails) for
456 patients with severe COVID-19. Altogether, we believe that SHINEv2 will be especially valuable
457 for community surveillance testing, as it combines user-friendly and equipment-free preparation
458 methods with sufficient sensitivity and the capacity to discriminate VOCs.

459
460 Further advances will be required for CRISPR-based diagnostic testing to take place in any
461 location, including at-home settings. Ideally, such a test would involve a few simple, ambient-
462 temperature steps and provide a fast and accurate visual readout without the need for specialized
463 equipment. To our knowledge, current nucleic acid diagnostics are not capable of meeting all
464 these requirements simultaneously. The combination of sample processing, nucleic acid
465 amplification and CRISPR-based detection into a single, ambient-temperature reaction could
466 remove liquid handling steps. The incorporation of solution-based, colorimetric readouts could
467 further simplify the assay and reduce the risk of contamination⁵¹. Moreover, with SHINEv2, we
468 have taken steps to eliminate all equipment needs, although additional advances will be required
469 to boost SHINEv2's performance at ambient temperature. The speed and sensitivity of ambient-
470 temperature CRISPR-Dx could be enhanced through the addition of auxiliary proteins or using
471 alternative isothermal amplification methods^{18,21,52}. Collectively, these improvements could greatly
472 enhance the accessibility of SHINEv2 and provide a critical tool in the fight against current and
473 future outbreaks. By reducing assay complexity and simplifying test distribution, without
474 sacrificing sensitivity or specificity, we have taken steps towards the development of such a
475 diagnostic tool.

476

477 **Methods**

478

479 *Clinical samples and ethics statement*

480 This study received a non-human subjects research determination from the Broad Institute Office
481 of Research Subject Protections (NHSR-4318). To conduct this research, de-identified
482 nasopharyngeal swab patient samples were purchased from Boca Biolistics (USA). Additional de-
483 identified clinical samples were obtained from studies evaluated and approved at the Center for
484 Disease Control and Prevention (CDC). This activity was reviewed by CDC and was conducted
485 consistent with applicable federal law and CDC policy (See e.g., 45 C.F.R. part 46; 21 definition
486 of research as defined in and was conducted consistent with C.F.R. part 56; 42 U.S.C. 241(d),
487 46.102(l) but did not involve human applicable federal law and CDC U.S.C. 552a, 44 U.S.C. 3501
488 et seq.)

489

490 *SARS-CoV-2 assay design and synthetic template information*

491 RPA primers were designed using ADAPT³⁶. The S gene, which contains SNPs of interest in
492 several SARS-CoV-2 VOCs, was extracted from an alignment of 308,315 SARS-CoV-2 genomes
493 downloaded from GISAID in January 2021^{53,54}. ADAPT was then run on the S gene alignment
494 with the following parameters: 35-65% GC content, max. 1 mismatch between the primer and
495 target, full coverage in >98% of the genomes and overlap allowed between amplicons but not
496 primers. Forward RPA primers were ordered with a T7 promoter sequence (5'-
497 GAAATTAATACGACTCACTATAGGG-3') appended upstream; and both forward and reverse
498 RPA primers were purchased from Integrated DNA Technologies (IDT) as single-stranded DNA
499 oligos.

500

501 The CRISPR RNAs (crRNAs) for SNP discrimination were designed using a generative sequence
502 design algorithm⁵⁵. This approach uses ADAPT's predictive model to predict the activity of
503 candidate crRNA sequences against on-target and off-target sequences³⁶. These predictions of
504 candidate crRNA activity guide the generative algorithm's optimization process, in which it seeks
505 to design crRNA probes that have maximal predicted on-target activity and minimal predicted off-
506 target activity. These crRNAs were purchased from IDT as Alt-R CRISPR guide RNAs.

507

508 Synthetic DNA targets with an upstream T7 promoter sequence were ordered as double-stranded
509 DNA (dsDNA) gene fragments from IDT, and were *in vitro* transcribed to generate synthetic RNA
510 targets. *In vitro* transcription was conducted as previously described²³. In brief, the dsDNA
511 template was transcribed using the HiScribe T7 High Yield RNA Synthesis Kit (New England
512 Biolabs (NEB)) for 2 hours at 37 °C. Transcribed RNA was then treated with RNase-free DNase
513 I (QIAGEN) according to the manufacturer's instructions. Finally, DNase-treated RNA was purified
514 using RNAClean SPRI XP beads using a 10:3:5 mixture of RNA solution, isopropanol and ethanol.

515

516 Sequence information for the synthetic targets, RPA primers, Cas13-crRNA and reporters is listed
517 in Supplementary Table 2.

518

519 *Full-genome synthetic RNA controls and viral seedstocks*

520 Synthetic SARS-CoV-2 RNA controls (controls 2, 14, 16, 17 and 23) were purchased from Twist
521 Biosciences and are referred to as full genome synthetic RNA standards.

522 Heat-inactivated viral stocks (2019-nCoV/USA-WA1/2020 as reference strain and
523 USA/CA_CDC_5574/2020 as Alpha VOC) were purchased from ATCC.

524

525 *Analysis of crRNA activity with Cas13a*

526 Initial measurements of crRNA activity were obtained under simplified SHINE conditions. That is,
527 crRNA activity was measured using 1X optimized reaction buffer (20 mM HEPES pH 8.0 with 60
528 mM KCl and 3.5% PEG-8000), 45 nM *LwaCas13a* protein, 125 nM polyU [*i.e.*, 6 uracils (6U) in
529 length] quenched FAM reporter, 1 U/ μ L murine RNase inhibitor, 22.5 nM crRNA and 14mM
530 magnesium acetate. Target RNA was added to each reaction at a ratio of 1:20 and fluorescence
531 was measured on a Biotek Cytation 5 plate reader (excitation: 485 nm, emission: 520 nm) at 37
532 °C every 5 minutes for up to 3 hours.

533

534 *Nuclease inactivation with FastAmp Lysis reagent*

535 A concentrated (5X) version of FastAmp Viral and Cell solution B for Covid-19 Testing was
536 purchased directly from Intact Genomics. This solution, referred to as FastAmp Lysis reagent,
537 was used at a final concentration of 1X. For nuclease inactivation experiments, 10% healthy nasal
538 fluid (Lee BioSolutions) in universal transport medium (UTM) was mixed with 1X lysis reagent and
539 either 1%, 2% or 5% (v/v) murine RNase inhibitor (NEB). HUDSON treatment was used as a
540 control for successful RNase inactivation and performed as previously described²³. In short, 100
541 mM TCEP (Thermo Fisher Scientific), 1 mM EDTA (Thermo Fisher Scientific) and 0.8 U/ μ L murine
542 RNase inhibitor were added to the nasal fluid/UTM mix and incubated at 40 °C for 5 minutes. PBS
543 was used instead of HUDSON reagents as a no-treatment control. All treated products were then
544 mixed 1:1 with 400 mM RNaseAlertv2 substrate (Thermo Fisher Scientific) in nuclease-free water
545 and incubated at 25°C, while measuring the fluorescence (excitation: 485 nm, emission: 520 nm)
546 every 5 minutes for up to 30 minutes using a Biotek Cytation 5 plate reader.

547

548 *Viral inactivation with Intact Genomics Lysis buffer*

549 2019-nCoV/USA-WA1-A12/2020 isolate of SARS-CoV-2 was obtained from the US Centers for
550 Disease Control and Prevention (CDC). At the Integrated Research Facility (IRF) - Frederick, the
551 virus was passaged by inoculating grivet kidney epithelial Vero cells (ATCC #CCL-81) at a
552 multiplicity of infection (MOI) of 0.01 under high containment (BSL-3) conditions. Infected cells
553 were incubated for 48 or 72 hours in Dulbecco's Modified Eagle Medium with 4.5g/L D-glucose,
554 L-glutamine, and 110 mg/L sodium pyruvate (DMEM, Gibco) containing 2% heat-inactivated fetal
555 bovine serum (SAFC Biosciences) in a humidified atmosphere at 37°C with 5% CO₂. The resulting
556 viral seedstock was harvested and quantified by plaque assay using Vero E6 cells (ATCC #CRL-
557 1586) with a 2.5% Avicel overlay and stained after 48 hours with a 0.2% crystal violet stain.

558

559 For testing the viral inactivation capacity of FastAmp Lysis reagent, the viral stock (10⁷ PFU/mL)
560 was mixed with 1X lysis buffer supplemented with 5% (v/v) murine RNase inhibitor and incubated
561 at ambient temperature for either A) 5 minutes, B) 20 minutes or C) 20 minutes followed by 10
562 minutes at 65°C. A no-treatment (PBS) control was also included. Treated viral stocks were then

563 cleaned and quantified by plaque assay using Vero E6 cells (ATCC #CRL-1586) with a 2.5%
564 Avicel overlay and stained after 48 hours with a 0.2% crystal violet stain.

565

566 *Single-step SARS-CoV-2 SHINE reactions.*

567 RPA primer and crRNA optimizations were performed using the following SHINE conditions: 1X
568 original SHINE buffer (20 mM HEPES pH 8.0 with 60 mM KCl and 3.5% PEG-8000), 45 nM
569 *LwaCas13a* protein resuspended in 1X SB (such that the resuspended protein is at 2.26 μ M), 125
570 nM polyU quenched FAM reporter, 2 mM of each rNTP, 1 U/ μ L murine RNase inhibitor, 1 U/ μ L
571 NextGen T7 RNA polymerase, 0.1 U/ μ L RNase H (NEB), 2 U/ μ L Invitrogen SuperScript IV (SSIV)
572 reverse transcriptase (Thermo Fisher Scientific), an assay specific concentration of forward and
573 reverse RPA primers (detailed below), and 22.5 nM crRNA. Once the master mix is created, it is
574 used to resuspend the TwistAmp Basic Kit RPA pellets (1 lyophilized pellet per 102 μ L final master
575 mix volume). After that, magnesium acetate (as the only magnesium cofactor) is added at a final
576 concentration of 14 mM to generate the final master mix. Finally, a synthetic RNA target or a
577 sample was added to the complete master mix at a ratio of 1:19. Fluorescence kinetics were
578 measured on a Biotek Cytation 5 plate reader (excitation: 485 nm, emission: 520 nm) at 37 °C
579 every 5 minutes for up to 3 hours.

580

581 For lateral flow readout, the quenched FAM reporter was exchanged for a biotinylated FAM
582 reporter at a final concentration of 1 μ M. After incubation at 37°C for 90 minutes, the SHINE
583 reaction was diluted 1:4 in 80 μ L HybriDetect Assay Buffer (Milenia Biotec), and a HybriDetect 1
584 lateral flow strip was added. Test images were collected 5 minutes after the addition of the strip
585 with a smartphone camera.

586

587 RPA primer concentrations varied between assays, but the ratio of forward to reverse primers
588 was always kept to 1:1. For each assay, the concentration of forward and reverse RPA primer
589 was the following: 120nM for ORF1a and 417 assays; 160nM for 452 assay and 180nM for S
590 gene, 69/70 deletion and 156-158 assays.

591

592 *SHINE tolerance to samples inactivated with lysis solution*

593 Contrived nasal samples (10% (v/v) in UTM) were inactivated with lysis solution (FastAmp lysis
594 reagent with 5% RNase inhibitor) for 5 minutes at ambient temperature. SHINE reactions were
595 generated as described above, except a variable amount of water was replaced with inactivated
596 contrived nasal samples. Synthetic RNA target (with the concentration specified in the figure) was
597 added to the modified SHINE reactions at a ratio of 1:19. Plate-based fluorescence detection was
598 performed as described above. In these reactions, the inactivated sample volume comprised 0%
599 - 40% of the total volume.

600

601 *Lyophilization optimization for SHINE*

602 The original SHINE buffer was optimized to retain SHINE's activity after lyophilization. For
603 lyophilization, we generated the master mix as described above, except the 1X SHINE buffer was
604 substituted for 1X lyophilization buffer (composition described below) and magnesium acetate
605 was omitted. The mastermix was aliquoted, flash frozen in liquid nitrogen and lyophilized
606 overnight. The lyophilization buffers tested had the following composition: 1) SHINE (20 mM

607 HEPES pH 8.0 with 60 mM KCl and 3.5% PEG-8000); 2) SHINE without PEG and KCl (20 mM
608 HEPES pH 8.0), 3) SHINE with sucrose and mannitol (20 mM HEPES pH 8.0 with 60 mM KCl,
609 3.5% PEG-8000, 5% (w/v) sucrose and 150mM mannitol); 4) LYO (20 mM HEPES pH 8.0 with
610 5% w/v sucrose and 150mM mannitol). Lyophilized pellets were resuspended with a buffer
611 composed of magnesium acetate (14 mM final concentration) and whichever reagent was left out
612 of the original SHINE buffer (e.g. 60 mM KCl, 3.5% PEG-8000 and 14 mM magnesium acetate
613 for the LYO resuspension buffer (RB)). After resuspension, synthetic RNA target was added to
614 the resuspended SHINE reactions at a ratio of 1:19. Plate-based fluorescence detection or lateral
615 flow based detection were performed as described above.

616
617 Lyophilized SHINE pellets for stability testing were prepared using the LYO buffer as described
618 above. SHINE pellets were stored at room temperature, 4°C or -20°C for 4,7,14,21 or 28 prior to
619 resuspension and testing. For SHINEv2 testing in Nigeria, SHINE pellets were lyophilized in the
620 United States, shipped in dry ice to Nigeria and then resuspended and tested after a total of 6
621 weeks in storage.

622
623 *Side-by-side clinical sample testing*
624 Clinical samples were thawed on ice. SHINEv2, BinaxNow and CareStart testing as well as RNA
625 extractions and RT-qPCR were performed side-by-side, according to the manufacturer's
626 instructions (see details below). 96 nasopharyngeal swab samples were tested in batches of 24
627 samples.

628
629 Information on clinical samples, including C_t values, can be found in Supplementary Table 1.
630 Clinical sample test results in Supplementary Fig. 8 - 10.

631
632 *Clinical sample extractions and RT-qPCR*
633 Clinical sample extractions and RT-qPCR were performed according to the CDC's recommended
634 protocol⁴¹. Nucleic acid extractions for samples S1 - S96 were performed using the QIAamp Viral
635 RNA Mini Kit (Qiagen). The starting volume for extraction was 100 µL and extracted nucleic acid
636 was eluted into 100 µL of nuclease-free water. Extracted RNA was used immediately or stored at
637 -80C. Extracted RNA was tested for the presence of SARS-CoV-2 RNA using the CDC's SARS-
638 CoV-2 RT-qPCR assay (2019-nCoV CDC EUA Kit, IDT). RT-qPCR cycling conditions were as
639 follows: hold at 25°C for 2 min, reverse transcription at 50 °C for 15 min, polymerase activation at
640 95 °C for 2 min and 45 cycles with a denaturing step at 95 °C for 3 s followed by annealing and
641 elongation steps at 55 °C for 30 s. RT-qPCR was run on a QuantStudio 6 (Applied Biosystems)
642 and data were analyzed using the Standard Curve (SC) module of the Applied Biosystems
643 Analysis Software.

644
645 *SHINEv2 testing - clinical samples*
646 SHINEv2 reactions were prepared the day before testing and consist of 1X optimized
647 lyophilization buffer (20 mM HEPES pH 8.0 with 5% w/v sucrose and 150mM mannitol), 45 nM
648 LwaCas13a protein resuspended in 1X SB (such that the resuspended protein is at 2.26 µM), 1
649 µM lateral flow biotinylated FAM reporter, 2 mM of each rNTP, 1 U/µL murine RNase inhibitor, 1
650 U/µL NextGen T7 RNA polymerase, 0.1 U/µL RNase H (NEB), 2 U/µL Invitrogen SuperScript IV

651 (SSIV) reverse transcriptase (Thermo Fisher Scientific), 180nM forward and reverse RPA primers
652 and 22.5 nM crRNA. Once the master mix was created, it was used to resuspend the TwistAmp
653 Basic Kit lyophilized reaction components (1 lyophilized pellet per 102 μ L final master mix
654 volume). The mastermix was aliquoted into individual reactions (20 μ L final), flash frozen in liquid
655 nitrogen and lyophilized overnight.

656
657 The following day, clinical samples were mixed with lysis reagent (diluted to 1X) with 5% RNase
658 inhibitor and incubated at ambient temperature for 5 minutes. In the meantime, the lyophilized
659 SHINEv2 pellets were resuspended with Resuspension Buffer to 1X (60 mM KCl, 3.5% PEG-
660 8000 and 14mM magnesium acetate). Inactivated clinical samples were added to the
661 resuspended SHINEv2 reactions at a ratio of 1:9. After incubation at 37C for 90 minutes, the
662 SHINEv2 reaction was diluted in 80 μ L HybriDetect Assay Buffer, and a HybriDetect 1 lateral flow
663 strip was added. Test images were collected 5 minutes after the addition of the strip. Tests were
664 considered positive when the intensity of the test band was higher than that of the negative
665 controls.

666
667 *BinaxNow COVID - 19 Antigen self-test - clinical samples*
668 BinaxNow COVID-19 Antigen self-tests (Abbott) were purchased from Walmart. Since we were
669 not able to procure dry swabs, the test was performed according to the manufacturers' protocol
670 for determining the analytical sensitivity with a liquid input (Instructions For Use submitted for FDA
671 EUA)⁵⁶. Briefly, 6 drops of BinaxNow liquid were added to the top hole of each test card. 20 μ L of
672 clinical samples were absorbed onto a clinical swab. The swab was then inserted into the bottom
673 hole of the test card and turned 3 times. Without removing the swab, the test card was closed and
674 sealed. The test was incubated at room temperature for at least 15 minutes before imaging with
675 a smartphone camera. Any faint colored lines in the test region were considered as positive.

676
677 *CareStart COVID-19 Antigen testing - clinical samples*
678 CareStart COVID-19 Antigen tests (Access Bio) were purchased from Medek Health. The test
679 was performed according to the manufacturer instructions for use⁵⁷. Briefly, clinical samples were
680 mixed 1:1 with the extraction buffer. 3 drops of that mix were added onto the sample well in a test
681 cartridge and incubated at ambient temperature for at least 10 minutes before imaging with a
682 smartphone camera. As suggested by the manufacturer, any faint colored line in the test region
683 was considered as positive.

684
685 *Contrived samples testing (VOCs)*
686 Contrived samples were created by diluting a known concentration of heat-inactivated viral
687 seedstocks (reference strain or Alpha VOC) 1:9 in UTM. Contrived samples were then inactivated
688 with lysis solution for 5 minutes at ambient temperature. Lyophilized SHINEv2 pellets were
689 prepared and resuspended as described above. Inactivated samples were added to the
690 resuspended SHINEv2 reactions at a ratio of 1:9. After incubation at 37°C for 90 minutes, the
691 SHINEv2 reaction was diluted in 80 μ L HybriDetect Assay Buffer, and a Milenia HybriDetect 1
692 lateral flow strip was added. Test images were collected 5 minutes after the addition of the strip.

693
694 *Extracted clinical samples testing (VOCs)*

695 Clinical sample extractions and RT-qPCR were performed according to the CDC's recommended
696 protocol⁴¹. Nucleic acid extraction for samples E6 - E10 were performed with the MagnaPure 96
697 instrument using the DNA and viral SV kit (Roche). The starting volume for extraction was 100 μ L
698 and extracted nucleic acid was eluted into 100 μ L of nuclease-free water. Extracted RNA was
699 used immediately or stored at -80C. Extracted RNA was tested for the presence of SARS-CoV-2
700 RNA using the CDC's SARS-CoV-2 RT-qPCR assay (2019-nCoV CDC EUA Kit, IDT). RT-qPCR
701 cycling conditions were as follows: hold at 25°C for 2 min, reverse transcription at 50 °C for 15
702 min, polymerase activation at 95 °C for 2 min and 45 cycles with a denaturing step at 95 °C for 3
703 s followed by annealing and elongation steps at 55 °C for 30 s. RT-qPCR was run on a
704 QuantStudio 6 (Applied Biosystems) and data were analyzed using the Standard Curve (SC)
705 module of the Applied Biosystems Analysis Software.

706
707 RNA extracted from clinical samples was added to resuspended SHINEv2 reactions at a ratio of
708 1:9, as described above. After incubation at 37°C for 90 minutes, the SHINEv2 reaction was
709 diluted in 80 μ L HybriDetect Assay Buffer, and a HybriDetect 1 lateral flow strip was added. Test
710 images were collected 5 minutes after the addition of the strip.

711
712 *Cas12-based RNase P assay*
713 Cas12-based SHINEv2 reactions were prepared as follows: 1X original SHINE buffer or LYO
714 buffer, 20 nM *Lba*Cas12a, 250nM polyC [*i.e.*, 5 cytosines (5C) in length] quenched HEX reporter,
715 1 U/ μ L murine RNase inhibitor, 90nM of forward and reverse RPA primers, and 22.5 nM Cas12a
716 crRNA were combined to create a master mix. The master mix was used to resuspend the
717 TwistAmp Basic Kit RPA pellets (1 lyophilized pellet per 102 μ L final master mix volume). For
718 lyophilization, the magnesium acetate was omitted, and individual reactions were aliquoted, flash
719 frozen and lyophilized overnight. Otherwise, magnesium acetate (as the only magnesium
720 cofactor) was added at a final concentration of 14 mM. Lyophilized Cas12 reactions were
721 reactivated with Resuspension Buffer, as described above. Finally, synthetic DNA target was
722 added to the complete master mix at a ratio of 1:19. Fluorescence kinetics were measured on a
723 Biotek Cytation 5 plate reader (excitation: 485 nm, emission: 520 nm) at 37 °C every 5 minutes
724 for up to 3 hours. For lateral flow readout, the quenched HEX reporter was exchanged for a 5C
725 biotinylated FAM reporter at a final concentration of 1 μ M. After incubation at 37°C for 90 minutes,
726 the Cas12-based reaction was diluted in 80 μ L HybriDetect Assay Buffer, and a HybriDetect 1
727 lateral flow strip was added. Test images were collected 5 minutes after the addition of the strip.

728
729 *Duplex SHINEv2*
730 Duplex SARS-CoV-2 and RNase P SHINEv2 reactions were prepared as follows: 1X original
731 SHINE buffer or 1X LYO buffer, 45 nM *Lwa*Cas13a, 20nM *Lba*Cas12a, 125 nM polyU quenched
732 FAM reporter, 250nM polyC quenched HEX reporter, 2 mM of each rNTP, 1 U/ μ L murine RNase
733 inhibitor, 1 U/ μ L NextGen T7 RNA polymerase, 0.1 U/ μ L RNase H, 2 U/ μ L Invitrogen SuperScript
734 IV (SSIV) reverse transcriptase, 90nM S gene RPA primers, 90nM RNase P RPA primers, 22.5
735 nM Cas12a crRNA and 22.5 nM Cas13a crRNA were combined to create a master mix. The
736 mastermix was then used to resuspend the TwistAmp Basic Kit RPA pellets (1 lyophilized pellet
737 per 102 μ L final master mix volume). After that, pre- and post-lyophilization reactions were
738 performed as previously described.

739

740 *PEG experiments*

741 To evaluate the effect of PEG composition and concentration on flow through the lateral flow strip,
742 SHINEv2 reactions were prepared as described above, except the 3.5% PEG-8000 present in
743 the original SHINE buffer was exchanged for 0 - 10% PEG-8000, PEG-1500 or a combination of
744 the two. Without adding synthetic targets and with or without diluting in HybriDetect Assay Buffer,
745 a Milenia HybriDetect 1 lateral flow strip was added to these modified reactions. Test images were
746 collected 5 minutes after the addition of the strip.

747

748 To evaluate the performance of SHINEv2 with modified PEG composition, SHINEv2 reactions
749 were performed as described above, except the composition of the original SHINE and
750 resuspension buffers was modified to be SHINE (20 mM HEPES pH 8.0 with 60 mM KCl, 0.35%
751 PEG-8000 and 3.5% PEG-1500) and resuspension buffer (60 mM KCl, 0.35% PEG-8000, 3.5%
752 PEG-1500 and 14mM magnesium acetate).

753

754 *Body heat experiments*

755 Lyophilized SHINEv2 pellets were prepared and resuspended as described above. Full genome
756 synthetic RNA standards were added to individual SHINEv2 reactions and incubated using body
757 heat or a heat block for 90 minutes. For body heat experiments, SHINEv2 reactions were closed,
758 inserted in a ziploc bag, taped to the underarm of one of the researchers and incubated for 90
759 minutes. The reactions were then diluted in 80 μ L HybriDetect Assay Buffer, and a HybriDetect 1
760 lateral flow strip was added to each reaction. Test images were collected 5 minutes after the
761 addition of the strip.

762

763 *Room temperature experiments*

764 Lyophilized SHINEv2 pellets were prepared and later resuspended as described above. Contrived
765 nasal samples were inactivated with lysis solution and added to the resuspended SHINEv2
766 reactions at a ratio of 1:9. Fluorescence kinetics were measured on a Biotek Cytation 5 plate
767 reader (excitation: 485 nm, emission: 520 nm) every 5 minutes for up to 3 hours, at either 37 °C
768 or 25 °C.

769

770 *Data analysis and schematic generation*

771 SHINE fluorescence values are reported as background-subtracted fluorescence, with the
772 fluorescence value collected before reaction progression (usually $t = 10$ minutes) subtracted from
773 the final fluorescence value (90 minutes, unless otherwise indicated). Since the SHINEv2 VOC
774 data was collected using different plate-readers, SHINE fluorescence in Fig. 3 was normalized.
775 For normalization, the maximal fluorescence value in an experiment is set to 1 and the
776 fluorescence values from that same experiment are set as ratios of the max. fluorescence value.

777

778 Schematics shown in Fig. 1a, 1d, 2a, 3a, 4c and Supplementary Fig. 5 were created using
779 www.biorender.com. Data panels were primarily generated using Prism 8 (GraphPad).

780

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921
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935 936 **Contributions**

937 J.A.-S. and C.A.F. conceived this study under the guidance of P.C.S. and C.M. J.A.-S., A.B.,
938 C.K.B., C.A.F., M.E.G., T.-S.F.K.-T. and G.K. performed initial experiments to improve the SHINE
939 technology. G.K. and R.G. performed viral inactivation experiments, under the guidance of L.E.H.
940 J.A.-S., A.B., C.K.B. and M.E.G performed experiments and data analysis related to the S gene
941 assay and the development of SHINEv2. J.N.U, P.E.E and O.G.J. performed SHINEv2
942 experiments in Nigeria, under the supervision of C.T.H. J.A.-S., A.B., N.L.W, P.P.P. and S.M.
943 designed and/or tested SHINEv2 assays for SARS-CoV-2 variant detection. J.S.L. provided
944 assistance in patient sample collection. J.A.-S. and A.B performed and analyzed experiments with
945 patient samples. Y.B.Z. designed and validated Cas12-based SHINEv2 assays. L.E.H., C.T.H.
946 and J.J. provided critical insights on protocols, the experimental results, and the work overall.
947 J.A.-S. wrote the paper with guidance from P.C.S. and C.M. P.C.S and C.M. jointly supervised
948 the project. All authors reviewed the manuscript.

949

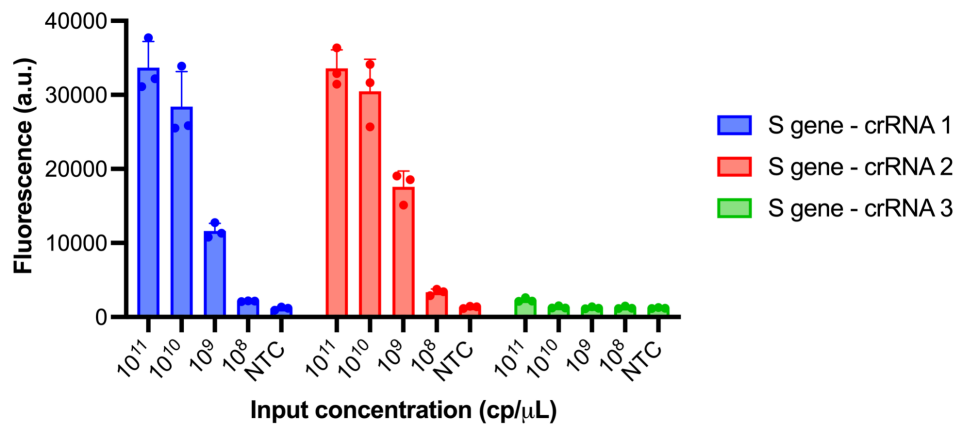
950 **Competing interests**

951 C.A.F., P.C.S., and C.M. are co-inventors on patent PCT/US2018/022764, which covers the
952 SHERLOCK and HUDSON technology for viral RNA detection held by the Broad Institute. J.A.-
953 S., C.A.F., P.C.S., and C.M. are co-inventors on a US Provisional Patent Application directed to
954 SHINE technology. P.C.S. is a co-founder of, shareholder in, and advisor to Sherlock Biosciences,
955 Inc., as well as a Board member of and shareholder in Danaher Corporation. All other authors
956 declare no competing interests.

957

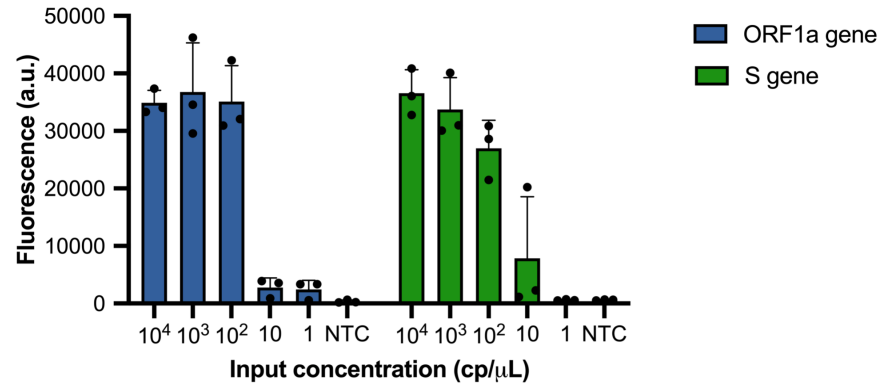
958 **Supplementary material**

959



960 **Supplementary Fig. 1 | Selection of most active S gene crRNA.** Cas13a detection of synthetic
961 RNA target (S gene) using different crRNAs. NTC, no target control. Centre = mean and error bar
962 = standard deviation (s.d.) for 3 technical replicates.

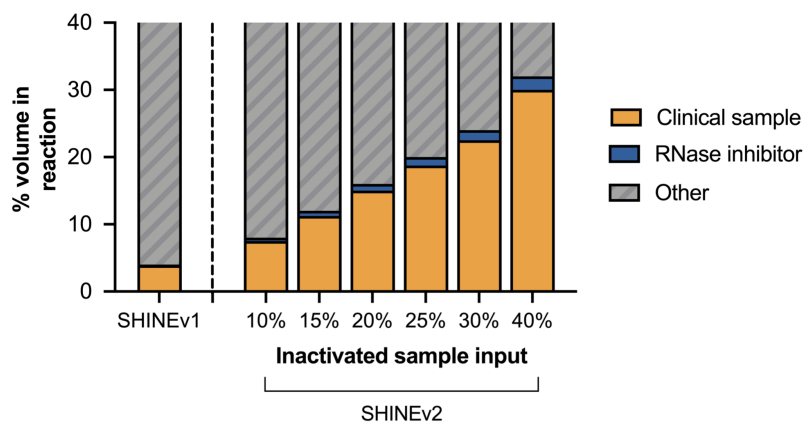
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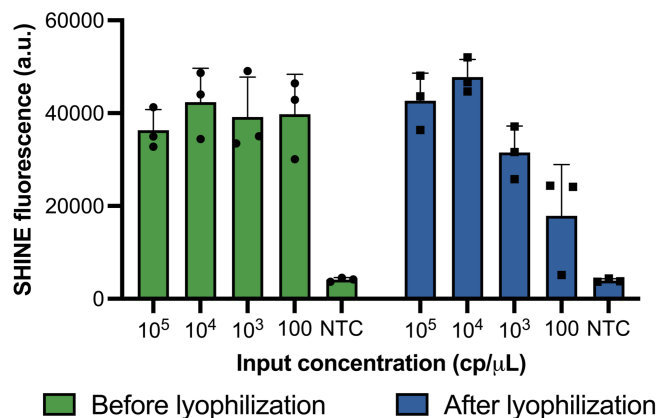
Supplementary Fig. 2 | SHINE assay comparison. Background-subtracted fluorescence of the ORF1a and S gene SHINE assays using synthetic SARS-CoV-2 RNA after 90 minutes. NTC, no target control. Center = mean and error bars = s.d. for 3 technical replicates.

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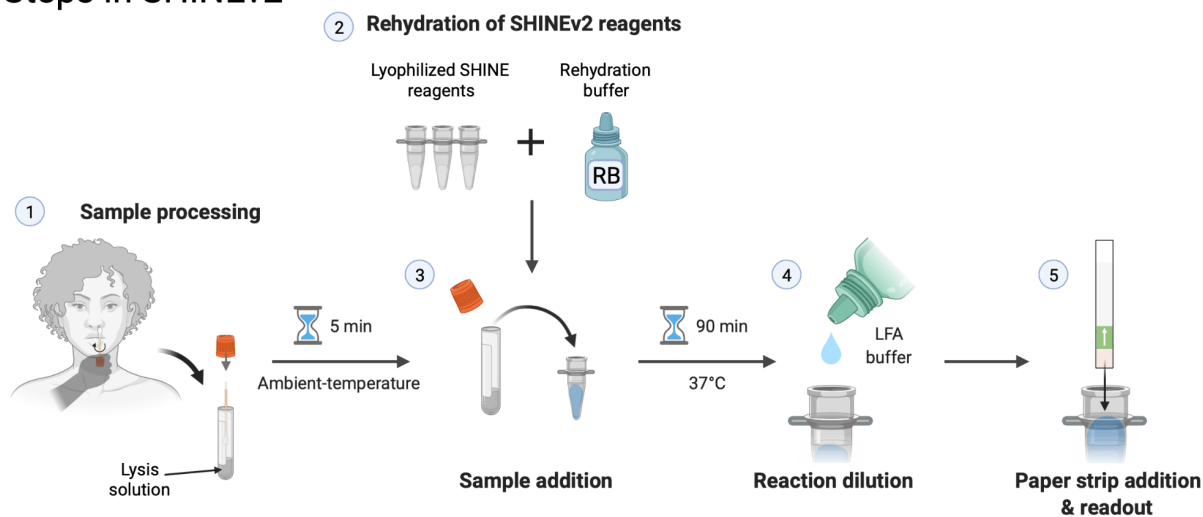
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Supplementary Fig. 3 | Inactivated sample input is increased in SHINEv2. Volume (%) of clinical sample and RNase inhibitors in the final reaction for SHINEv1 and SHINEv2 as a function of inactivated sample input.



976
977 **Supplementary Fig. 4 | The activity of SHINE is preserved after lyophilization.** SHINE
978 fluorescence on synthetic RNA target before and after lyophilization. Fluorescence measured
979 after 90 minutes. NTC, no target control. Center = mean and error bars = s.d. for 3 technical
980 replicates.

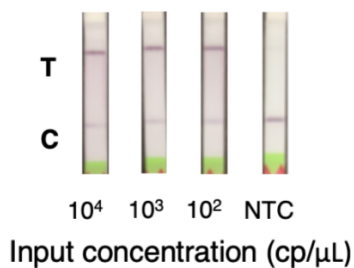
Steps in SHINEv2



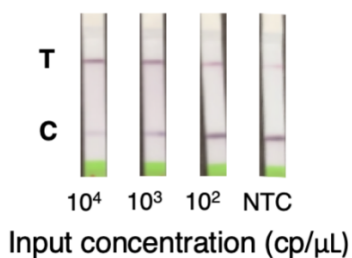
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982 **Supplementary Fig. 5 | SHINEv2 requires 5 user manipulations.** Schematic of the SHINEv2
983 workflow, including sample inactivation, rehydration of pellets, sample addition, reaction dilution
984 after incubation and result readout.
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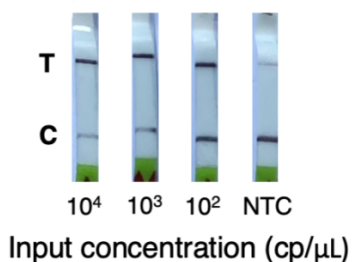
Broad Institute, USA. T = 0



Broad Institute, USA. T = 6 weeks



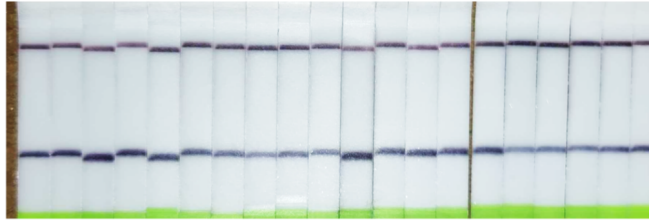
ACEGID, Nigeria. T = 6 weeks



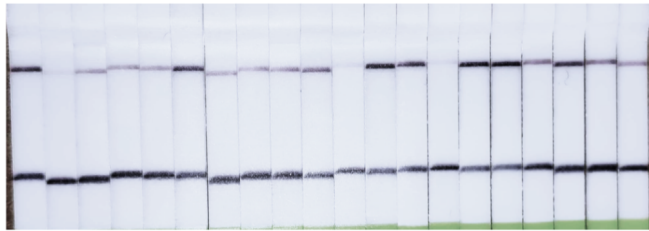
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Supplementary Fig. 6 | SHINEv2 can be transported overseas and is stable for at least 6 weeks. Lateral-flow detection of full-genome synthetic RNA standards in lysis solution-treated UTM using SHINEv2, before and after transportation. Incubated for 90 minutes. C = control band; T = test band; NTC = no target control.

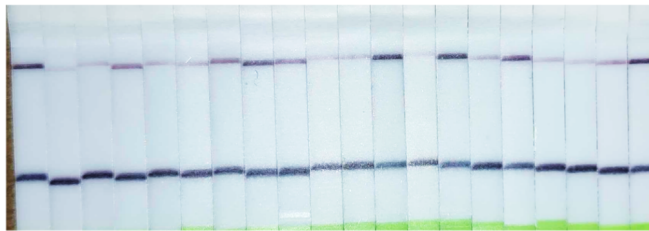
Target concentration: 500 cp/ μ L



Target concentration: 100 cp/ μ L



Target concentration: 50 cp/ μ L



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
























































































Supplementary Fig. 7 | SHINEv2 has an analytical sensitivity of 200cp/ μ L. Determination of analytical limit of detection with 20 replicates of SHINEv2 at different concentrations of SARS-CoV-2 RNA from lysis solution-treated contrived samples. Incubated for 90 minutes. Results for 200cp/ μ L sample input shown in Fig. 1i.

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Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
Test																
Sample	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32
Test																
Sample	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48
Test																
Sample	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61	S62	S63	S64
Test																
Sample	S65	S66	S67	S68	S69	S70	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80
Test																
Sample	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	S96
Test																

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Supplementary Fig. 8 | SHINEv2 results on clinical samples. Images of positive and negative test results with SHINEv2 on unextracted nasopharyngeal (NP) swab samples. Samples were incubated for 90 minutes at 37°C.

Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Test										
Sample	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
Test										
Sample	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30
Test										
Sample	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40
Test										
Sample	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50
Test										
Sample	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60
Test										
Sample	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70
Test										
Sample	S71	S72	S73	S74	S75	S76	S77	S78	S79	S80
Test										
Sample	S81	S82	S83	S84	S85	S86	S87	S88	S89	S90
Test										
Sample	S91	S92	S93	S94	S95	S96				
Test										

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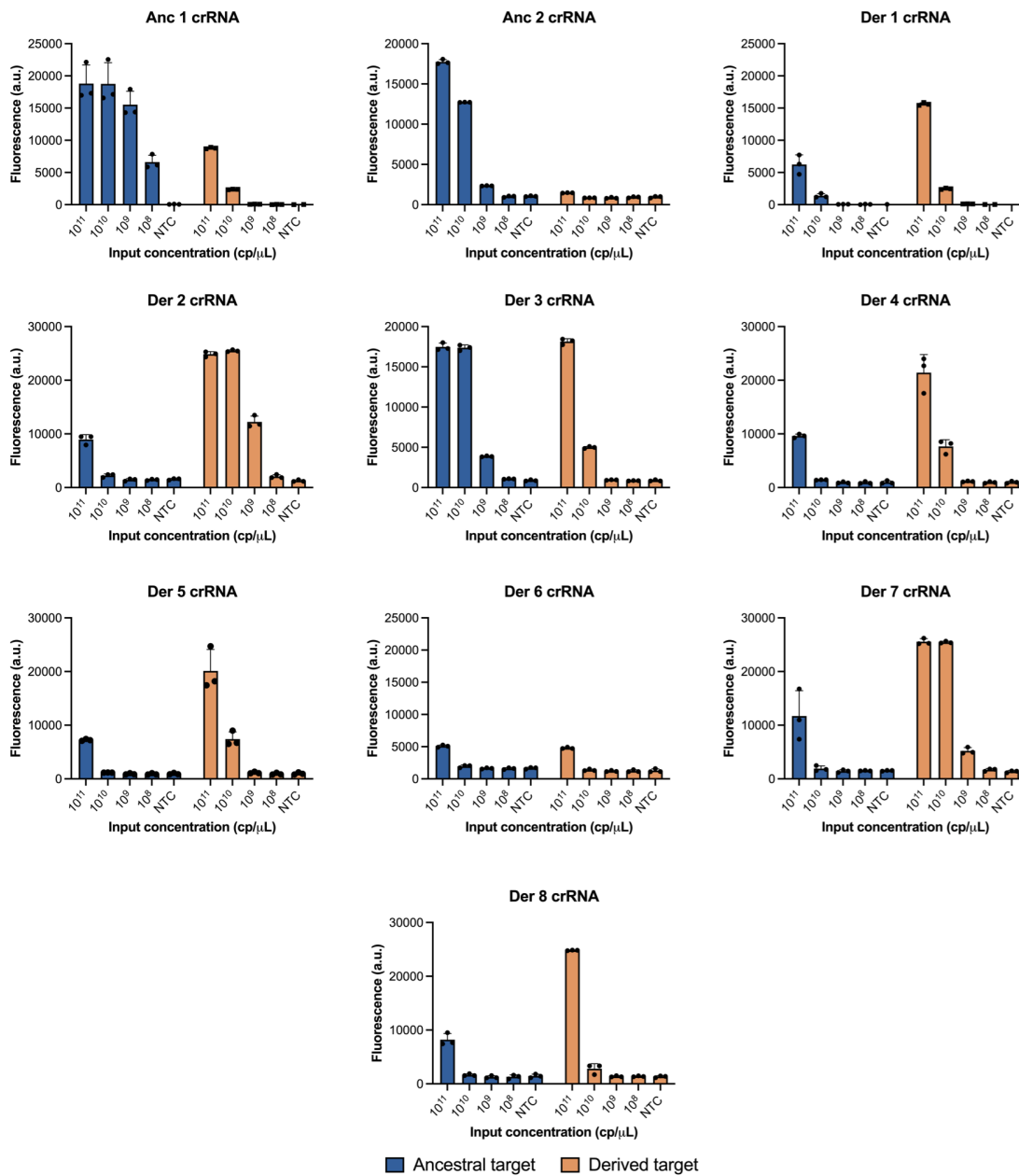
Supplementary Fig. 9 | BinaxNow results on clinical samples. Images of positive and negative test results with BinaxNow on unextracted NP swab samples.

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Sample	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
Test												
Sample	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24
Test												
Sample	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36
Test												
Sample	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48
Test												
Sample	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60
Test												
Sample	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71	S72
Test												
Sample	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83	S84
Test												
Sample	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95	S96
Test												

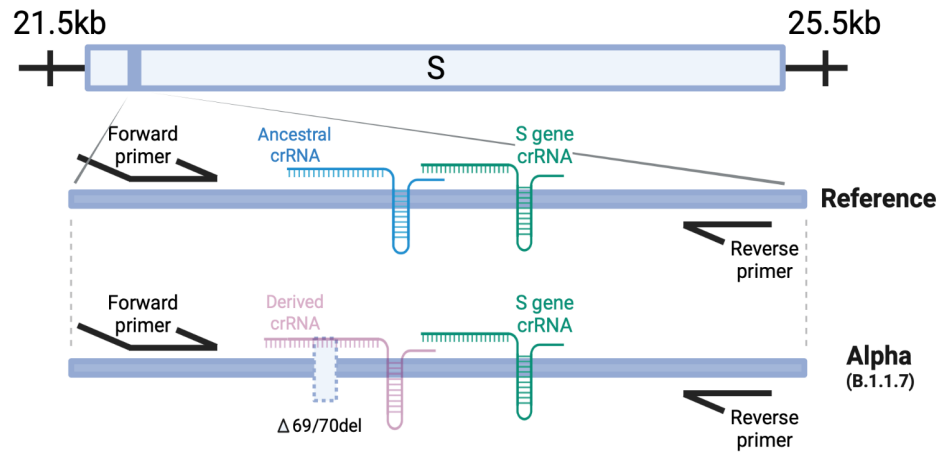
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Supplementary Fig. 10 | CareStart results on clinical samples. Images of positive and negative test results with CareStart on unextracted nasopharyngeal NP swab samples.



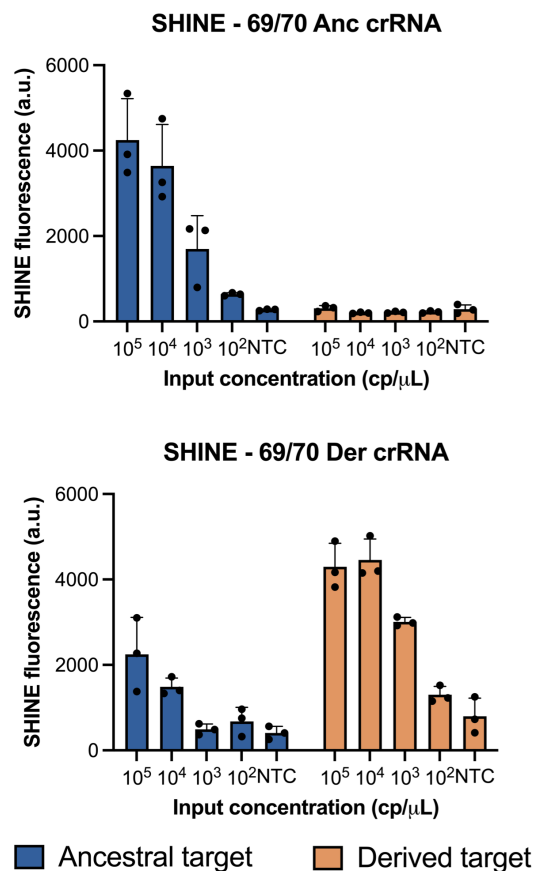
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 1015 **Supplementary Fig. 11 | Selection of ancestral (anc) and derived (der) crRNAs for the S**
 1016 **gene 69/70 deletion assay.** Cas13a detection of ancestral and derived synthetic RNA targets
 1017 with different anc and der crRNAs. NTC, no target control. Centre = mean and error bar = standard
 1018 deviation (s.d.) for 3 technical replicates.
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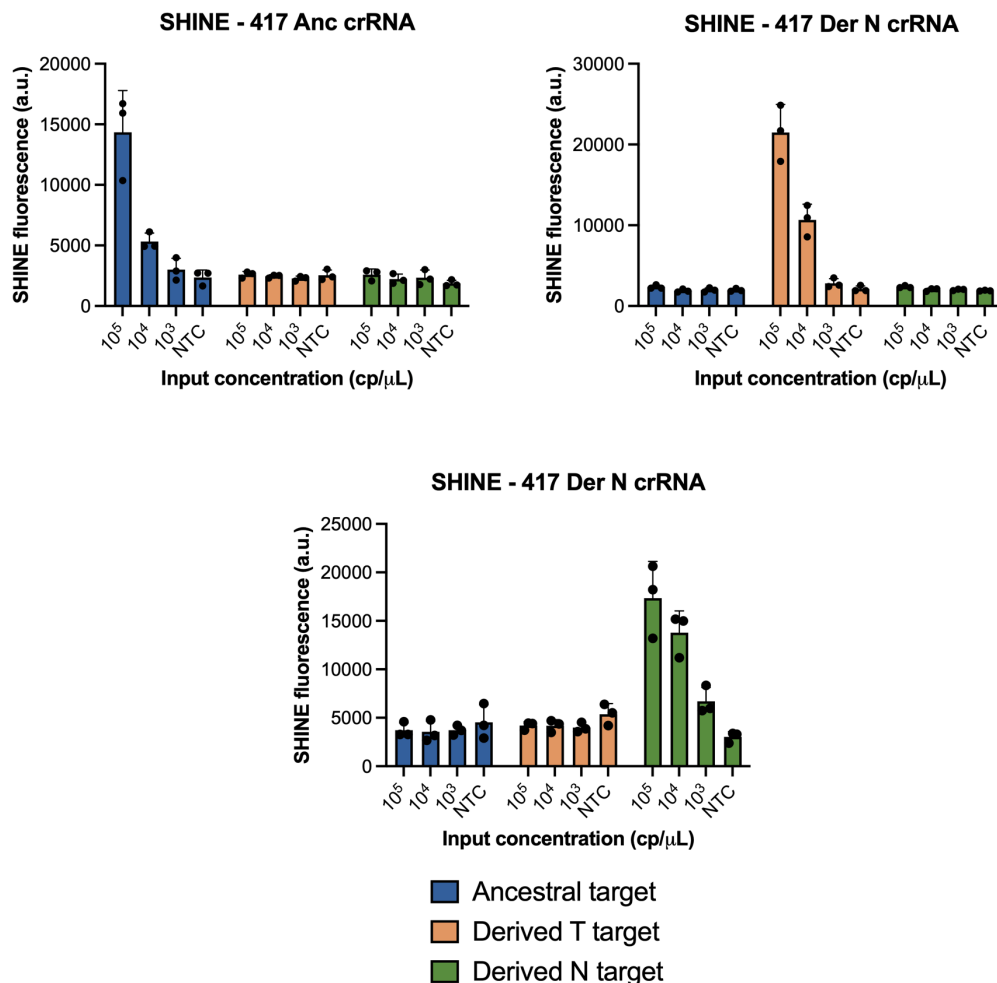


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Supplementary Fig. 12 | Location of the SHINEv2 assay for the 69/70 deletion detection. Schematic of location of the S gene and 69/70 deletion SHINEv2 assays within the S gene of SARS-CoV-2 for the reference strain and Alpha VOC. Dashed rectangle, deletion at amino acid positions 69 and 70 ($\Delta 69/70\text{del}$).



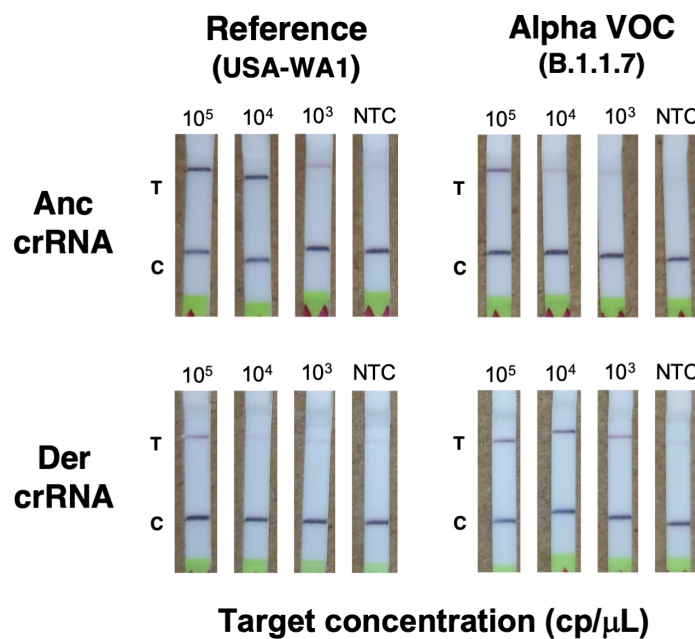
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1028 **Supplementary Fig. 13 | Performance of 69/70 deletion assay on synthetic RNA targets.**
1029 SHINE fluorescence of the 69/70 ancestral (anc) and derived (der) assays on synthetic SARS-
1030 CoV-2 RNA targets after 90 minutes. NTC, no target control. Center = mean and error bars = s.d.
1031 for 3 technical replicates.
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Supplementary Fig. 14 | Performance of 417 SNP detection assay on synthetic RNA targets. SHINE fluorescence of the 417 ancestral (anc), derived T (der T) and derived N (der N) assays on synthetic SARS-CoV-2 RNA targets after 90 minutes. NTC, no target control. Center = mean and error bars = s.d. for 3 technical replicates.

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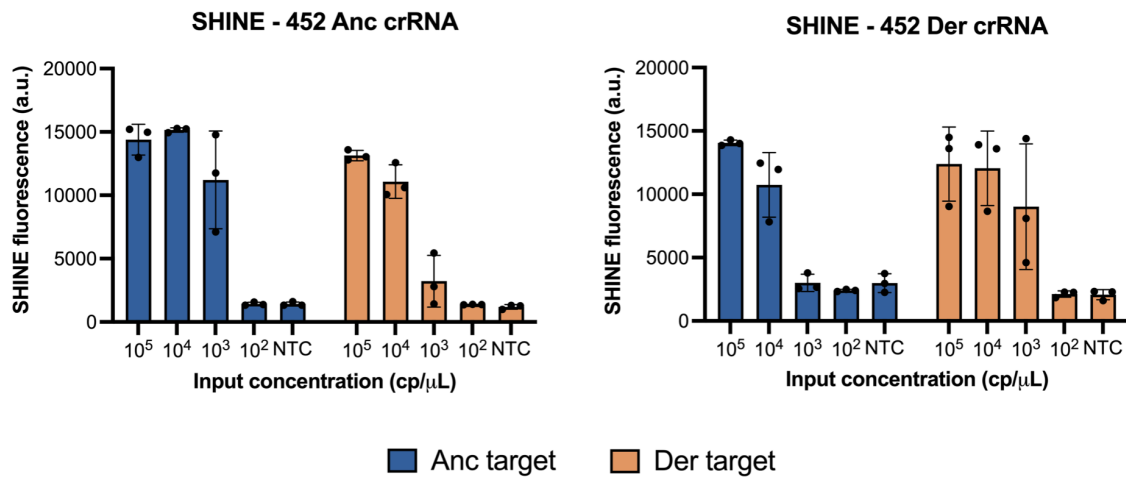


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1042 **Supplementary Fig. 15 | Lateral-flow detection of Alpha VOC in contrived clinical samples.**
1043 Colorimetric lateral flow based detection of SARS-CoV-2 RNA in contrived clinical samples using
1044 the 69/70 SHINEv2 assay. SHINEv2 incubation time: 90 minutes. NTC, no-target control. T, test
1045 line; C, control line.

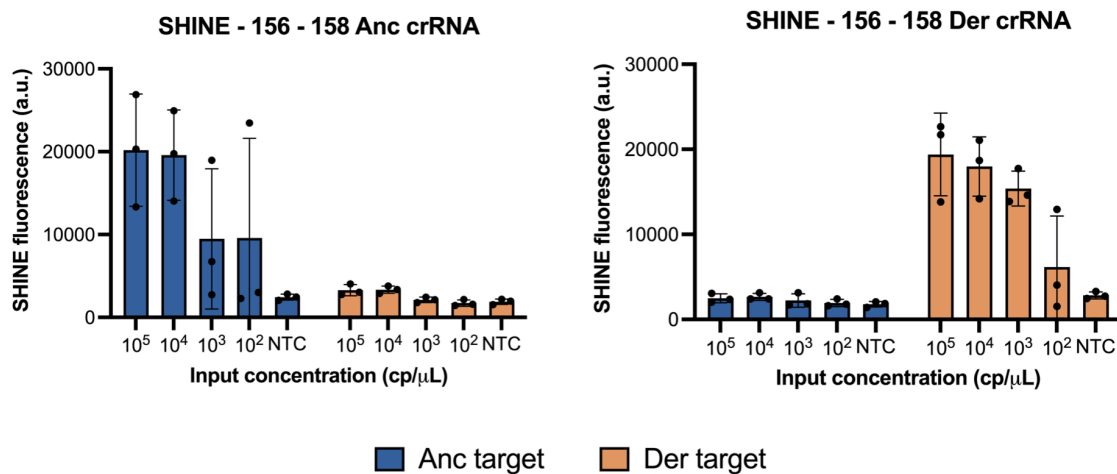
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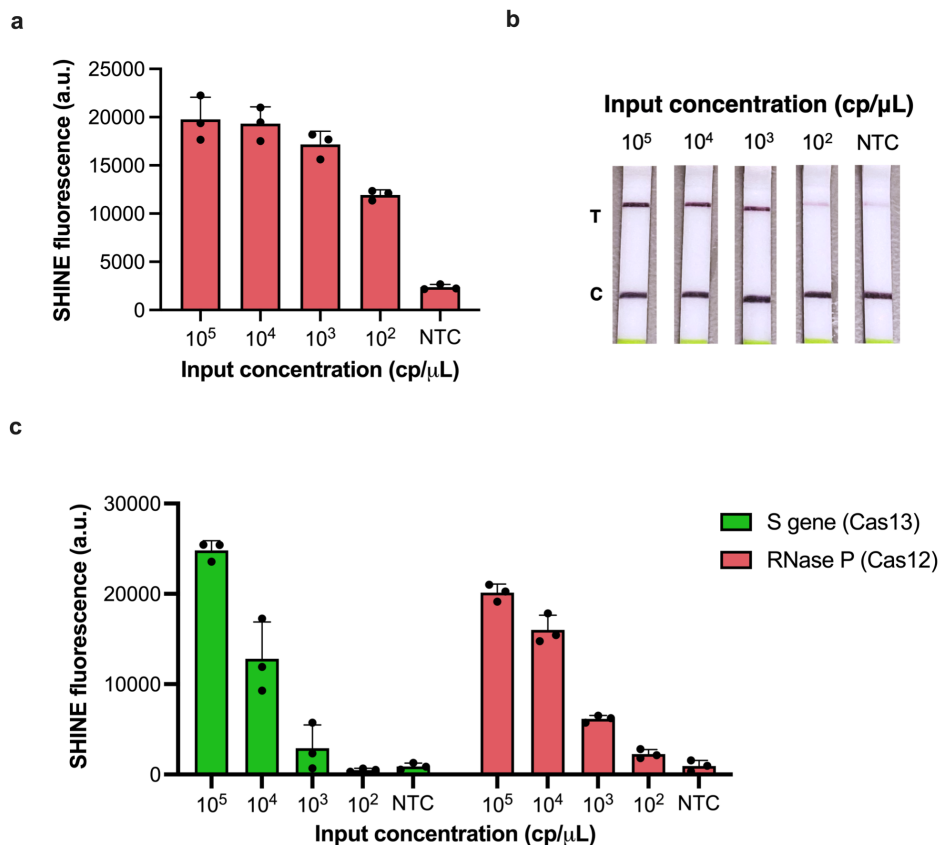
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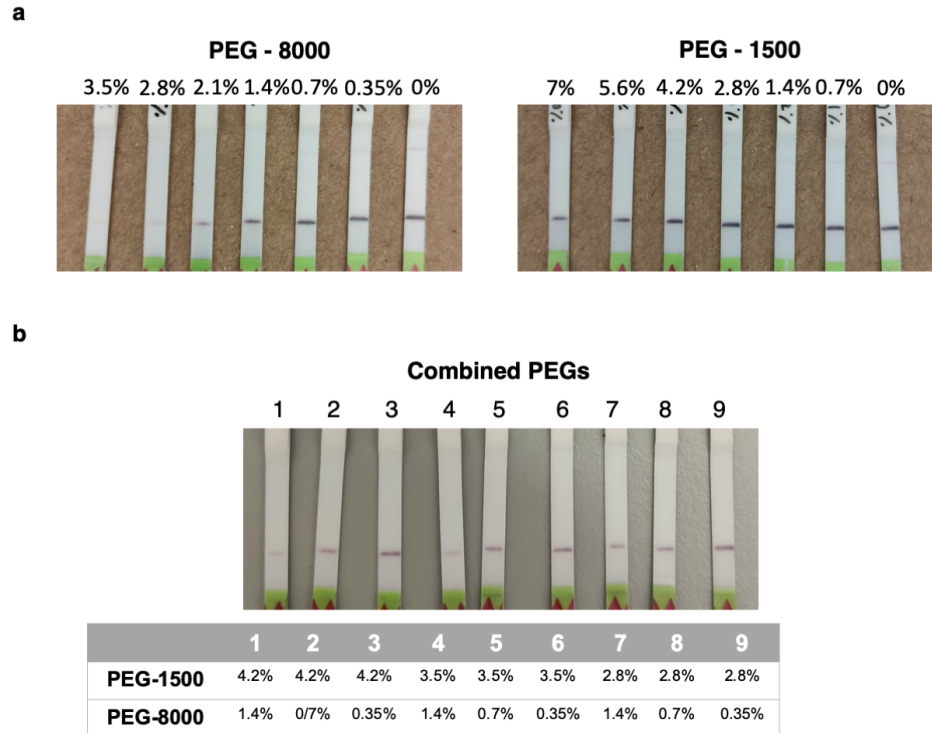
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Supplementary Fig. 16 | Performance of SARS-CoV-2 Delta assays on full genome synthetic RNA standards. a,b. SHINE fluorescence of the (a) 452 and (b) 156 - 158 ancestral (anc) and derived (der) assays on full genome synthetic RNA standards after 90 minutes. NTC, no target control. Center = mean and error bars = s.d. for 3 technical replicates.



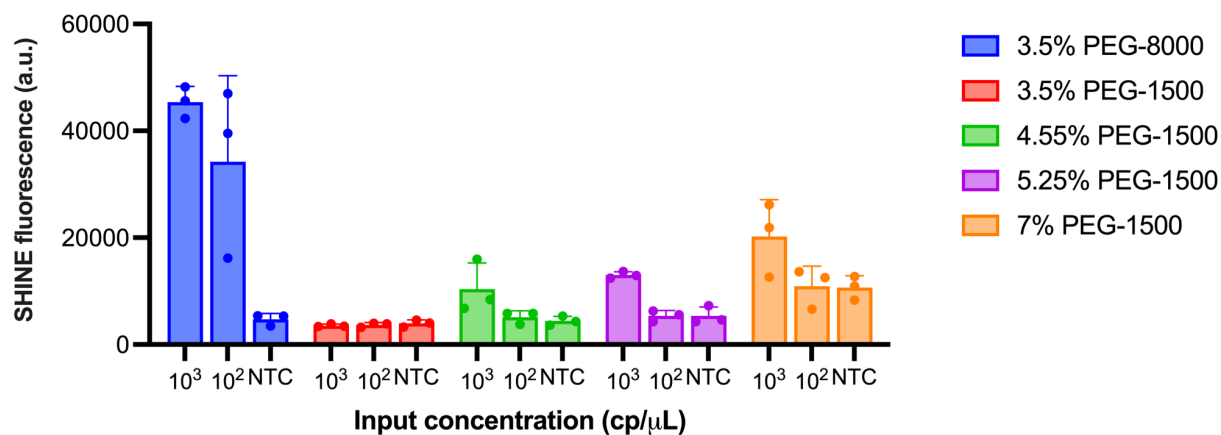
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1058 **Supplementary Fig. 17 | Development of Cas12-based SHINE assay for RNase P detection.**
1059 **a.** SHINE fluorescence of the RNase P assay on synthetic DNA target after 90 minutes; NTC =
1060 no target control **b.** Lateral-flow detection of synthetic DNA target using Cas12-based SHINE
1061 assay, after 90 minute incubation; NTC = no target control C = control band; T = test band **c.**
1062 SHINE fluorescence of the duplex SARS-CoV-2 S gene and RNase P assays on synthetic nucleic
1063 acid targets after 90 minutes. NTC = no target control. For **a** and **c**, center = mean and error
1064 bars = s.d. for 3 technical replicates.



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Supplementary Fig. 18 | Effect of PEG on flow through the strip. **a** and **b**, images of lateral flow strips from SHINEv2 reactions with PEGs of different molecular weights and concentrations (**a**) separately and (**b**) in combination, in the absence of an RNA target.

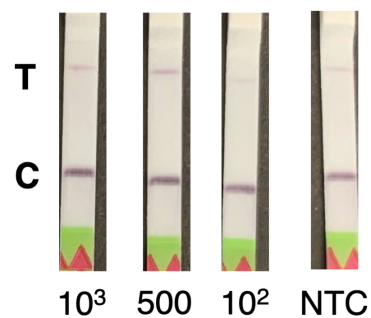
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Supplementary Fig. 19 | Effect of PEG concentration on SHINE performance. SHINE fluorescence on synthetic SARS-CoV-2 RNA targets (S gene) relative to PEG molecular weight and concentration. Reactions incubated for 90 minutes. NTC, no target control. Center = mean and error bars = s.d. for 3 technical replicates.

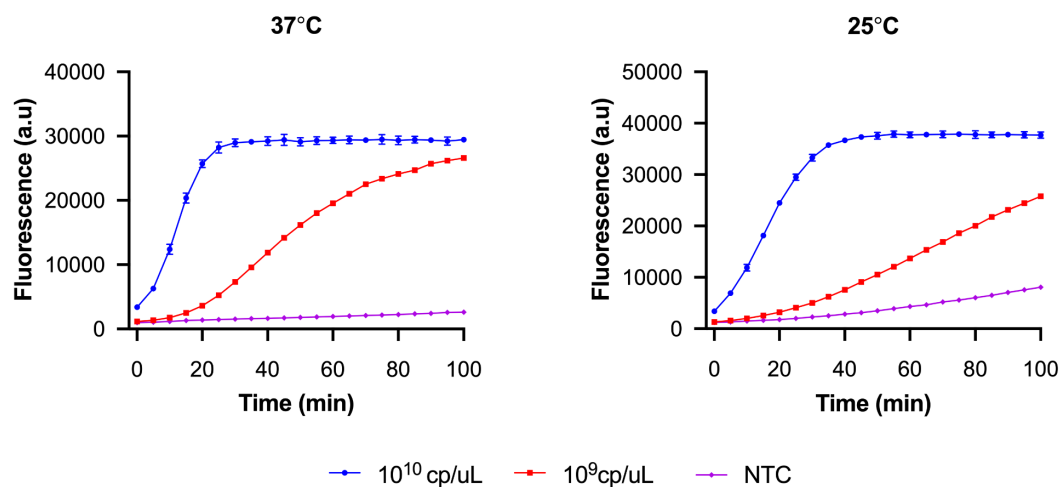
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Input concentration (cp/μL)

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1081 **Supplementary Fig. 20 | Performance of equipment-free SHINEv2 at room temperature.**
1082 Lateral flow detection of full genome synthetic RNA standards in lysis solution-treated UTM using
1083 SHINEv2, after a 90 minute incubation at 25°C. C = control band; T = test band; NTC = no target
1084 control.
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Supplementary Fig. 21 | Temperature has a limited effect on Cas13-based detection. Cas13-detection of synthetic RNA target (S gene) at 37°C and 25°C. NTC, non target control. Centre = mean and error bar = standard deviation (s.d.) for 3 technical replicates.

1093 **Supplementary Table 1 | Patient sample information.**

1094 Samples S1 - S96 were used without extraction, whereas RNA was extracted from samples E1 -
 1095 E20 before use. See *methods* for details. UTM, universal viral transport medium; VTM, viral
 1096 transport medium; RP, RNase P.

SHINEv2 sample ID	Sample matrix	N1 viral titer (copies/ μ L)	N1 Ct	N2 Ct	RP Ct	Figures
S1	VTM	883	27.8	29.3	29.7	Supplementary Fig. 8-10
S2	VTM	1078	27.5	29.0	26.9	Supplementary Fig. 8-10
S3	VTM	7	35.6	37.8	31.5	Supplementary Fig. 8-10
S4	VTM	194575	19.4	20.3	28.9	Supplementary Fig. 8-10
S5	VTM	107	31.0	32.7	31.9	Supplementary Fig. 8-10
S6	VTM	109	31.0	33.0	30.0	Supplementary Fig. 8-10
S7	VTM	328	29.0	31.1	24.6	Supplementary Fig. 8-10
S8	VTM	34	33.0	35.1	29.5	Supplementary Fig. 8-10
S9	VTM	778	28.0	29.8	30.0	Supplementary Fig. 8-10
S10	VTM	4739	25.2	26.9	29.3	Supplementary Fig. 8-10
S11	VTM	33	33.0	35.6	29.4	Supplementary Fig. 8-10
S12	VTM	599754	17.7	18.6	29.5	Fig. 2b and Supplementary Fig. 8-10
S13	VTM	271	29.4	31.6	29.5	Supplementary Fig. 8-10
S14	VTM	931	27.7	29.2	29.4	Supplementary Fig. 8-10
S15	VTM	540	28.6	30.8	29.4	Supplementary Fig. 8-10
S16	VTM	3281	25.7	27.9	29.1	Supplementary Fig. 8-10
S17	VTM	739	28.1	29.8	24.6	Fig. 2b and Supplementary Fig. 8-10
S18	Saline	-	-	-	29.7	Supplementary Fig. 8-10
S19	Saline	232	29.6	32.2	28.2	Supplementary Fig. 8-10
S20	Saline	-	-	-	29.0	Supplementary Fig. 8-10
S21	Saline	1	37.7	-	26.4	Supplementary Fig. 8-10
S22	Saline	3	37.0	38.5	27.3	Supplementary Fig. 8-10
S23	VTM	188	29.9	32.1	30.3	Supplementary Fig. 8-10
S24	UTM	3739	25.5	27.8	28.9	Supplementary Fig. 8-10
S25	UTM	-	-	-	32.3	Supplementary Fig. 8-10
S26	UTM	-	-	-	29.8	Supplementary Fig. 8-10
S27	UTM	-	-	-	30.6	Supplementary Fig. 8-10

S28	UTM	-	-	-	28.8	Supplementary Fig. 8-10
S29	UTM	-	-	-	30.2	Supplementary Fig. 8-10
S30	UTM	-	-	-	29.8	Supplementary Fig. 8-10
S31	UTM	-	-	-	31.1	Supplementary Fig. 8-10
S32	UTM	-	-	-	34.1	Supplementary Fig. 8-10
S33	UTM	-	-	-	30.5	Supplementary Fig. 8-10
S34	UTM	-	-	-	31.7	Supplementary Fig. 8-10
S35	UTM	-	-	-	28.4	Fig. 2b and Supplementary Fig. 8-10
S36	UTM	-	-	-	30.1	Supplementary Fig. 8-10
S37	UTM	-	-	-	27.4	Supplementary Fig. 8-10
S38	UTM	-	-	-	28.8	Supplementary Fig. 8-10
S39	UTM	-	-	-	29.6	Supplementary Fig. 8-10
S40	UTM	-	-	-	28.0	Supplementary Fig. 8-10
S41	UTM	-	-	-	30.6	Supplementary Fig. 8-10
S42	UTM	-	-	-	29.7	Supplementary Fig. 8-10
S43	UTM	-	-	-	29.2	Supplementary Fig. 8-10
S44	UTM	-	-	-	31.0	Supplementary Fig. 8-10
S45	UTM	-	-	-	30.0	Supplementary Fig. 8-10
S46	UTM	-	-	-	30.8	Supplementary Fig. 8-10
S47	UTM	-	-	-	29.1	Supplementary Fig. 8-10
S48	UTM	-	-	-	27.7	Supplementary Fig. 8-10
S49	UTM	3	36.8	38.7	30.0	Supplementary Fig. 8-10
S50	VTM	-	-	-	27.7	Supplementary Fig. 8-10
S51	VTM	653	28.0	30.0	26.1	Supplementary Fig. 8-10
S52	VTM	193	29.8	31.8	31.3	Supplementary Fig. 8-10
S53	VTM	31094	22.0	23.4	31.9	Supplementary Fig. 8-10
S54	VTM	1130	27.1	29.4	29.6	Supplementary Fig. 8-10
S55	VTM	527	28.3	30.7	31.8	Supplementary Fig. 8-10
S56	VTM	2323088	15.4	16.6	28.6	Supplementary Fig. 8-10
S57	VTM	-	-	37.7	32.2	Supplementary Fig. 8-10
S58	VTM	129	30.2	33.1	31.6	Supplementary Fig. 8-10
S59	VTM	-	-	38.6	30.6	Supplementary Fig. 8-10
S60	VTM	34	32.6	35.0	30.3	Fig. 2b and

						Supplementary Fig. 8-10
S61	VTM	82	31.2	33.4	31.3	Supplementary Fig. 8-10
S62	VTM	10	34.5	36.7	32.0	Supplementary Fig. 8-10
S63	VTM	1224	27.0	29.0	29.1	Supplementary Fig. 8-10
S64	VTM	4	36.0	38.3	28.2	Supplementary Fig. 8-10
S65	UTM	-	-	-	29.9	Supplementary Fig. 8-10
S66	UTM	-	-	-	30.2	Supplementary Fig. 8-10
S67	UTM	-	-	-	27.8	Supplementary Fig. 8-10
S68	UTM	2	37.2	39.3	31.4	Supplementary Fig. 8-10
S69	UTM	4	35.9	38.5	26.4	Supplementary Fig. 8-10
S70	UTM	3	36.0	38.6	29.6	Supplementary Fig. 8-10
S71	VTM	3706009	14.7	15.8	27.3	Supplementary Fig. 8-10
S72	VTM	3118	25.6	27.5	29.7	Supplementary Fig. 8-10
S73	VTM	13588	23.1	24.4	28.8	Supplementary Fig. 8-10
S74	VTM	7	34.9	37.0	25.8	Supplementary Fig. 8-10
S75	VTM	1471	26.5	27.9	27.8	Supplementary Fig. 8-10
S76	VTM	3	36.5	38.2	30.3	Supplementary Fig. 8-10
S77	VTM	12	34.4	37.3	25.8	Supplementary Fig. 8-10
S78	VTM	9	34.6	38.0	29.5	Supplementary Fig. 8-10
S79	VTM	2925	25.5	27.2	28.1	Supplementary Fig. 8-10
S80	VTM	345	28.8	30.2	32.1	Supplementary Fig. 8-10
S81	VTM	5	35.6	37.5	28.3	Supplementary Fig. 8-10
S82	VTM	20694	22.4	23.4	29.5	Supplementary Fig. 8-10
S83	VTM	46	32.0	33.8	30.1	Supplementary Fig. 8-10
S84	VTM	14	33.8	36.6	29.1	Supplementary Fig. 8-10
S85	VTM	187051	18.9	19.9	26.1	Supplementary Fig. 8-10
S86	VTM	21	33.3	35.1	28.8	Supplementary Fig. 8-10
S87	VTM	13	34.2	36.6	30.8	Supplementary Fig. 8-10
S88	VTM	412032	17.7	18.8	27.4	Supplementary Fig. 8-10
S89	VTM	10	34.4	37.7	29.4	Supplementary Fig. 8-10
S90	VTM	172	30.0	32.3	28.1	Supplementary Fig. 8-10
S91	VTM	23	33.4	35.8	26.4	Supplementary Fig. 8-10
S92	VTM	386	28.7	29.7	30.9	Supplementary Fig. 8-10
S93	VTM	3907	25.0	26.3	28.9	Supplementary Fig. 8-10

S94	VTM	16	33.8	35.0	31.8	Supplementary Fig. 8-10
S95	VTM	-	-	-	30.3	Supplementary Fig. 8-10
S96	VTM	3	36.9	38.2	32.6	Supplementary Fig. 8-10
E1	VTM	65806	20.9	20.0	28.2	Fig. 3e
E2	UTM	23588	22.1	23.2	27.9	Fig. 3e
E3	VTM	1213	26.8	25.8	30.6	Fig. 3e
E4	VTM	3237	25.5	26.8	31.2	Fig. 3e
E5	VTM	753	27.3	29.3	32.1	Fig. 3e
E6	VTM	738	27.6	27.2	29.8	Fig. 3e
E7	Unknown	7804	24.1	24.2	31.2	Fig. 3e
E8	Unknown	4495	24.9	24.2	29.1	Fig. 3e
E9	Unknown	291	29.0	28.6	30.0	Fig. 3e
E10	Unknown	12954	23.3	23.3	33.5	Fig. 3e
E11	UTM	-	-	-	32.0	Fig. 3e
E12	UTM	-	-	-	30.2	Fig. 3e
E13	UTM	-	-	-	30.7	Fig. 3e
E14	UTM	-	-	-	29.9	Fig. 3e
E15	UTM	-	-	-	30.4	Fig. 3e
E16	UTM	-	-	-	29.5	Fig. 3e
E17	UTM	-	-	-	30.2	Fig. 3e
E18	UTM	-	-	-	33.8	Fig. 3e
E19	UTM	-	-	-	30.6	Fig. 3e
E20	UTM	-	-	-	31.1	Fig. 3e

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1104 **Supplementary Table 2 | Oligonucleotides used in this study.**

Name	Oligo type	Sequence	Gene - Assay
ORF1a crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACCUUCUUCUUCAGGUUGAAGAGCAGCAG AA	ORF1ab
S gene crRNA - 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACUGGUAGGACAGGGUUAUCAAAACCUCU UA	S
S gene crRNA - 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACACAGGUUAUCAAAACCUCUAGUACC AU	S
S gene crRNA - 3	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACACCAUUUAAUGAUGGUGUUUUAUUUUG CU	S
S - 69/70 anc crRNA 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACACCAUUGGUCCAGAGACAUGUAUAG CA	S - 69/70
S - 69/70 anc crRNA 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACUCCCAGAGACAUGUAUAGCAUGGAAC CA	S - 69/70
S - 69/70 der crRNA 1	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACCUUAGUACCAUUGGUCCAGAUUAG CA	S - 69/70
S - 69/70 der crRNA 2	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACUCCCAGAUUAGCAUGGAACCAAGUA C	S - 69/70
S - 69/70 der crRNA 3	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACCAGAUUAGCAUGGAACCAAGUAACAU U	S - 69/70
S - 69/70 der crRNA 4	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACGGUCCCAGAUUAGCAUGGAACCAAG UA	S - 69/70
S - 69/70 der crRNA 5	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACUUGGUCCCAGAUUAGCAUGGAACCA AG	S - 69/70
S - 69/70 der crRNA 6	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU AAAACCAUUGGUCCCAGAUUAGCAUGGAAC CA	S - 69/70
S - 69/70 der	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACU	S - 69/70

crRNA 7		AAAACACCAUUGGUCCCAGAUUAGCAUGGAC	
S - 69/70 der crRNA 8	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACAGTACCATTGGTCCCAGATATAGCATGG	S - 69/70
S - 417 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGCATTCTCTCCAGTTTGCCCTGGAGCGA	S - 417
S - 417 derN crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUUAUUAUUAUUAUCAGCAACAUCUCCGCA	S - 417
S - 417 derT crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUUAUUAUCAGCAGUCGUACCAGUUUGCUA	S - 417
S - 452 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGUUCCUAAACAUCUAUACAGGUAAUCA	S - 452
S - 452 der crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCUAUAGCUGUAAUUAUAAUUACCACCAA	S - 452
S - 156-158 anc crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAAACUCUGAACUCACUUUCCAUCCAAAC	S - 156-158
S - 156-158 der crRNA	crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACUAAACUCCACUUUCCAUCCAACUUUUGU	S - 156-158
RNase P - crRNA	Cas12a crRNA	UAAUUUCUACUAAGUGUAGAUAGGCCAGCUGGCCCGCUGC	RNase P
ORF1a fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGCCAAGGTAACCTTTGGAATTTGGTGCCAC	ORF1ab
ORF1a rev primer	RPA primer	ACTATCATCATCTAACCAATCTTCTTCTTG	ORF1ab
S/S - 69/70 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGCAACTCAGGACTTGTCTTACCTTTCTTTCC	S & S - 69/70
S/S - 69/70 rev primer	RPA primer	AAGCAAATAAACACCATCATTAAT	S & S - 69/70
S - 417 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGTCTATGCAGATTCATTTGTAATTAGAGGTG	S - 417
S - 417 rev primer	RPA primer	ATAACGCAGCCTGTAAATCATCTGGTAAT	S - 417

S - 452 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGAGCTTGG AATTCTAACAATCTTGATTCT	S - 452
S - 452 rev primer	RPA primer	AGTTGAAATATCTCTCTCAAAGGTTTGA	S - 452
S - 156-158 fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGTTTTTGGG TGTTTATTACCACAAAAACA	S - 156-158
S - 156-158 rev primer	RPA primer	AGAAAAGGCTGAGAGACATATTCAAAG	S - 156-158
RNase P Fwd primer	RPA primer	GAAATTAATACGACTCACTATAGGGGTGGAAT ACACCCTTAGGAAAAGGCTTC	RNase P
RNase P Rev primer	RPA primer	CAAGCCGTGAATGTAGATCTCAGAGCAC	RNase P
ORF1a synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGGTGAGTTT AAATTGGCTTCACATATGTATTGTTCTTTCTAC CCTCCAGATGAGGATGAAGAAGAAGGTGATTG TGAAGAAGAAGAGTTTGAGCCATCAACTCAAT ATGAGTATGGTACTGAAGATGATTACCAAGGT AAACCTTTGGAATTTGGTGCCACTTCTGCTGCT CTTCAACCTGAAGAAGAGCAAGAAGAAGATTG GTTAGATGATGATAGTCAACAACTGTTGGTCA ACAAGACGGCAGTGAGGACAATCAGACA ACTA CTATTCAAACAATTGTTGAGGTTCAACCTCAAT TAGAGATGGAACCTTACACCAGTTGTTCAACT ATTGAAGTGAATAGTTTTAGTGGTTATTTAAAA CTTACTGACAATGTATACATTA AAAATGCAGAC ATTGTGGAAGAAGCTAAAAAGGTAAAACCAAC AGTGGTTGTTAATGCAGCCAATGTTTACCTTAA ACATGGAGGAGG	ORF1ab
S - 69/70 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTGACAAAGT TTTCAGATCCTCAGTTTTACATTCAACTCAGGA CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGG TTCCATGCTATACATGTCTCTGGGACCAATGG TACTAAGAGGTTTGATAACCCTGTCCTACCATT TAATGATGGTGTATTATTTGCTTCCACTGAGAA GTCTAACATAATAAGAGGCTGGATTTTTGGTAC TACTTTAGATTGGAAGACCCAGTCCCTACTTAT TGTTAATAACGCTACTAATGTTGTTATTAAGT CTGTGAATTTCAATTTTGTAAATGATCC	S - 69/70
S - 69/70 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTGACAAAGT TTTCAGATCCTCAGTTTTACATTCAACTCAGGA CTTGTTCTTACCTTTCTTTTCCAATGTTACTTGG TTCCATGCTATATCTGGGACCAATGGTACTAA GAGGTTTGATAACCCTGTCCTACCATTTAATGA TGGTGTATTTTGTCTTCCACTGAGAAGTCTAA	S - 69/70

		CATAATAAGAGGCTGGATTTTTGGTACTACTTT AGATTTCGAAGACCCAGTCCCTACTTATTGTTAA TAACGCTACTAATGTTGTTATTAAAGTCTGTGA ATTTCAATTTTGAATGATCC	
S - 417 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACCTGGAAAGATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGAATGGTGTGGAAGGT TTTAATTGTTACTTTCTTTTACAATCATATGTT TCCAACCCACTAATGGTGTGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 417 derN synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACCTGGAAATATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGAATGGTGTGGAAGGT TTTAATTGTTACTTTCTTTTACAATCATATGTT TCCAACCCACTAATGGTGTGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 417 derT synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGTCTATGCAG ATTCATTTGTAATTAGAGGTGATGAAGTCAGAC AAATCGCTCCAGGGCAAACCTGGAACGATTGCT GATTATAATTATAAATTACCAGATGATTTTACAG GCTGCGTTATAGCTTGGAAATTCTAACAATCTTG ATTCTAAGGTTGGTGGTAATTATAATTACCTGT ATAGATTGTTTAGGAAGTCTAATCTCAAACCTT TTGAGAGAGATATTTCAACTGAAATCTATCAGG CCGGTAGCACACCTTGAATGGTGTGGAAGGT TTTAATTGTTACTTTCTTTTACAATCATATGTT TCCAACCCACTAATGGTGTGTTACCAACCA TACAGAGTAGTAGTACTTTCTTTTGAACTTCTA CATGCACCAGCAA	S - 417
S - 452 anc synthetic RNA	gBlock	GAAATTAATACGACTCACTATAGGGTCTATGCA GATTCATTTGTAATTAGAGGTGATGAAGTCAGA	S - 452

target		CAAATCGCTCCAGGGCAAACCTGGAAAGATTGC TGATTATAATTATAAATTACCAGATGATTTTACA GGCTGCGTTATAGCTTGGAATTCTAACAATCTT GATTCTAAGGTTGGTGGTAATTATAATTACCTG TATAGATTGTTTAGGAAGTCTAATCTCAAACCT TTTGAGAGAGATATTTCAACTGAAATCTATCAG GCCGGTAGCACACCTTGTAAATGGTGTGGAAGG TTTTAATTGTTACTTTTCTTTACAATCATATGGT TTCCAACCCACTAATGGTGTGTTGGTTACCAACC ATACAGAGTAGTAGTACTTTCTTTTGAACCTTCT ACATGCACCAGCAA	
S - 452 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGTCTATGCA GATTCATTTGTAATTAGAGGTGATGAAGTCAGA CAAATCGCTCCAGGGCAAACCTGGAAAGATTGC TGATTATAATTATAAATTACCAGATGATTTTACA GGCTGCGTTATAGCTTGGAATTCTAACAATCTT GATTCTAAGGTTGGTGGTAATTATAATTACCGG TATAGATTGTTTAGGAAGTCTAATCTCAAACCT TTTGAGAGAGATATTTCAACTGAAATCTATCAG GCCGGTAGCAAACCTTGTAAATGGTGTGGAAGG TTTTAATTGTTACTTTTCTTTACAATCATATGGT TTCCAACCCACTAATGGTGTGTTGGTTACCAACC ATACAGAGTAGTAGTACTTTCTTTTGAACCTTCT ACATGCACCAGCAA	S - 452
S - 156-158 anc synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGCTACTTAT TGTTAATAACGCTACTAATGTTGTTATTAAGT CTGTGAATTTCAATTTTGTAAATGATCCATTTTG GGTGTTTATTACCACAAAAACAACAAAAGTTGG ATGGAAAGTGAGTTCAGAGTTTATTCTAGTGC GAATAATTGCACTTTTGAATATGTCTCTCAGCC TTTTCTTATGGACCTTGAAGGAAAACAGGGTAA TTTCAAAAATCTTAGGGAATTTGTGTTAAGAA T	S - 156-158
S - 156-158 der synthetic RNA target	gBlock	GAAATTAATACGACTCACTATAGGGCTACTTAT TGTTAATAACGCTACTAATGTTGTTATTAAGT CTGTGAATTTCAATTTTGTAAATGATCCATTTTG GGTGTTTATTACCACAAAAACAACAAAAGTTGG ATGGAAAGTGGAGTTTATTCTAGTGCGAATAAT TGCACTTTTGAATATGTCTCTCAGCCTTTTCTT ATGGACCTTGAAGGAAAACAGGGTAATTTCAA AAATCTTAGGGAATTTGTGTTAAGAAT	S - 156-158
RNase P synthetic DNA target	gBlock	GAAATTAATACGACTCACTATAGGGGTGCTGT GGAGGCTGAACTGGATCCAGTGAATACACC CTTAGGAAAAGGCTTCCAGCCGCCTGCCCC GGAGACCCAATGACATTTATGTCAACATGAAG	RNase P

		ACGGACTTTAAGGCCAGCTGGCCCGCTGCC AGAAGCTGCTGGACGGAGGGGCCCGGGTCA GAACGCGTGCTCTGAGATCTACATTCACGGCT TGGGCCTGGCCATCAACCGCGCCATCAACATC GCGCTGCAGCTGCAGGCGGGCAGCTTCGGGT CCTTGCAGGTGGCTGCCAATACCTCCACCGTG GAGCTTGTGATGAGCTGGAGCCAGAGACCG ACACACGGGAGCCACTGACTCGGATCCGCAA CAACTCAGCCATCCACATCCGAGTCTTCAGGG TCACACCCAAGTAATTGAAAAGACACTCCTCC ACTTATCCCCTCCGTGATATGGCTCTTCGCAT GCTGAGTA	
5C-HEX-reporter	Fluorescence DNA reporter	5' - /5HEX/CCCCC/3IABkFQ/-3'	-
6U-FAM-reporter	Fluorescence RNA reporter	5'- /56-FAM/rUrUrUrUrU/3IABkFQ/-3'	-
FAMBio 5C LFA reporter	LFA DNA reporter	/56-FAM/CCCCCCCCCCCC/3Bio/	-
FAMBio 14U LFA reporter	LFA RNA reporter	/5BiosG/rUrUrUrUrUrUrUrUrUrUrU/36- FAM/	-

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