

1 **Practical strategies for SARS-CoV-2 RT-PCR testing in resource-**  
2 **constrained settings**

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12  
13 **Running Title:** SARS-CoV-2 testing strategies

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32 **ABSTRACT**

33 **Background** Standard nasopharyngeal swab testing for SARS-CoV-2 detection by PCR is not  
34 always feasible due to limitations in trained personnel, personal protective equipment, swabs,  
35 PCR reagents, and access to cold chain and biosafety hoods.

36 **Methods** We piloted the collection of nasal mid-turbinate swabs amenable to self-testing,  
37 including both standard polyester flocked swabs as well as 3D printed plastic lattice swabs,  
38 placed into either viral transport media or an RNA stabilization agent. Quantitative SARS-CoV-2  
39 viral detection by RT-qPCR was compared to that obtained by nasopharyngeal sampling as the  
40 reference standard. Pooling specimens in the lab versus pooling swabs at the point of collection  
41 was also evaluated.

42 **Results** Among 275 participants, flocked nasal swabs identified 104/121 individuals who were  
43 PCR-positive for SARS-CoV-2 by nasopharyngeal sampling (sensitivity 87%, 95% CI 79-92%),  
44 mostly missing those with low viral load ( $<10^3$  viral copies/uL). 3D-printed nasal swabs  
45 showed similar sensitivity. When nasal swabs were placed directly into an RNA stabilizer, the  
46 mean 1.4 log decrease in viral copies/uL compared to nasopharyngeal samples was reduced to  
47  $<1$  log, even when samples were left at room temperature for up to 7 days. Pooling sample  
48 specimens or swabs both successfully detected samples  $>10^2$  viral copies/uL.

49 **Conclusions** Nasal swabs are likely adequate for clinical diagnosis of acute infections to help  
50 expand testing capacity in resource-constrained settings. When collected into an RNA  
51 preservative that also inactivates infectious virus, nasal swabs yielded quantitative viral loads  
52 approximating those obtained by nasopharyngeal sampling.

53

54 **BACKGROUND**

55 Since the start of the SARS-CoV-2 pandemic, testing has been a cornerstone of the public health  
56 response. The de facto standard for clinical testing is PCR from nasopharyngeal (NP) swabs.

57 However, nasopharyngeal sampling must be performed by trained staff using personal  
58 protective equipment (PPE). Shortages in both, as well as NP swabs themselves, often manifest  
59 when case counts climb. A wide array of strategies amenable to self-collection have been  
60 piloted to expand testing capacity, including the collection of nasal swabs, oropharyngeal and  
61 tongue swabs, saliva, and oral rinses (1–3). The volume of tests conducted can also become  
62 burdensome and lengthen turnaround time, spurring interest in pooled testing strategies in low

63 prevalence and settings (4–8). Finally, regarding sample storage and transport, viral specimens  
64 are typically placed in viral transport medium, and CDC recommends maintenance of cold chain  
65 prior to processing (1), but this may not be possible in all settings.

66 In order to implement a household transmission study in the early phases of the epidemic in  
67 North Carolina, when shortages of PPE and swabs were prevalent, we adopted a strategy of  
68 self-collected nasal swabs from household members during follow-up. Here we compare this  
69 strategy to concurrently collected nasopharyngeal swabs at enrollment in our study population.  
70 We piloted different types of swabs stored in different media. Given interest in pooling  
71 strategies for high throughput testing, we also used our cohort to test two different pooling  
72 strategies: pooling swabs at the point of collection or pooling sample lysate in the lab. Our  
73 findings provide confidence in using self-collected nasal swabs, preferably stored in an RNA  
74 stabilizer, when nasopharyngeal sampling is not feasible.

75

## 76 **METHODS**

### 77 **Clinical samples**

78 Clinical samples were collected as part of a SARS-CoV-2 household transmission study  
79 conducted in the Piedmont region of North Carolina. The study received ethical approval from  
80 the Institutional Review Board at the University of North Carolina-Chapel Hill and is registered  
81 at [clinicaltrials.gov](https://clinicaltrials.gov) (NCT04445233). Participants were enrolled if they were adults that tested  
82 positive for SARS-CoV-2 by PCR at the UNC Respiratory Diagnostic Center and shared a living  
83 space with one or more persons who also agreed to participate. At enrollment, a standard  
84 clinician-collected nasopharyngeal (NP) swab was performed, followed by up to two other nasal  
85 swabs (on different sides) that were either collected by study staff or self-collected by the  
86 participant or their guardian with guidance from study staff (**Figure S1**). For nasal sampling,  
87 participants were instructed to insert the swab about 1-2 inches into one nostril , then swirl 5  
88 times while slowly withdrawing the swab before placing it into the collection tube. All samples  
89 were placed into a cooler on ice prior to processing in a BSL2+ laboratory space.

90

### 91 **Sample collection strategies**

92 Flocked NP swabs were collected into 3mL of Becton Dickinson’s co-packaged universal viral  
93 transport system. Two types of nasal swabs designed for mid-turbinate sampling (NMT) were

94 used: flocked NMT swabs (COPAN, Murrietta CA) and 3D-printed lattice NMT swabs (Resolution  
95 Medical, Fridley MN) (**Figures S1**). Both were collected into 3mL viral transport media (VTM)  
96 prepared using CDC SOP# DSR-052-05. Upon sample receipt in the laboratory, 1mL of the  
97 collected sample was combined with 1mL 2X DNA/RNA Shield, a nucleic acid preservation agent  
98 and lysis buffer (Zymo Research), and stored at -80°C until extraction. RNA was extracted from  
99 200uL of the lysate using the Quick-RNA Viral 96 Kit (Zymo Research) and eluted in 20uL of  
100 water. We also evaluated the effect of storage media by collecting flocked NMT swabs directly  
101 into 3mL of 1X DNA/RNA Shield (Shield), with aliquots either frozen immediately upon return to  
102 the lab or left at room temperature for 4 or 7 days before being stored at -80°C. RNA was  
103 extracted from 100 uL of the lysate using the same extraction and elution protocols.

104

#### 105 **qRT-PCR viral quantification**

106 Samples were tested using a CDC RT-qPCR protocol authorized for emergency use that consists  
107 of three unique assays: two targeting regions of the virus' nucleocapsid gene (N1, N2) and one  
108 targeting human RNase P gene (RP) (Catalog # 2019-nCoV-EUA-01, Integrated DNA  
109 Technologies) (9). 5uL of extracted RNA was added to 15uL of each assay's reaction mixture  
110 containing TaqPath 1-Step RT-qPCR Master Mix, CG (Thermofisher Scientific) and the  
111 corresponding primer-probe set (IDT), followed by the recommended thermocycler protocol.  
112 Plasmid DNA containing the human RPP30 gene and SARS-CoV-2 in vitro transcribed RNA  
113 control (nCoVPC, IDT) were used as positive controls. Water was used as a negative extraction  
114 control. Samples were designated positive if all three PCRs were positive (N1 and N2 for virus,  
115 RP for adequate sampling). If the N1 and N2 PCRs were negative, but the RP assay had a Ct  
116 value  $\geq 30$  or was negative, suggesting inadequate sampling, then the sample was re-extracted.  
117 The second result was reported if the RP Ct value was  $< 30$  or if both N1 and N2 PCRs were  
118 positive regardless of RP Ct value.

119 The viral load of each sample, in copies/uL, was extrapolated from standard curves generated  
120 for each viral assay (N1 and N2) using serial dilutions of nCoVPC (2 to 100,000 viral RNA  
121 copies/uL). The average copies/uL between the N1 and N2 assays was used as the final  
122 quantitative viral load. Based on the sample collection and RNA extraction volumes as well as  
123 volume of template RNA used in the RT-qPCR (5uL), this viral load represents the number of  
124 viral RNA copies per 5 uL of VTM or Shield sample.

## 125 **Pooling strategies**

126 The efficacy of pooling NMT samples was examined through two different approaches: pooling  
127 swabs at the point of care into the same collection vessel and pooling individual sample lysates  
128 prior to extraction. For the first strategy, self-collected 3D-printed lattice NMT swabs from each  
129 member of a household of three or more were collected and pooled together in 5mL of VTM.  
130 This was done at one or more of the study visits for each household. 200uL of the sample lysate  
131 was extracted and quantified as above. Results were compared to the self-collected individual  
132 flocked NMT swab collected at the same visit. In the second pooling strategy, one qRT-PCR  
133 positive sample lysate from a flocked NMT swab (pre-RNA extraction) was pooled with sample  
134 lysate from negative individuals to construct pool sizes of 5, 10, 15, and 20. The Ct values of  
135 twelve samples with viral copies/uL ranging from  $10^1$  to  $10^7$  were compared to the Ct values of  
136 their corresponding pools.

137

## 138 **Statistical analysis**

139 A probit analysis of results from the nCoVPC plasmid control concentrations (ranging from 2 to  
140 100,000 copies/uL as part of standard curves generated in every RT-qPCR run) by parametric  
141 curve fitting to hit rate data was used to determine the limit of detection (LOD) of the N1 and  
142 N2 qRT-PCR assays. Samples that were positive in both N1 and N2 assays, but with an average  
143 viral load that fell below the LOD were categorized as indeterminate. The sensitivity and  
144 specificity of different swab types for RT-qPCR detection of SARS-CoV-2 was calculated using  
145 flocked NP swabs as the reference standard. Additionally, the difference in the quantitative  
146 viral load was compared for different collection strategies. Comparisons were made on the log  
147 scale and analyzed using Wilcoxon matched-pairs signed rank testing with a p-value<0.05  
148 considered significant. Statistical analyses were performed using GraphPad Prism 8 and SAS 9.4  
149 (Cary, NC).

150

## 151 **RESULTS**

152 We report data from 644 swab samples collected from 275 participants (91 households) at  
153 enrollment, 24 pools collected at follow-up or enrollment, and 44 pools constructed from  
154 participant samples in the lab. Participants ranged in age from 1-77 years old, with 71% >18  
155 years of age.

## 156 Limit of detection of RT-qPCR assay

157 Probit analysis of nCoVPC plasmid control concentrations tested in 33 RT-qPCR runs yielded a  
158 limit of detection (LOD) for the N1 and N2 assays of 9 and 13 copies/uL, respectively (**Table S1**).  
159 The average LOD between the two assays, 11 copies/uL, was used as the cutoff for sample  
160 positivity. A sample was deemed positive if the average viral load derived from the cycle  
161 threshold (Ct) values of N1 and N2 corresponded to a concentration  $\geq 11$  copies/uL,  
162 indeterminate if  $< 11$  copies/uL, and negative if either assay failed to amplify. Altogether,  
163 21/702 (3.0%) samples tested fell into the indeterminate category. Another 33 (4.7%) samples  
164 only amplified in one assay (N1 or N2 assay), but with a Ct value corresponding to a viral load  
165 that fell below the LOD. Only 2 samples (0.3%) were discordant between the N1 and N2 assays  
166 (positive in one but not the other).

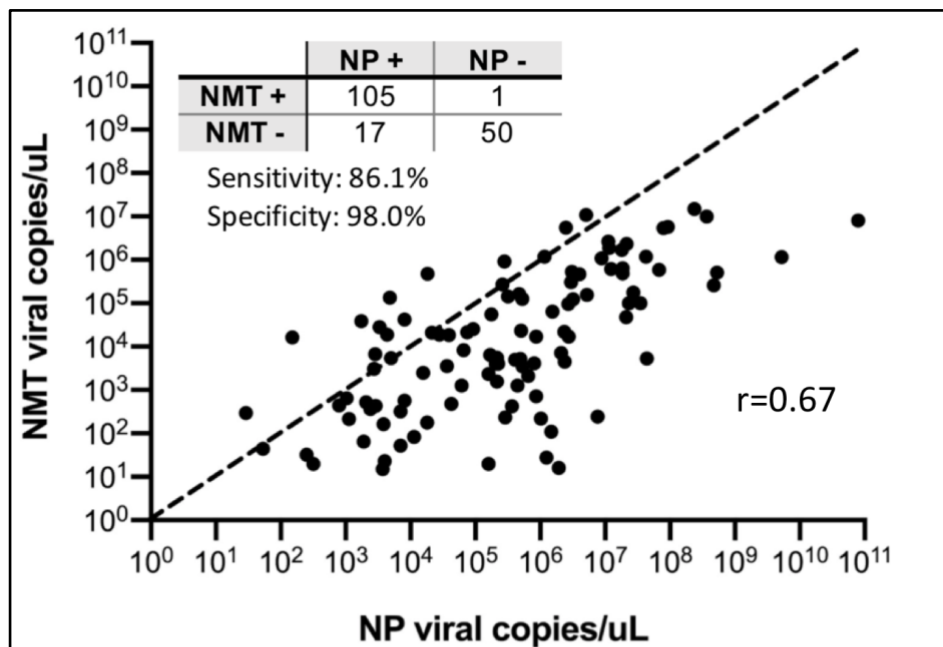
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## 168 Comparison of collection swabs and storage medium

169 Compared to

170 nasopharyngeal sampling,  
171 flocked nasal mid-  
172 turbinate (NMT) swabs  
173 displayed slightly  
174 decreased sensitivity, but  
175 were well-accepted by the  
176 participants and yielded  
177 adequate sampling.

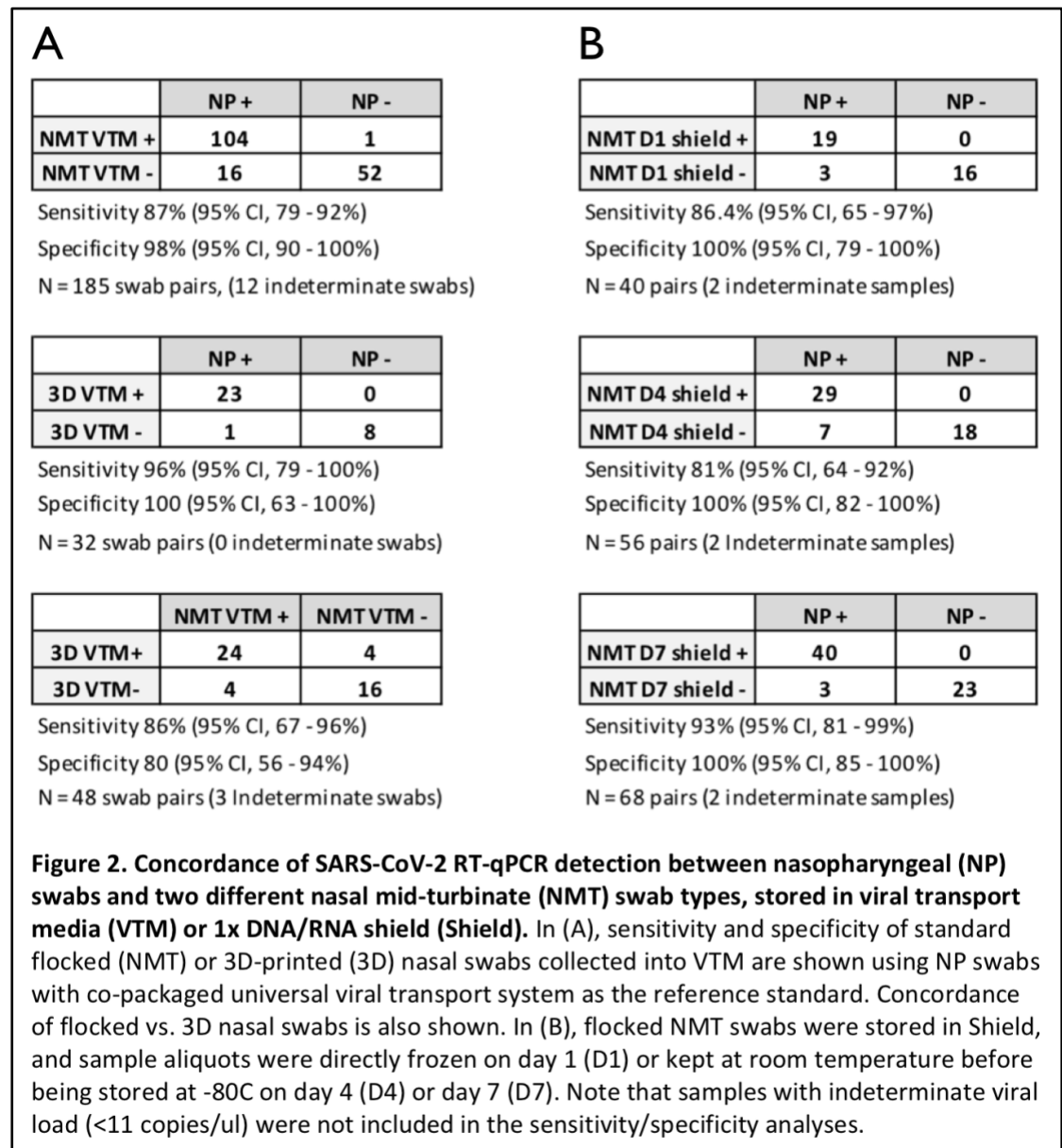
178 Altogether, at enrollment,  
179 275 study participants  
180 completed 226 NP swabs  
181 and 418 NMT swabs (255  
182 flocked and 51 3D-printed  
183 in VTM, 112 flocked in  
184 Shield) (**Figure S1**). Of the  
185 49 participants that  
186 declined to do NP swabs,



**Figure 1. Concordance and comparison of SARS-CoV-2 viral loads from paired nasopharyngeal (NP) and nasal mid-turbinate (NMT) swabs.**

Paired NP and NMT swabs from 173 participants showed overall good concordance, with most discordances (15/16) arising from positive NP/negative NMT samples. Quantitative viral loads derived from the average of N1 and N2 qRT-PCR assays favored NP swabs compared to NMT swabs. A  $y=x$  dashed line is drawn for reference.

187 46 agreed to at least one type of NMT swab. Inadequate sampling, as defined by negative N1  
 188 and N2 PCRs in concert with a negative human RP PCR or Ct  $\geq 30$ , occurred in small numbers of  
 189 flocked  
 190 swabs, but a  
 191 substantial  
 192 proportion of  
 193 3D-printed  
 194 plastic lattice  
 195 swabs: 1/226  
 196 (0.4%) of NP  
 197 swabs,  
 198 14/343 (4.1%)  
 199 of flocked  
 200 NMT swabs,  
 201 and 11/51  
 202 (21.6%) of  
 203 3D-printed  
 204 plastic lattice  
 205 swabs.

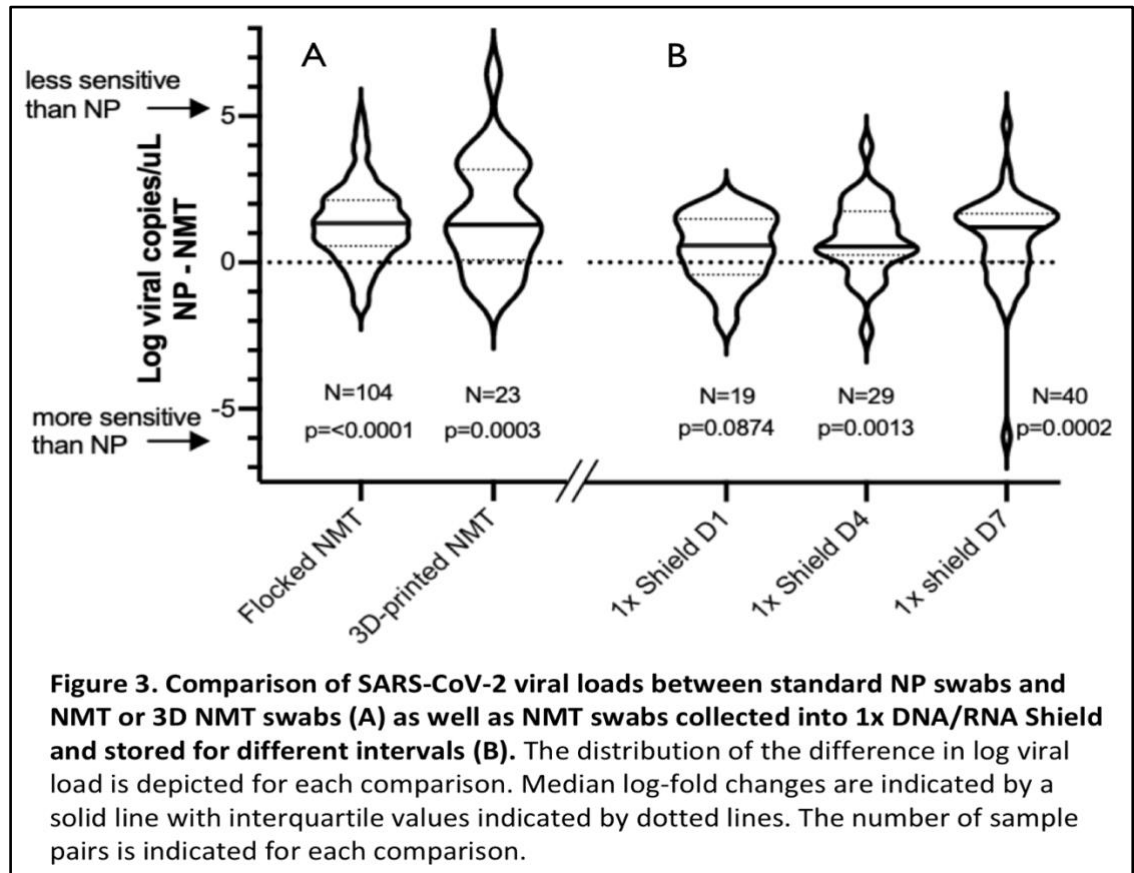


212 swabs  
 213 showed excellent specificity (98%, 95% CI 90-100%) but slightly decreased sensitivity (87%, 95%  
 214 CI 79-92%) for SARS-CoV-2 detection by RT-qPCR (**Figures 1&2**). Of 173 NP-NMT swab pairs, 104  
 215 were both positive, 52 both negative, and 10% (17/173) were discordant. Three of these  
 216 discordances were likely due to inadequate sampling (1 NP, 2 NMT swabs with RP Ct value  $\geq 30$ ),  
 217 while 71% of the rest (10/14) occurred in samples with low viral loads (<10<sup>3</sup> viral copies

218 detected in the NP swab). In the 104 positive swab pairs, NMT samples displayed lower average  
219 viral loads (Spearman correlation coefficient=0.67, **Figure 1**), with a mean 1.3 log decrease in  
220 viral copies/uL (IQR 0.6 - 2.1 log viral copies/ul) compared to NP sampling ( $p<0.0001$ ) (**Figure**  
221 **3A**). This was at least partly due to a sampling difference, as NMT swabs also showed on  
222 average 3.1 cycles higher Ct values in the human RP PCR (**Figure S2**).

223

224 Though the  
225 3D-printed  
226 plastic  
227 lattice NMT  
228 swabs were  
229 more likely  
230 to lead to  
231 inadequate  
232 sampling,  
233 positive  
234 samples  
235 showed  
236 quantitative  
237 viral loads  
238 similar to  
239 flocced  
240 NMT swabs



241 (**Figure 3A**). This was true despite on average 1.2 higher Ct values for the human RP assay in the  
242 3D vs. flocced swabs. Compared to NP sampling, 3D-printed NMT swabs displayed 95.7%  
243 sensitivity (95% CI 78.1%-99.9%) and 100% specificity (95% CI 63.1-100%) among 48 swab pairs  
244 (**Figure 2**).

245

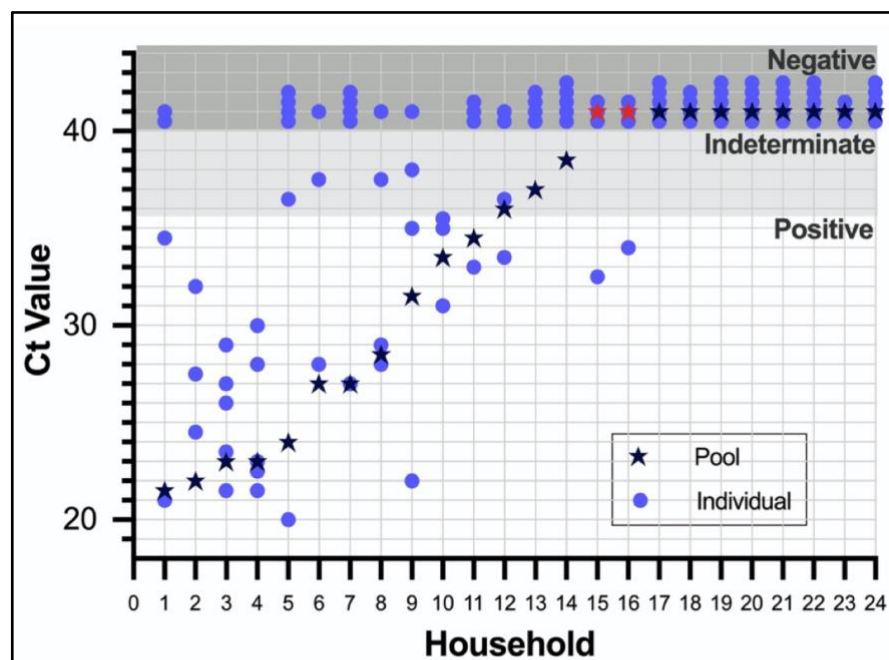
246 Placing flocced NMT swabs directly into 1x DNA/RNA Shield did not improve the sensitivity of  
247 detection, but did result in viral loads comparable to those obtained by NP sampling. Aliquots  
248 of Shield samples were either directly stored at -80C (similar to other samples collected on day



249 1), or left out at room temperature for 4 or 7 days prior to freezing and processing. All NMT  
250 Shield samples showed a specificity of 100% compared to NP swabs, while sensitivity ranged  
251 86%, 78%, and 91% for the samples frozen at day 1, 4, and 7, respectively (**Figure 2**).  
252 Altogether, regardless of how many days the Shield samples were left out, the overall  
253 sensitivity was 85% (95% CI 77-92%). While sensitivity for detection was slightly diminished,  
254 quantitative viral loads derived from NMT Shield aliquots frozen on day 1 were comparable to  
255 NP viral loads (mean decrease of 0.5 log viral copies/uL (IQR -0.3-1.4),  $p=0.09$ ) (**Figure 3B**). For  
256 aliquots left at room temperature until day 4 and day 7, we observed a mean decrease that was  
257 still  $<1$  log viral copies/uL compared to NP sampling (mean 0.8 and 0.8 log viral copies/ul,  
258 respectively ( $p=0.001$  and  $p=0.0002$ ) (**Figure 3B**).

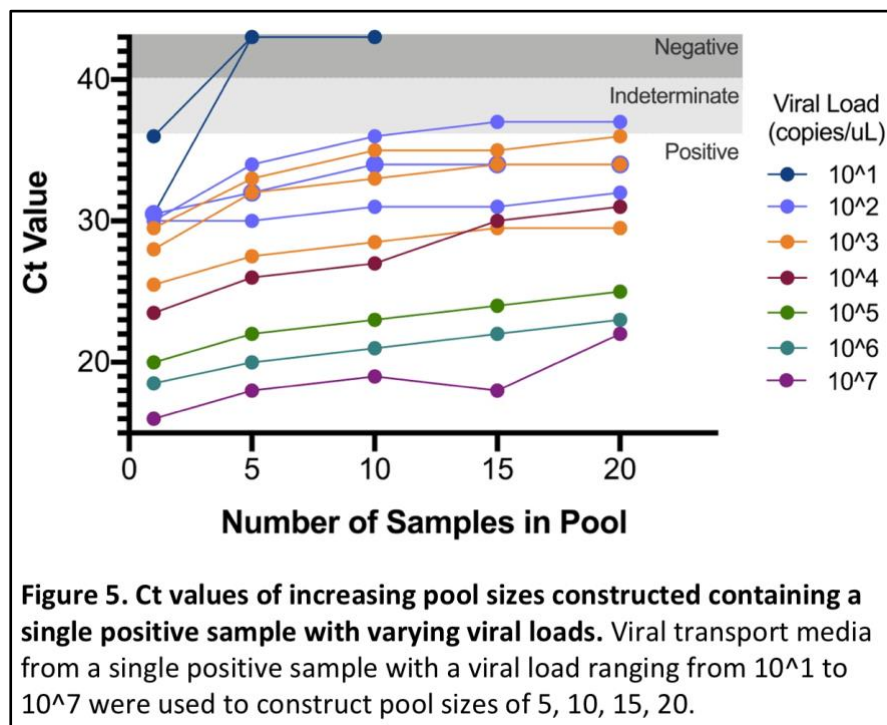
### 260 Pooling strategies

261 The pooling strategies  
262 implemented were sufficient  
263 for detecting samples with  
264 viral loads  $>10^2$  copies/uL  
265 but were not as sensitive as  
266 individual swabs for detecting  
267 samples with lower viral loads.  
268 Of the 24 pools of 3D-NMT  
269 swabs pooled at the point of  
270 care, 3 were indeterminate,  
271 and 2 (8%) yielded discordant  
272 results (depicted as red stars  
273 in **Figure 4**). Under the  
274 assumption that the  
275 concurrently collected  
276 individual flocked NMT swabs  
277 were accurate, the two



**Figure 4. Comparison of Ct values from nasal mid-turbinate (NMT) swabs pooled from households of 3-5 persons at the point of care vs. concurrently collected individual NMT swabs.** Among the pools collected from 24 households (listed along the x axis in order of decreasing viral loads), 2 pools with discordant results from individual swabs are depicted as red stars. Viral loads derived from the Ct values for each sample and the corresponding pool are found in Table S2.

278 discordant results were false  
279 negative pools where the  
280 individual swab had a viral  
281 load <100 copies/uL, close to  
282 the limit of detection (Table  
283 S2). Of the 22 concordant  
284 pools, 8 were negative and 11  
285 were positive, mostly with  
286 individual swab viral loads  
287  $\geq 10^2$  copies/uL.



288  
289 Similarly, when individual  
290 sample lysates were pooled  
291 in the lab at varying pool  
292 sizes, none of the 2 sets of experimental pools containing a sample with a viral load of  $10^1$   
293 copies/uL were positive (Figure 5). Of the 3 sets of pools containing a sample with a viral load of  
294  $10^2$  copies/uL, 2 were positive at every pool size, while the remaining set was positive within  
295 pools of 5 and 10 samples, but indeterminate when the pool size was increased to 15 and 20  
296 samples. The remaining pools constructed with samples with a viral load  $>10^2$  copies/uL were  
297 positive across all pool sizes. The average total Ct value increase for the pools that remained  
298 positive at a pool size of 20 samples was 5.1 cycles, close to the expected 4.3 cycle increase for  
299 a sample diluted 1:20 using a PCR with 100% amplification efficiency.

## 300 301 DISCUSSION

302 In a highly exposed outpatient cohort, we found nasal swabs to be reasonably sensitive,  
303 capturing 87% of SARS-CoV-2 infections diagnosed by nasopharyngeal sampling. This estimate  
304 is similar to most other outpatient studies showing  $>85\%$  concordance between self-collected  
305 nasal swabs (either nasal mid-turbinate or anterior nasal swabs) and clinician-collected  
306 nasopharyngeal sampling (2,10–13). Not all studies are consistent however, likely due to  
307 heterogeneity in testing environments, and inclusion of non-acute samples collected during  
308 follow-up (14,15).

309 By calculating quantitative SARS-CoV-2 viral loads, our study gives clarity on where sensitivity is  
310 diminished (16). For the majority of participants in which nasal sampling failed to detect virus,  
311 the NP viral load was <1000 copies/uL, at a level that is likely non-infectious. Of these  
312 participants, 7/11 were antibody-positive at the time of sampling (unpublished data), and for  
313 the 8/11 participants still reporting symptoms, the average duration of reported symptoms was  
314 6.5 days. Thus nasal samples are likely adequate for clinical diagnosis of acute infections to help  
315 expand testing capacity, but insensitivity to low viral load infections should be taken into  
316 consideration. On average, the decreased sensitivity of NMT swabs led to a little over a log  
317 decrease in viral copies/uL compared to NP swabs.

318  
319 Our pragmatic approach of “show one, then do one” meant that nasal swabs were both  
320 clinician and self-collected. Also, since we often collected two nasal swabs per person, one from  
321 each nostril, our sampling strategy may have slightly underperformed relative to other studies  
322 that sample both nostrils with the same swab. It should be noted that we tested flocced and  
323 3D-printed lattice swabs, but did not test dry swabs or non-flocced cotton swabs. Where high  
324 quality swabs are not available, but other swab types are plentiful, a strategy of combining oral  
325 and nasal samples appears promising (17).

326  
327 3D-printed plastic swabs may also help address supply chain shortages (18,19). We first  
328 acquired prototype NMT lattice swabs from Resolution Medical in anticipation of shortage of  
329 supplies for our research study. In our limited testing, the prototype 3D-printed NMT lattice  
330 swabs showed high categorical concordance with NP swabs and also yielded similar viral loads  
331 compared to flocced NMT swabs. Similar high concordance has been demonstrated for 3D-  
332 printed nasopharyngeal swabs (18–20). Anecdotally, the prototype 3D-printed were observed  
333 to be more uncomfortable for study participants compared to flocced NMT swabs, a sentiment  
334 shared by other studies (18). This may have contributed to the higher proportion of samples  
335 deemed as inadequate sampling.

336  
337 Labs also face VTM shortages requiring alternate transport media (21,22). Reagents which can  
338 inactivate virus and also keep samples stable at ambient temperature may be particularly apt  
339 substitutes (23). We used 1xDNA/RNA shield (Zymogen), an RNA preservation agent that has

340 been widely used to inactivate SARS-CoV-2 and other respiratory viruses in various sample  
341 types and is now part of saliva and NMT Shield collection kits that have received FDA  
342 emergency use authorization (24–26). In our hands, storage of nasal swab samples in Shield did  
343 not improve their overall diagnostic sensitivity, but positive NMT swabs stored in Shield  
344 maintained quantitative viral loads more similar to those detected in concurrently collected NP  
345 swabs.

346  
347 Pooling specimens in the lab is a well-documented strategy to accelerate SARS-CoV-2 testing in  
348 high-throughput settings (4–6). As in previous studies, we found that although Ct values do  
349 increase with pooling, the strategy can be broadly successful (27–31). Samples with viral loads  
350 at or near the limit of detection (31), or  $<10^3$  viral copies/uL in the CDC EUA assay we adopted,  
351 may go undetected as pool sizes increase. This was even more apparent when pooling swabs at  
352 the point of collection, which we piloted as unsupervised self-collection of 3D-printed swabs  
353 into the same conical tube containing 5mL of VTM.

354  
355 Our findings add to the evidence base for nasal swabs as an adequate substitute for PCR-based  
356 clinical diagnosis of SARS-CoV-2 infection in outpatient settings where nasopharyngeal sampling  
357 is challenging. Viral recovery can be maintained even when immediate cold chain is not possible  
358 by storing swabs in an RNA preservation agent that also deactivates infectious virus. Combined  
359 with pooling specimens in the lab, these practical strategies can help expand testing in  
360 resource-constrained settings.

361

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370

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475

## 476 FIGURE LEGENDS

477 **Figure 1. Concordance and comparison of SARS-CoV-2 viral loads from paired nasopharyngeal**  
478 **(NP) and nasal mid-turbinate (NMT) swabs.** Paired NP and NMT swabs from 173 participants  
479 showed overall good concordance, with most discordances (15/16) arising from positive  
480 NP/negative NMT samples. Quantitative viral loads derived from the average of N1 and N2 qRT-  
481 PCR assays favored NP swabs compared to NMT swabs. A  $y=x$  dashed line is drawn for  
482 reference.

483

484 **Figure 2. Concordance of SARS-CoV-2 RT-qPCR detection between nasopharyngeal (NP) swabs**  
485 **and two different nasal mid-turbinate (NMT) swab types, stored in viral transport media**  
486 **(VTM) or 1x DNA/RNA shield (Shield).** In (A), sensitivity and specificity of standard flocked



487 (NMT) or 3D-printed (3D) nasal swabs collected into VTM are shown using NP swabs with co-  
488 packaged universal viral transport system as the reference standard. Concordance of flocked vs.  
489 3D nasal swabs is also shown. In (B), flocked NMT swabs were stored in Shield, and sample  
490 aliquots were directly frozen on day 1 (D1) or kept at room temperature before being stored at  
491 -80C on day 4 (D4) or day 7 (D7). Note that samples with indeterminate viral load (<11  
492 copies/ul) were not included in the sensitivity/specificity analyses.

493

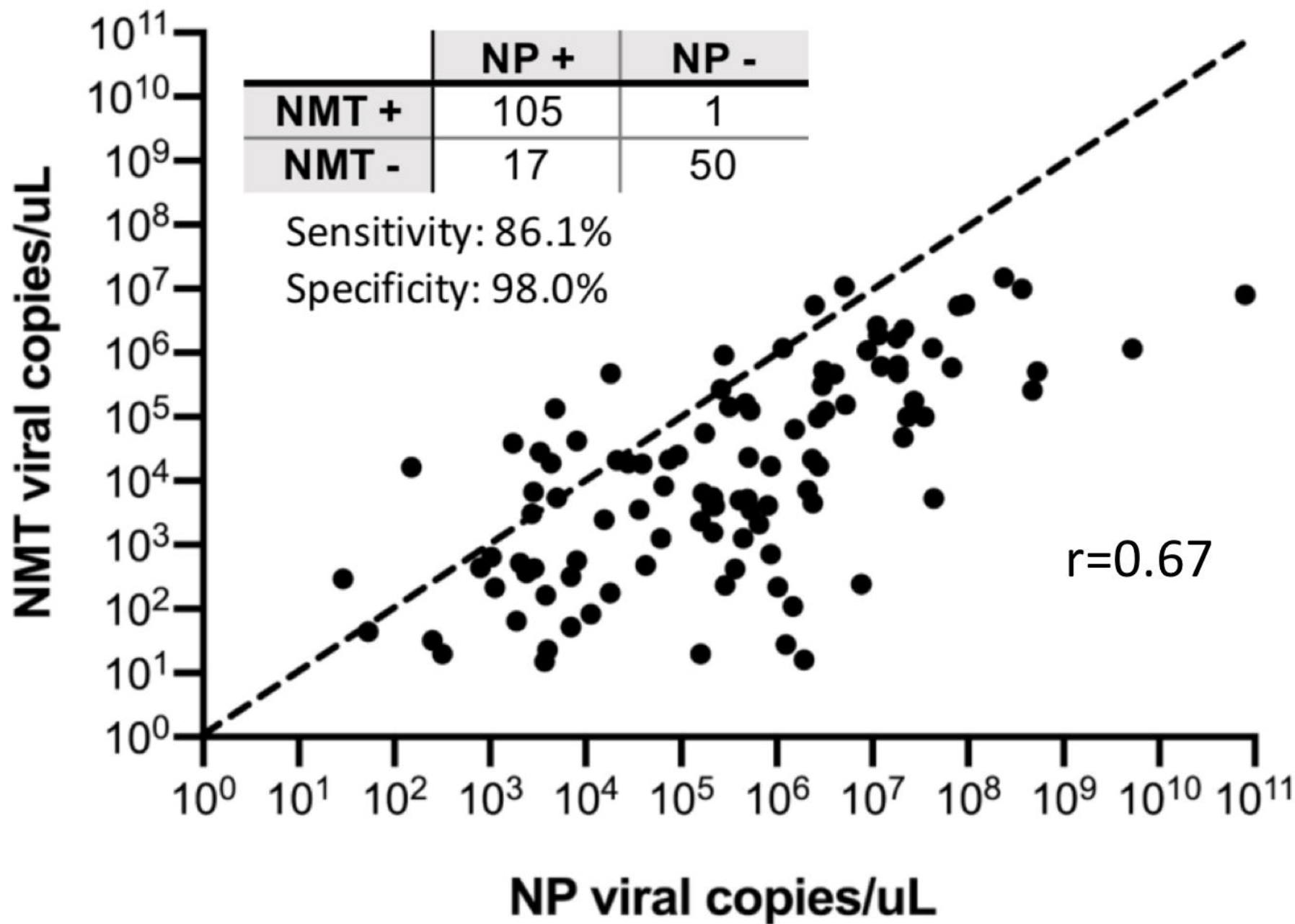
494 **Figure 3. Comparison of SARS-CoV-2 viral loads between standard NP swabs and NMT or 3D**  
495 **NMT swabs (A) as well as NMT swabs collected into 1x DNA/RNA Shield and stored for**  
496 **different intervals (B).** The distribution of the difference in log viral load is depicted for each  
497 comparison. Median log-fold changes are indicated by a solid line with interquartile values  
498 indicated by dotted lines. The number of sample pairs is indicated for each comparison.

499

500 **Figure 4. Comparison of Ct values from nasal mid-turbinate (NMT) swabs pooled from**  
501 **households of 3-5 persons at the point of care vs. concurrently collected individual NMT**  
502 **swabs.** Among the pools collected from 24 households (listed along the x axis in order of  
503 decreasing viral loads), 2 pools with discordant results from individual swabs are depicted as  
504 red stars. Viral loads derived from the Ct values for each sample and the corresponding pool are  
505 found in Table S2.

506

507 **Figure 5. Ct values of increasing pool sizes constructed containing a single positive sample**  
508 **with varying viral loads.** Viral transport media from a single positive sample with a viral load  
509 ranging from  $10^1$  to  $10^7$  were used to construct pool sizes of 5, 10, 15,



**A**

	NP +	NP -
<b>NMT VTM +</b>	<b>104</b>	<b>1</b>
<b>NMT VTM -</b>	<b>16</b>	<b>52</b>

Sensitivity 87% (95% CI, 79 - 92%)

Specificity 98% (95% CI, 90 - 100%)

N = 185 swab pairs, (12 indeterminate swabs)

	NP +	NP -
<b>3D VTM +</b>	<b>23</b>	<b>0</b>
<b>3D VTM -</b>	<b>1</b>	<b>8</b>

Sensitivity 96% (95% CI, 79 - 100%)

Specificity 100 (95% CI, 63 - 100%)

N = 32 swab pairs (0 indeterminate swabs)

	NMT VTM +	NMT VTM -
<b>3D VTM+</b>	<b>24</b>	<b>4</b>
<b>3D VTM-</b>	<b>4</b>	<b>16</b>

Sensitivity 86% (95% CI, 67 - 96%)

Specificity 80 (95% CI, 56 - 94%)

N = 48 swab pairs (3 Indeterminate swabs)

**B**

	NP +	NP -
<b>NMT D1 shield +</b>	<b>19</b>	<b>0</b>
<b>NMT D1 shield -</b>	<b>3</b>	<b>16</b>

Sensitivity 86.4% (95% CI, 65 - 97%)

Specificity 100% (95% CI, 79 - 100%)

N = 40 pairs (2 indeterminate samples)

	NP +	NP -
<b>NMT D4 shield +</b>	<b>29</b>	<b>0</b>
<b>NMT D4 shield -</b>	<b>7</b>	<b>18</b>

Sensitivity 81% (95% CI, 64 - 92%)

Specificity 100% (95% CI, 82 - 100%)

N = 56 pairs (2 Indeterminate samples)

	NP +	NP -
<b>NMT D7 shield +</b>	<b>40</b>	<b>0</b>
<b>NMT D7 shield -</b>	<b>3</b>	<b>23</b>

Sensitivity 93% (95% CI, 81 - 99%)

Specificity 100% (95% CI, 85 - 100%)

N = 68 pairs (2 indeterminate samples)

