

Shedding of Infectious SARS-CoV-2 Despite Vaccination

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Short title: SARS-CoV-2 RNA levels in breakthrough and unvaccinated Delta infections

28 Abstract

29 The SARS-CoV-2 Delta Variant of Concern is highly transmissible and contains mutations that confer partial im-
30 mune escape. The emergence of Delta in North America caused the first surge in COVID-19 cases after SARS-
31 CoV-2 vaccines became widely available. To determine whether individuals infected despite vaccination might
32 be capable of transmitting SARS-CoV-2, we compared RT-PCR cycle threshold (Ct) data from 20,431 test-pos-
33 itive anterior nasal swab specimens from fully vaccinated (n = 9,347) or unvaccinated (n=11,084) individuals
34 tested at a single commercial laboratory during the interval 28 June – 1 December 2021 when Delta variants
35 were predominant. We observed no significant effect of vaccine status alone on Ct value, nor when controlling
36 for vaccine product or sex. Testing a subset of low-Ct (<25) samples, we detected infectious virus at similar rates,
37 and at similar titers, in specimens from vaccinated and unvaccinated individuals. These data indicate that vac-
38 cinated individuals infected with Delta variants are capable of shedding infectious SARS-CoV-2 and could play
39 a role in spreading COVID-19.

40

41 Main text

42 Introduction

43 The SARS-CoV-2 Delta variant was initially characterized in March 2021 and was associated with increased
44 infection incidence in North America beginning in the summer of 2021. In Wisconsin, Delta-lineage viruses were
45 first detected on 12 April 2021, and within 10 weeks accounted for more than 90% of sequenced viruses. Delta
46 viruses were highly transmissible and contained mutations that confer partial immune escape. The “surge” in
47 cases attributable to Delta-lineage viruses represented the first substantial increase in SARS-CoV-2 infection
48 incidence after vaccines had become widely available in the United States. By July 2021, SARS-CoV-2 infec-
49 tion incidence was low in the United States ([https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/
50 past-reports/05212021.html#print](https://www.cdc.gov/coronavirus/2019-ncov/covid-data/covidview/past-reports/05212021.html#print)) [1], and national and local public health agencies were loosening require-
51 ments for face coverings and other non-pharmaceutical interventions to reduce virus transmission [1–3]. A key
52 question in developing these policies was whether persons infected with SARS-CoV-2 despite vaccination could
53 transmit infection to others.

54
55 By late July 2021, outbreak investigations suggested that vaccinated persons who became infected could spread
56 Delta-lineage SARS-CoV-2 [4,5]. To determine whether individuals with vaccine breakthrough infections could
57 shed Delta viruses at levels consistent with potential transmission, we compared the SARS-CoV-2 RNA burden
58 in nasal swab specimens from vaccinated and unvaccinated individuals tested at a single commercial laboratory.
59 We also attempted virus isolation and determined infectious viral titers from a subset of samples from vaccinat-
60 ed and unvaccinated individuals. We focus here on samples collected between 28 June 2021 and 1 December
61 2021, an interval that spans the time when Delta virus first accounted for at least 90% of sequenced specimens
62 in Wisconsin and the first detection of an Omicron sequence on 4 December 2021 ([https://www.dhs.wisconsin.
63 gov/news/releases/120421.htm](https://www.dhs.wisconsin.gov/news/releases/120421.htm)).

64 Methods

65 Study design

66 To estimate nasal viral RNA burden, we compared RT-PCR cycle threshold (Ct) data from 30,101 test-positive
67 anterior nasal swab specimens from fully vaccinated (n =9,347) or unvaccinated (n = 11,084) individuals. Sam-
68 ples were collected using the same collection kits from multiple clinic locations. All viral RNA extraction and
69 RT-PCR was performed at a single commercial testing provider (Exact Sciences, Madison, WI) using the same
70 protocol. Because this is a cross-sectional study analyzing specimens submitted for clinical testing, we are only
71 able to analyze a single timepoint from most individuals in our cohort. The estimated prevalence of Delta in Wis-
72 consin was 60% at the start of the study on 28 June 2021, reached 95% by 23 July 2021, and remained at or
73 above 95% until 12 December, 2021 (outbreak.info). The cutoff date was chosen to exclude samples containing
74 the Omicron variant, which was first detected in Wisconsin 4 December 2021.

75

76 **RT-PCR assay**

77 The Flu-SC2 Multiplex Assay (<https://www.cdc.gov/coronavirus/2019-ncov/lab/multiplex.html>) as implemented
78 by Exact Sciences was used to determine Ct values. This RT-PCR assay can simultaneously detect nucleic acid
79 from SARS-CoV-2, as well as Influenza A and B from anterior nasal swabs. RNA extraction was conducted us-
80 ing Exact Sciences Corporation's proprietary extraction procedure on the Hamilton STARlet liquid handler. The
81 oligonucleotide primers and probe for detection of SARS-CoV-2 were selected from an evolutionarily conserved
82 region of the 3' terminus of SARS-CoV-2 genome and also cover part of the 3'-terminal portion of the nucleocap-
83 sid (N) gene. RNA isolated from anterior nasal swab specimens was reverse transcribed into cDNA and amplified
84 using the ThermoFisher TaqPath 1-Step RT-qPCR Master Mix and Applied Biosystems 7500 Fast Dx Real-Time
85 PCR Instrument with SDS version 1.4.1 software. Controls included a no-template control, a positive extraction
86 control containing human RNase P, and an internal control for RNase P.

87 **Defining vaccination status**

88 Individuals were considered fully vaccinated at the time of testing if vaccine registry or self-reported data indi-
89 cated receiving a final vaccine dose at least 14 days prior to submitting the specimen that tested positive for
90 SARS-CoV-2 and was used in our analysis. We used validated public health vaccine registries for the State of
91 Wisconsin where possible. Self-reported vaccination status was included with sample metadata submitted by
92 testing providers to the Exact Sciences laboratory; when individuals' vaccination status was not available in
93 public health databases, we used self-report data to determine status. Comparing self-reporting to data from
94 vaccine registries determined that under-reporting of full vaccination status was more common than over-report-
95 ing (**Supplemental Figure 1**).

96

97 Specimens from individuals who were partially vaccinated (i.e., had not received a complete vaccine series, were
98 tested <14 days after the final dose, or those whose vaccination dates were after the sample collection date)
99 were excluded. We also excluded 430 samples from individuals who received a booster vaccine dose prior to
100 the sample collection date, since these individuals represented a small fraction of the total number of available
101 samples and booster effects could confound our analyses.

102 **Virus isolation and plaque assay**

103 With an initial set of specimens with Ct values <25, we assessed the presence of infectious virus by inoculating
104 residual specimens onto a monolayer of Vero E6/TMPRSS2 cells and monitoring for the presence of cytopathic
105 effects over 5 days. Specimens were selected by N1 Ct-matching between fully vaccinated and unvaccinated
106 persons. Specimens from individuals with unknown vaccine status were excluded from this assay. With a sec-
107 ond set of samples, we determined virus titer, expressed as plaque-forming units (PFU) per ml specimen, by
108 using a 10-fold dilution series along with undiluted samples to infect a monolayer of Vero E6/TMPRSS2 cells
109 (100 μ l per well) for 30 minutes at 37°C. The cells were washed once to remove unbound virus, then overlaid
110 with 1% methylcellulose for four days at which time plaques were counted.

111 **Statistical analysis**

112 We used analysis of variance (ANOVA) to evaluate how Ct values varied between age groups, sexes, and by

113 vaccine product, as well as two-way interactions between these factors. Raw Ct values were not normally dis-
114 tributed, so we log-transformed all Ct values prior to ANOVA, and confirmed normality by plotting residuals and
115 normal probability (**Supplemental Figure 2**). We report least square means along with the corresponding 95%
116 confidence intervals (CIs). Tukey's Honestly Significant Difference Method (HSD) was used to control the type
117 I error when conducting multiple comparisons between groups. Because our dataset included individuals with
118 varying amounts of time between vaccination and SARS-CoV-2 infection, it is possible that waning levels of
119 immunity could impact susceptibility to infection and/or viral loads after vaccination. To determine whether there
120 was a relationship between time since vaccination and Ct values in infected persons, we conducted additional
121 regression analyses that included months since completion of vaccination as a vaccine manufacturer-specific
122 continuous predictor variable. Months since completion of vaccination was defined as the number of days since
123 completion divided by 30.44, the average number of days per month.

124

125 In order to quantify and interpret differences between groups, we calculated standardized differences (Cohen's
126 effect size d), defined as the mean differences between groups divided by the pooled standard deviations. Effect
127 sizes of $d < 0.2$ were considered to indicate either no difference or a negligible difference between populations. An
128 effect size of 0.2 to 0.5 indicated a small difference, 0.5 to 0.8 was a moderate difference, and > 0.8 was a large
129 difference. The proportions of subjects with Ct values < 25 were compared between groups using a chi-square
130 test.

131

132 The results of the primary comparisons were confirmed by conducting nonparametric analyses. Specifically, the
133 nonparametric Wilcoxon rank sum test was used to conduct comparisons between Ct values between the two
134 groups, and the nonparametric Kruskal-Wallis test was used to conduct the comparisons of Ct values between
135 more than two groups. Statistical analyses were conducted using SAS software (SAS Institute, Cary NC), ver-
136 sion 9.4, figures were plotted using the R package ggplot2 [6] or from Prism version 9.3.1.

137

138 Results

139 Individuals infected with SARS-CoV-2 despite vaccination have low Ct values.

140 SARS-CoV-2 RT-PCR Ct values <25 had previously been associated with shedding of infectious SARS-CoV-2
141 [7,8]. We observed Ct values <25 in 6,253 of 9,347 fully vaccinated (67%) and 6,739 of 11,084 (61%) unvacci-
142 nated individuals (**Figure 1A**). Because of the very large number of samples, very small differences in outcome
143 variables may nonetheless reach statistical significance when using p values with a traditional alpha set to 0.05.
144 That is, we may find small differences between groups that are statistically significant ($p < 0.05$), but have a
145 negligible effect ($d < 0.2$). In order to quantify the magnitude of differences between groups, we calculated stan-
146 dardized differences (Cohen's effect size d), defined as the mean differences between the groups divided by
147 the pooled standard deviations. A value of $d < 0.2$ indicates negligible effects of the analyzed variables on the
148 outcome variable. Here we report values for both p and d for completeness. We observed no significant effect of
149 vaccination status on Ct values in infected persons (Cohen's $d=0.14$, $p<0.0001$; **Table 1**). Low Ct values were
150 detected in vaccinated people whether or not they reported symptoms at the time of testing (**Figure 1B**), with Ct
151 values <25 detected in 65% (95% CI:63-66%) of unvaccinated symptomatic individuals and in 70% (95% CI:69-
152 71%) of fully vaccinated symptomatic individuals ($p<0.0001$). Notably, for symptomatic individuals, time from
153 symptom onset to testing did not vary by vaccination status. Both vaccinated and unvaccinated individuals in our
154 population reported a median time of 2.4 days between symptom onset and testing. 92% of individuals in our
155 dataset sought testing within 6 days of symptom onset. Together these results suggest that our observations are
156 not confounded by biases in test-seeking behavior between vaccinated and unvaccinated persons (Two-sided
157 K-S test: $p=0.0012$; medians 2.40d unvaccinated, 2.42d vaccinated, **Supplemental Figure 3**).

158

159 Table 1: *Vaccinated vs. Unvaccinated*

	Means	95% CI	Effect size d	p-value
160 Unvaccinated (N=11,084)	22.9	22.8-23.0	0.14	<0.0001
162 Vaccinated (N=9,347)	22.1	22.0-23.2		

163

164 *Interpretation of effect size d: ($d<0.2$ no difference/negligible difference, 0.2-0.5 small difference, 0.5-0.8 moder-*
165 *ate difference, >0.8 large difference)*

166 Individuals infected with SARS-CoV-2 despite vaccination shed infectious virus.

167 Previous studies focusing primarily on unvaccinated individuals suggested that RT-qPCR Ct values <25 may be
168 strongly associated with the shedding of infectious SARS-CoV-2 [8,9]. To determine whether vaccinated persons
169 with potentially high viral burdens might be capable of shedding infectious virus, we inoculated a subset of resid-
170 ual specimens with Ct values <25 onto a monolayer of Vero E6/TMPRSS2 cells and monitored for the presence
171 of cytopathic effects over 5 days. Specimens were selected by N1 Ct-matching between fully vaccinated and
172 unvaccinated persons. Specimens from individuals with unknown vaccine status were excluded from this assay.
173 37 of 39 specimens from vaccinated individuals contained culturable SARS-CoV-2, as compared with 15 of 17
174 specimens from unvaccinated persons (**Supplemental Figure 4**). We therefore performed virus titration on a

175 second set of samples with Ct < 25 and found no difference in infectious virus titer between samples from vacci-
176 nated vs. unvaccinated individuals (**Figure 1C**).

177

178 **Ct value in breakthrough infection is not strongly affected by vaccine product,** 179 **age, or sex.**

180

181 We considered whether different vaccine products affected Ct values observed in individuals with breakthrough
182 infections. Vaccination had negligible effects on mean Cts in vaccinated as compared with unvaccinated individu-
183 als, regardless of the manufacturer, (Janssen (JNJ-78436735) effect size $d=0.18$, $p<0.0001$; Moderna (mRNA-
184 1273) effect size $d=0.07$, $p=0.0052$; Pfizer (BNT162b2) effect size $d=0.17$, $p<0.0001$; **Supplemental Figure 5A**;
185 see also **Supplemental Table 1**). Low-Ct samples were found in similar proportions among all groups, Janssen
186 68% Ct<25, Moderna 64% Ct<25 and Pfizer 68% Ct<25.

187

188 Vaccine effectiveness, particularly against symptomatic, test-positive SARS-CoV-2 infection, wanes with time af-
189 ter vaccine receipt [10–21]. We therefore asked whether Ct values decreased as a function of time between last
190 vaccination and the time at which individuals tested positive for SARS-CoV-2 infection. Indeed, when considering
191 all vaccine products combined, there was a small, but statistically significant decrease in Ct values (consistent
192 with higher levels of SARS-CoV-2 RNA in swab specimens) as the time between last vaccination and positive
193 test increased (Slope: -0.18 , 95% CI: $-0.26 - 0.10$; p -value <0.0001 ; **Supplemental Figure 5B**). However, when
194 we stratify individuals according to vaccine product received, we find that this effect seems to be driven principal-
195 ly by high Ct values among Pfizer vaccine recipients infected in the first month after vaccination, as the slopes
196 of Ct value vs time between vaccination and infection are not significantly different from zero for recipients of the
197 other two products (**Supplemental Figure 5B**).

198

199 Age and male sex have been considered risk factors for COVID-19 disease [22–26]. While one might hypothe-
200 size that older individuals and/or males might have higher SARS-CoV-2 burdens and therefore lower Ct values
201 at the time of testing, evidence for this is mixed, with some studies reporting lower Ct values in older individuals
202 [24,27], others in younger individuals [28], and still others finding no difference by age [20,29–34]. We therefore
203 stratified groups based on age and compared Ct values by age group. Vaccination status had negligible effects
204 on Ct values ($d<0.2$) for all age groups considered except those aged 0-11 years (**Supplemental Table 2**). In
205 this group, there were very few vaccinated individuals (N=7), as would be expected because vaccines had not
206 been approved for those 11 and under for most of our study period. Therefore, despite the significant effect size
207 ($d=0.79$, $p=0.0466$), we do not believe our data strongly support the notion that vaccination status has a strong
208 effect on Ct value in children under 12. When comparing Ct values between unvaccinated and vaccinated within
209 males and females, negligible differences were observed (female: $d=0.14$, male: $d=0.15$; **Supplemental Table**
210 **3**).

211

212 Discussion

213 The emergence of Delta variants in the United States led to the first wave of increasing case burdens following
214 the widespread availability of SARS-CoV-2 vaccines. At the time, prevailing public health recommendations were
215 that vaccinated persons need not use face coverings in indoor settings. These recommendations were based in
216 part on the fact that vaccines demonstrated remarkable effectiveness against test-positive SARS-CoV-2 infec-
217 tion in initial clinical trials conducted in 2020 [35–40], suggesting that vaccinated persons might play negligible
218 roles in SARS-CoV-2 transmission. However, the initial vaccine effectiveness studies were conducted when
219 ancestral variants predominated, prior to the emergence of variants of concern. Here we conducted a compre-
220 hensive retrospective analysis of RT-PCR Ct values in persons infected with SARS-CoV-2 during the time when
221 Delta variants predominated, to determine whether individuals infected with Delta variants despite vaccination
222 could be involved in community spread of SARS-CoV-2. Combined with other studies [41,42] our data indicate
223 that vaccinated as well as unvaccinated individuals infected with SARS-CoV-2 Delta variants can shed, and po-
224 tentially transmit, infectious virus [43,44]. We find low Ct values in substantial proportions of both unvaccinated
225 and vaccinated individuals who tested positive for SARS-CoV-2 during the time when Delta variants predominat-
226 ed, in agreement with other recent reports [41,44–47]. The occurrence in our dataset of positive samples from
227 multiple Wisconsin counties without a linking outbreak (more than 80% of samples were not associated with an
228 outbreak known to public health) indicate that Delta-lineage SARS-CoV-2 can achieve low Ct values consistent
229 with transmissibility in fully vaccinated individuals across a range of environments. Importantly, we also show that
230 infectious SARS-CoV-2 is found at similar titers in vaccinated and unvaccinated persons.

231
232 An important limitation of our study is that we analyzed only single specimens from each infected individual, so
233 our data cannot determine whether vaccinated individuals control virus replication in the upper respiratory tract
234 more quickly than unvaccinated persons, as other studies have suggested [42]. We also note that the duration
235 and level of infectious virus shedding varies widely among individuals [48], and that Ct values are an imperfect
236 proxy for shedding of infectious virus. However, the vast majority of individuals included in our study were tested
237 within 6 days of symptom onset (**Supplemental Figure 3**), a time before viral loads diverged in vaccinated and
238 unvaccinated persons tested daily in a previous study [42]. Our cross-sectional, laboratory-based study was also
239 not designed to detect or quantify differences in the relative roles of vaccinated and unvaccinated persons in
240 spreading SARS-CoV-2 in the community.

241
242 We find that a substantial proportion of individuals infected with Delta viruses despite vaccination had low Ct
243 values consistent with the potential to shed infectious virus. Our findings support the notion that persons infected
244 despite vaccination can transmit SARS-CoV-2. Therefore, preventing infection is critical to preventing trans-
245 mission. Vaccinated and unvaccinated persons should be tested when symptomatic or after close contact with
246 someone with suspected or confirmed COVID-19. Continued adherence to non-pharmaceutical interventions
247 during periods of high community transmission to mitigate spread of COVID-19 remains important for both vac-
248 cinated and unvaccinated individuals.

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Figure 1

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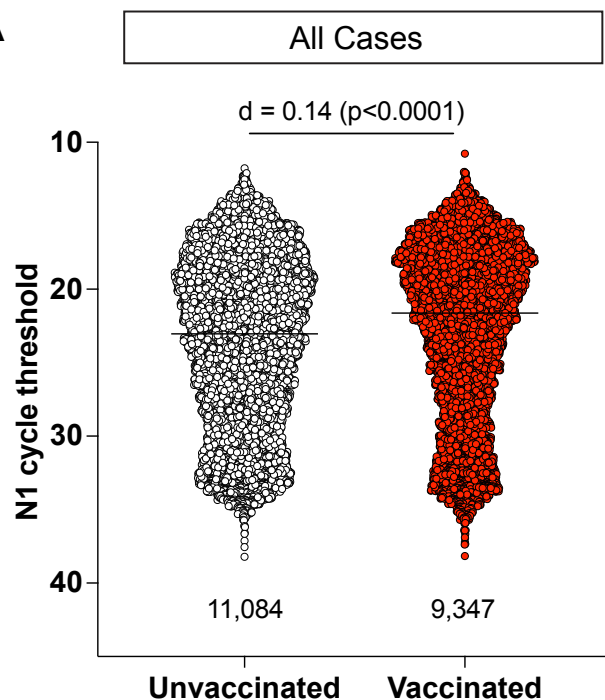
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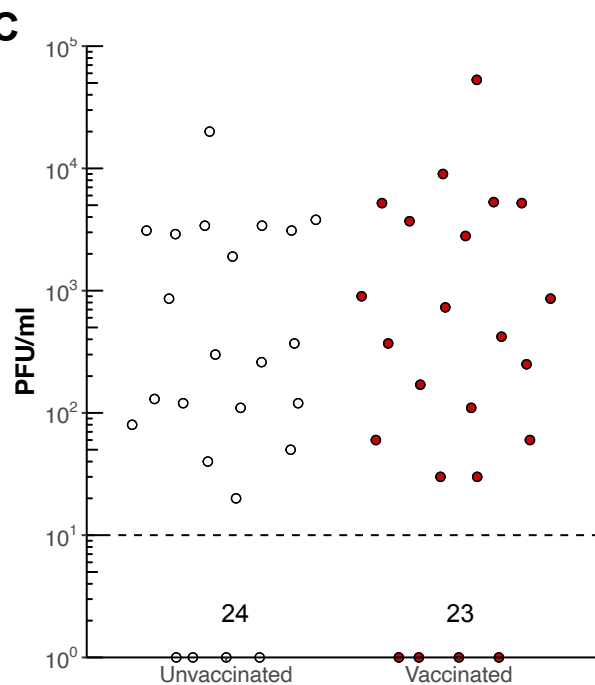
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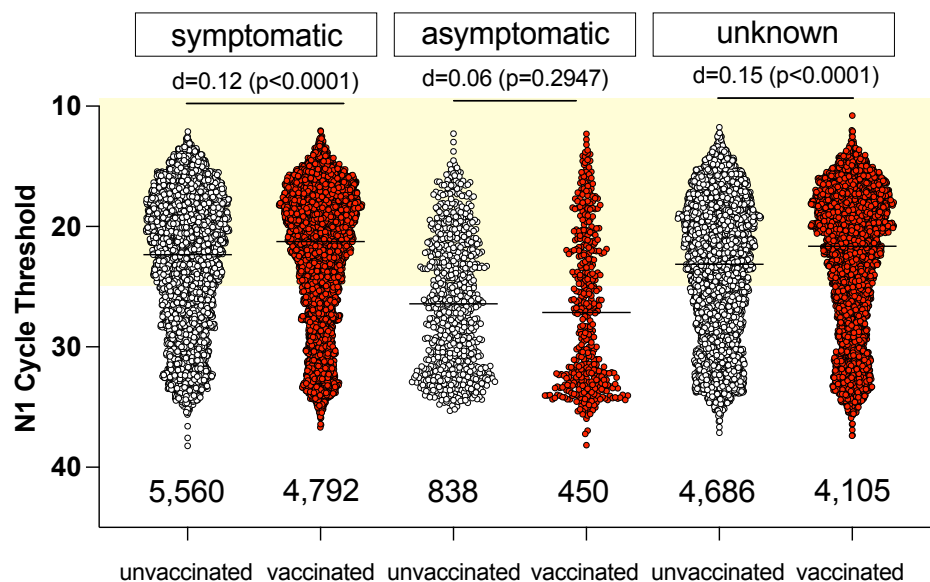
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Figure 1. Individuals infected with SARS-CoV-2 despite full vaccination have low Ct values and shed similar amounts of infectious virus as unvaccinated individuals. A. N1 Ct values for SARS-CoV-2-positive specimens were grouped by vaccination status. RT-PCR was performed by Exact Sciences Corporation, responsible for over 10% of all PCR tests in Wisconsin during this period, using a qualitative diagnostic assay targeting the SARS-CoV-2 N gene (oligonucleotides identical to CDC's N1 primer and probe set) that has been authorized for emergency use by FDA (<https://www.fda.gov/media/138328/download>). See also **Table 1**. An effect size of $d < 0.2$ is negligible. The number of samples in each group is listed under the dot plot. **B.** N1 Ct values for SARS-CoV-2-positive specimens grouped by vaccination status for individuals who were symptomatic or either asymptomatic or did not have any information, at the time of testing. Light yellow box indicates Ct values < 25 . **C.** We

292 performed plaque assays on Vero E6 TMRSS2 cells on a subset of specimens. Specimens were selected by
293 N1 Ct-matching between fully vaccinated and unvaccinated persons. Specimens from individuals with unknown
294 vaccination status were excluded from the analysis. Infectious titers are expressed as plaque-forming units
295 (PFU) per milliliter of specimen. Specimens underwent a freeze-thaw cycle prior to virus titration.

296

297 **Supplemental materials**

298 **Supplemental Table 1: Comparisons between vaccine type**

	Mean	95% CI	p-value [‡]	p-value ¹	p-value ²	p-value ³	p-value ⁴	p-value ⁵	p-value ⁶
Unvaccinated	22.9	22.8-23.0	<0.0001	<0.0001	0.0052	<0.0001	0.0064	0.9870	0.0001
Janssen	21.9	21.6-22.2							
Moderna	22.5	22.3-22.7							
Pfizer	22.0	21.8-22.1							

304 ‡ comparisons between all groups

305 ¹: comparison Unvaccinated vs. Janssen (adjusted for multiple comparisons using Tukey's HSD method)

306 ²: comparison Unvaccinated vs. Moderna (adjusted for multiple comparisons using Tukey's HSD method)

307 ³: comparison Unvaccinated vs. Pfizer (adjusted for multiple comparisons using Tukey's HSD method)

308 ⁴: comparison Janssen vs. Moderna (adjusted for multiple comparisons using Tukey's HSD method)

309 ⁵: comparison Janssen vs. Pfizer (adjusted for multiple comparisons using Tukey's HSD method)

310 ⁶: comparison Moderna vs. Pfizer (adjusted for multiple comparisons using Tukey's HSD method)

311

312 **Supplemental Table 2: Comparison of Ct values in vaccinated and unvaccinated persons, stratified by age group (there is a significant interaction between age group and vaccination status, p<0.0001)**

	Not Vaccinated		Vaccinated		Effect size <i>d</i>	p-value
	Mean	95% CI	Mean	95% CI		
0-11 yr	23.9	23.7-24.1	19.8	16.5-23.8	0.79	0.0466
12-18 yr	23.0	22.8-23.3	23.9	22.5-23.5	0.00	0.9242
19-35 yr	22.4	22.2-22.6	23.0	22.1-22.6	0.00	0.8846
36-60 yr	22.3	22.1-22.5	21.9	21.8-22.1	0.07	0.0080
>61 yr	22.3	21.9-22.8	22.1	21.8-22.3	0.05	0.3239

321

322 **Supplemental Table 3: Comparison of Ct values in vaccinated and unvaccinated persons, stratified by sex.**

	Unvaccinated		Vaccinated		Effect size <i>d</i>	p-value
	Mean	95% CI	Mean	95% CI		
Female	23.0	22.9-23.2	22.3	22.1-22.4	0.14	<0.0001
Male	22.8	22.6-22.9	22.0	21.8-22.1	0.15	<0.0001

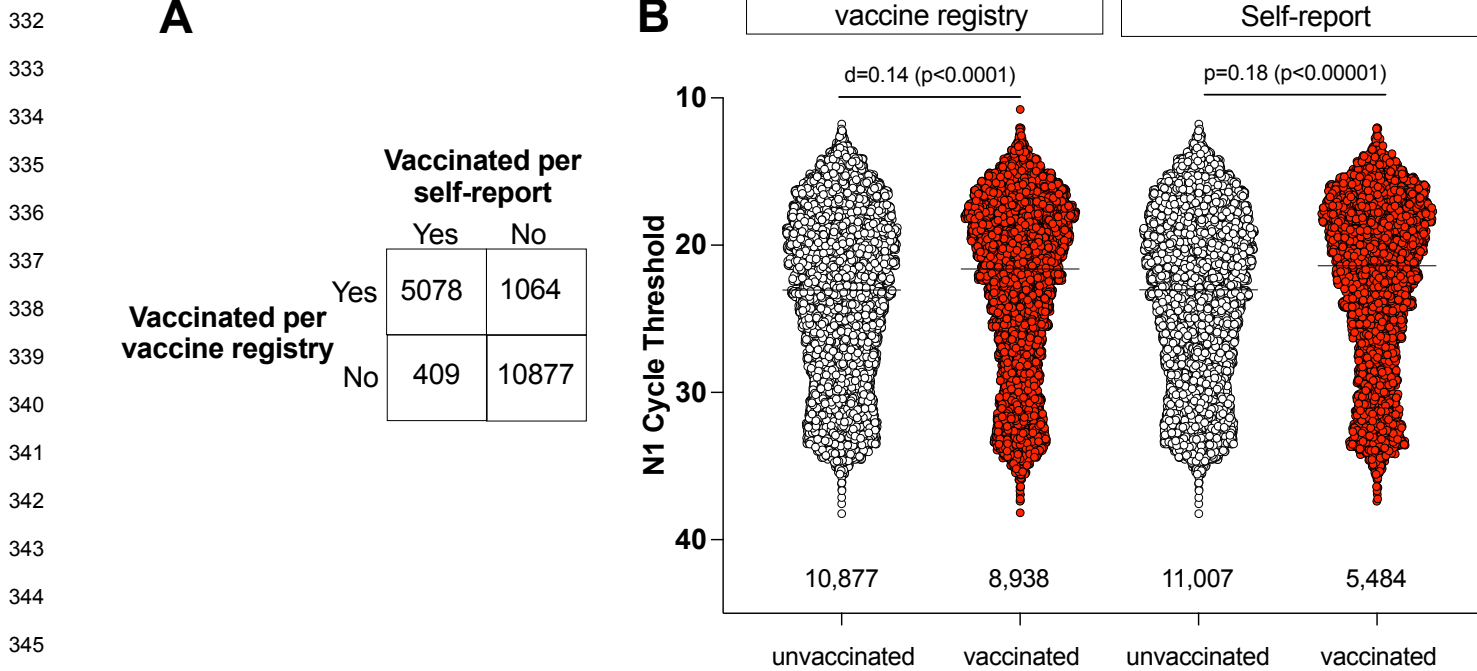
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331 **Supplemental Figure 1**

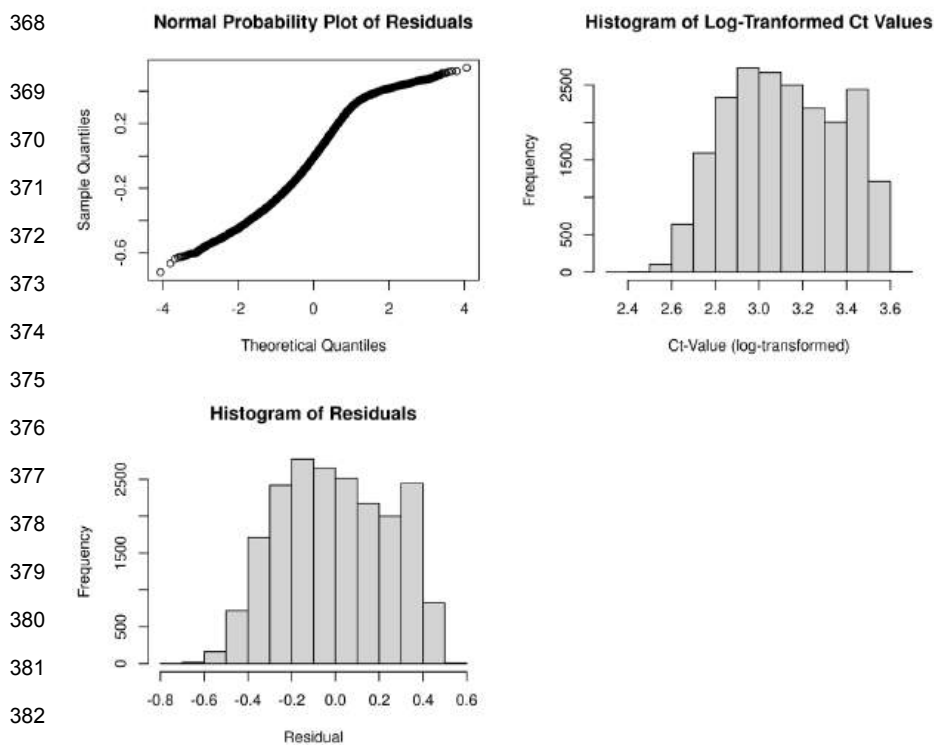


348 **Supplemental Figure 1. Concordance between self-reported vaccination status and records in public**
 349 **health vaccine registries.** Individuals were considered fully vaccinated based on vaccine registry (WIR/WEDSS)
 350 data if the registries indicated receipt of a final vaccine dose at least 14 days prior to submitting the sample used
 351 in our analysis. For individuals whose vaccination status could not be verified in the registry, self-reported data
 352 collected at the time of testing were used. Individuals were considered unvaccinated based on self-report only
 353 if there was an explicit declaration of unvaccinated status in the self-reported data. Individuals were considered
 354 fully vaccinated based on self-report if they fulfilled all of the following criteria: (1) indicated that they had received
 355 a COVID vaccine prior to testing; (2) indicated that they did not require another vaccine dose; and (3) reported a
 356 date of last vaccine dose that was at least 14 days prior to testing.

357

358 Specimens lacking data on vaccination status were excluded from the study. Specimens from partially vaccinat-
 359 ed individuals (incomplete vaccine series, or <14 days post-final dose) were also excluded. Specimens from indi-
 360 viduals who received a booster prior to sample collection were also excluded as non-equivalent to those fulfilling
 361 the criteria to be considered fully vaccinated. **A.** Of 20,431 specimens with vaccination status available from at
 362 least one source, 5,078 specimens had data available from both sources. Under-reporting of full vaccination
 363 status in self-reports 1,064/6,142 or 17%) was more common than over-reporting (409/5,487 or 7.4%). **B.** N1
 364 Ct values for SARS-CoV-2-positive specimens grouped by vaccination status for individuals whose vaccination
 365 status was determined by vaccine registry or by self-reported data.

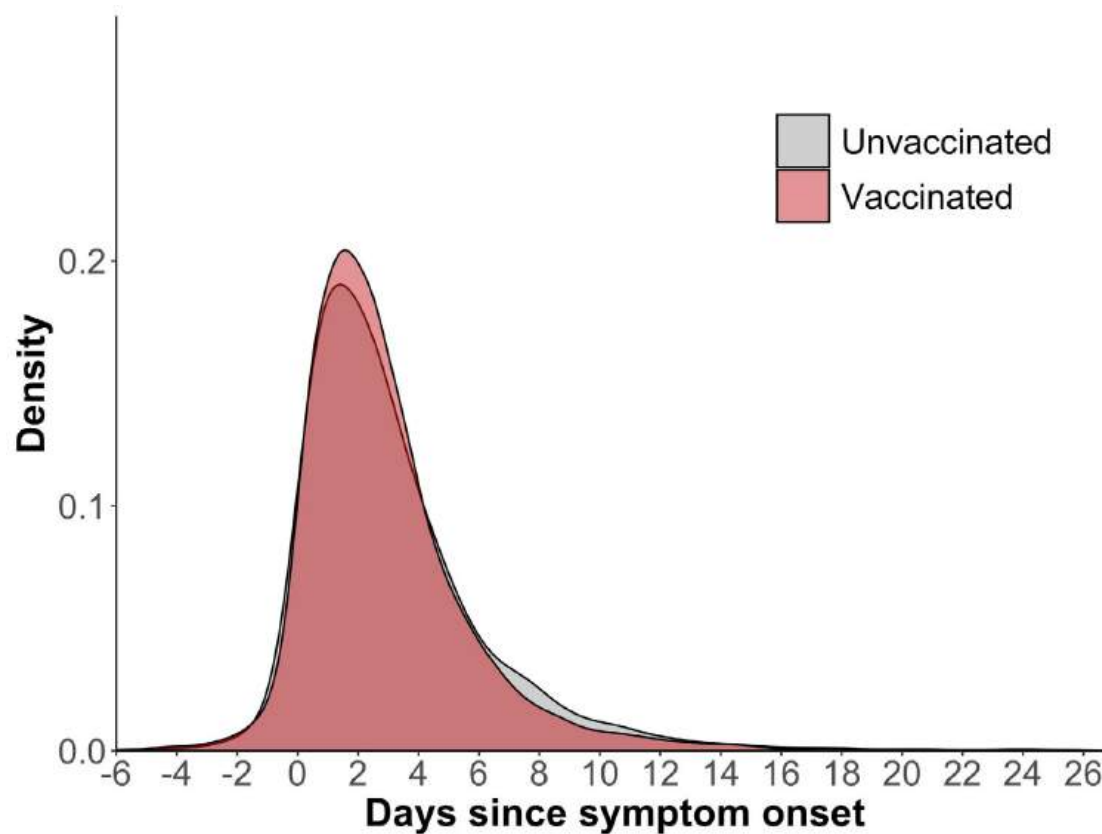
367 Supplemental Figure 2



385 **Supplemental Figure 2. Log transformation of raw Ct values results in normally distributed residuals.**
386 Raw Ct values were not normally distributed, so we log-transformed all Ct values prior to ANOVA, and confirmed
387 normality by plotting residuals and normal probability.

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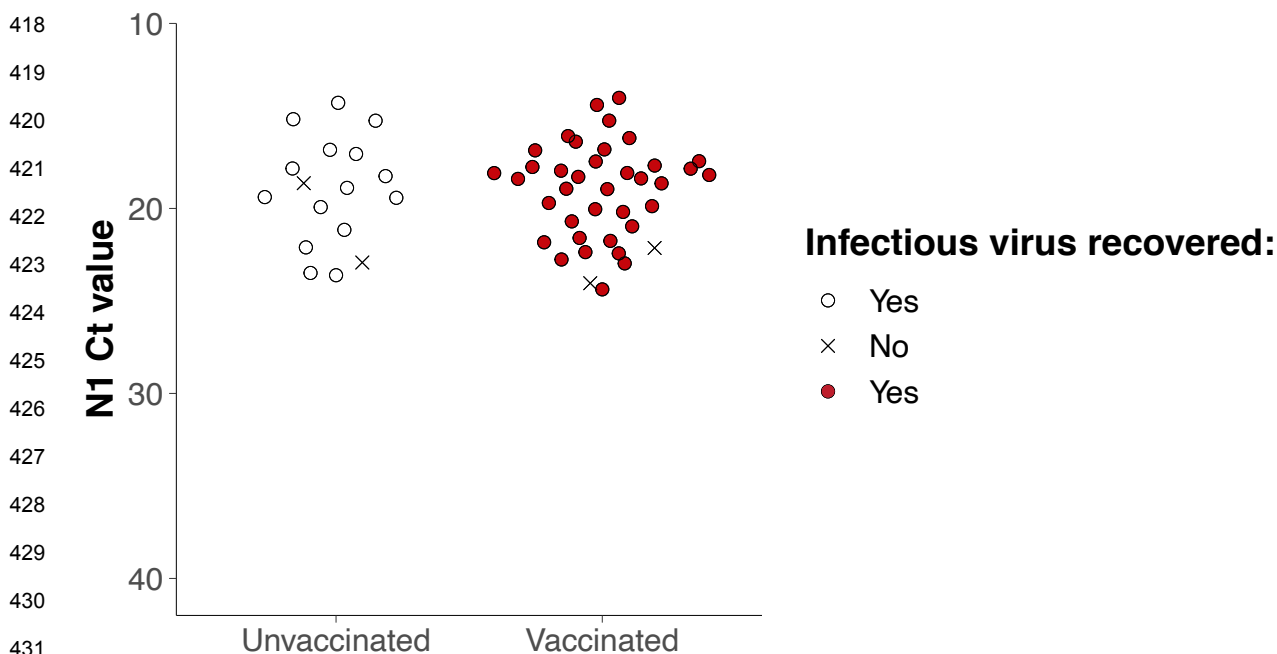
389 Supplemental Figure 3



411 **Supplemental Figure 3. Density distributions of unvaccinated and vaccinated specimen collection dates**
412 **by day since symptom onset.** Day 0 on the x-axis denotes self-reported day of symptom onset. Negative
413 values for days indicate specimen collection prior to symptom onset. Symptom onset data were available for
414 $n=6,871$ unvaccinated cases and $n=5,522$ vaccinated cases. Two-sided K-S test: $p=0.0012$; median days since
415 symptom onset were 2.4 for both unvaccinated and vaccinated cases.

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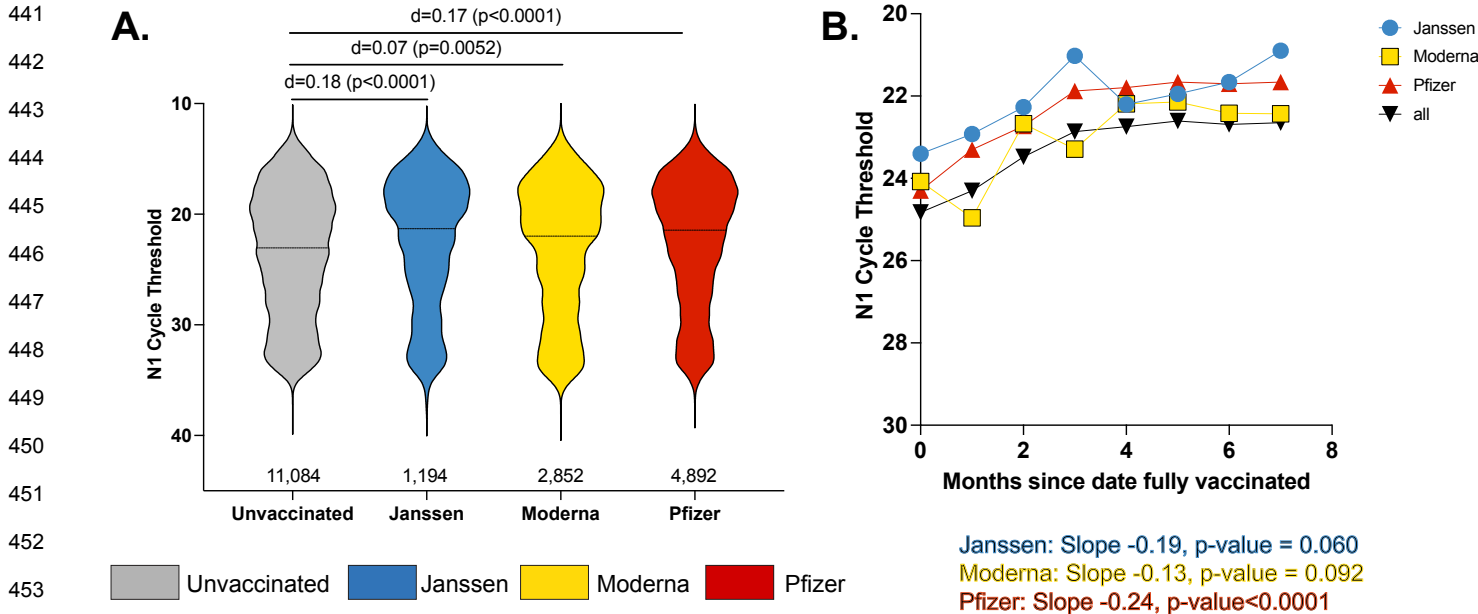
417 **Supplemental Figure 4**



434 **Supplemental Figure 4. Infectious SARS-CoV-2 detected in the majority of fully vaccinated individuals**
435 **with low Ct values.** Infectiousness was determined for a subset of N1 Ct-matched specimens with Ct <25 by in-
436 oculation onto Vero E6 TMRSS2 cells, then determining presence or absence of cytopathic effects (CPE) after
437 5 days in culture. Specimens with unknown vaccination status were excluded from the analysis. Circles indicate
438 presence of CPE; 'X' indicates no CPE detected.

439

440 **Supplemental Figure 5**



460 **Supplemental Figure 5. Ct values do not differ substantially by vaccine type.** **A.** Comparison of mean N1
 461 Ct values in all specimens, stratified by vaccine type shows negligible effect ($d < 0.2$) of vaccine type on Ct val-
 462 ue at time of positive test, relative to unvaccinated persons. **B.** The time analysis showed a decrease in N1 Ct
 463 values with time over 7 months. Combining all three vaccines, there was a significant decrease over the first 7
 464 months, with a slope of -0.18 (95% CI: -0.26 - 0.10), p value < 0.0001. Individually, Janssen had a slope -0.19
 465 (95% CI: -0.38 to -0.001, p-value=0.060), Moderna had a slope of -0.13 (95% CI: -0.28 - 0.02, p-value=0.092),
 466 Pfizer had a slope of -0.24 (95% CI: -0.24 to -0.13, p-value<0.0001).

467

468 **Conflict of interest**

469 The authors declare no conflicting interests.

470 **Ethics statement**

471 The University of Wisconsin-Madison Institutional Review Board deemed that this project qualifies as public
472 health surveillance activities as defined in the Common Rule, 45 CFR 46.102(l)(2). As such, the project is not
473 deemed to be research regulated under the Common Rule and therefore, does not require University of Wiscon-
474 sin-Madison IRB review and oversight.

475

476 The opinions expressed by authors contributing to this journal do not necessarily reflect the opinions of the Cen-
477 ters for Disease Control and Prevention or the institutions with which the authors are affiliated.

478 **Data availability**

479 Data and processing workflows are available at <https://go.wisc.edu/p22116>. To protect potentially personally
480 identifiable information, the publicly available dataset contains only PCR Ct values, vaccine status, age, sex,
481 manufacturer, symptom status, virus culture status, and days from symptom onset to testing for each specimen.

482 References

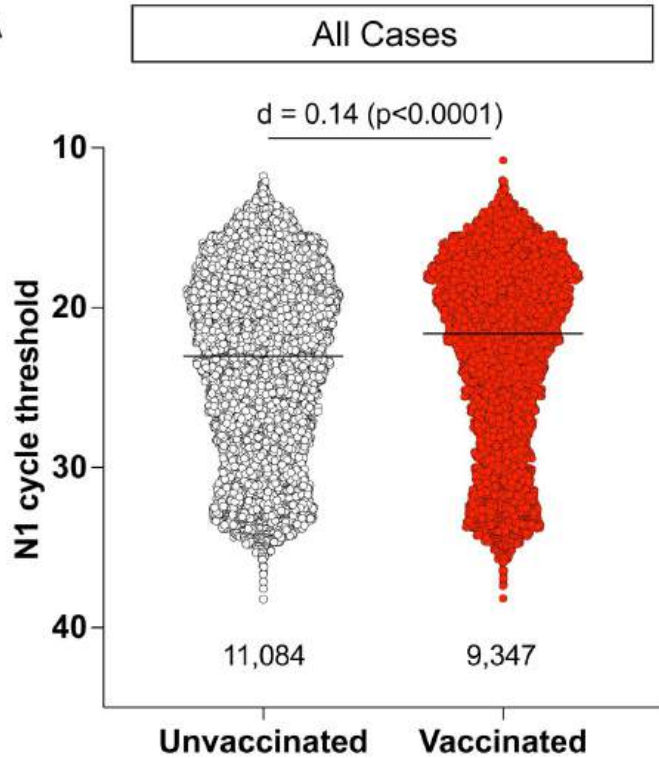
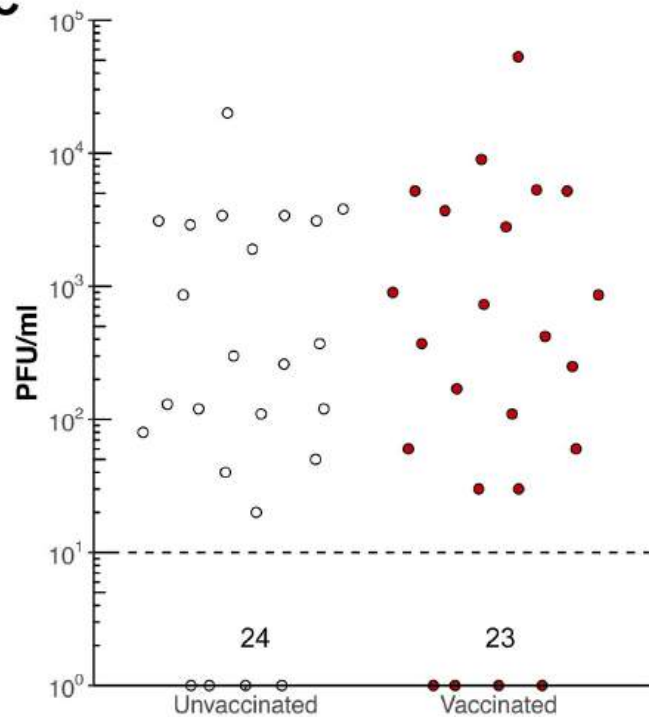
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