

- 1 **Comparison of upper respiratory viral load distributions in asymptomatic and**
2 **symptomatic children diagnosed with SARS-CoV-2 infection in pediatric hospital testing**
3 **programs**
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34 analysis.
35
36 Manuscript word count: 3057
37
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48

49

50 **Abstract**

51 The distribution of upper respiratory viral loads (VL) in asymptomatic children infected with
52 SARS-CoV-2 is unknown. We assessed PCR cycle threshold (Ct) values and estimated VL in
53 infected asymptomatic children diagnosed in nine pediatric hospital testing programs.
54 Records for asymptomatic and symptomatic patients with positive clinical SARS-CoV-2 tests
55 were reviewed. Ct values were adjusted by centering each value around the institutional median
56 Ct value from symptomatic children tested with that assay, and converted to estimated VL
57 (copies/mL) using internal or manufacturer data.

58 Adjusted Ct values and estimated VL for asymptomatic versus symptomatic children (118 vs.
59 197 ages 0-4; 79 vs 97 ages 5-9; 69 vs 75 ages 10-13; 73 vs 109 ages 14-17) were compared.
60 The median adjusted Ct value in asymptomatic children was 10.3 cycles higher than for
61 symptomatic children ($p < 0.0001$), and VL 3-4 logs lower ($p < 0.0001$); differences were
62 consistent ($p < 0.0001$) across all four age brackets. These differences were consistent across all
63 institutions and by sex, ethnicity, and race. Asymptomatic children with diabetes (OR 6.5, $p =$
64 0.01), recent contact (OR 2.3, $p = 0.02$), and testing for surveillance (OR 2.7, $p = 0.005$) had
65 higher estimated risk of having a Ct value in the lowest quartile than children without, while
66 immunocompromise had no effect.

67 Children with asymptomatic SARS-CoV-2 infection had lower levels of virus in the
68 nasopharynx/oropharynx than symptomatic children, but timing of infection relative to diagnosis
69 likely impacted levels in asymptomatic children. Caution is recommended when choosing
70 diagnostic tests for screening of asymptomatic children.

71

72 **Introduction**

73 Compared to adults, children have been less impacted by the COVID-19 pandemic in terms of
74 both disease incidence and severity (1); this has held true even in populations known to be at
75 high risk of complications from other respiratory viruses, such as infants (2) and
76 immunocompromised children (3). Early reports suggested that children were not major
77 contributors to SARS-CoV-2 spread (4), but shelter-in-place advisories and school closures early
78 in pandemic responses may have limited opportunities for spread among children in the
79 community. More recent data suggest that children can transmit SARS-CoV-2 to adults and other
80 children, although transmission rates and the impact of age are unclear (5, 6). Despite our
81 evolving understanding of COVID-19 epidemiology and transmission in children, many
82 questions remain.

83
84 Upper respiratory viral loads are associated with transmission risk for other respiratory viruses
85 (7, 8), but the association between SARS-CoV-2 upper respiratory viral load and transmission is
86 unknown. Among those with non-severe symptomatic COVID-19 illness, children have similar
87 nasopharyngeal (NP) viral loads to adults (9), and young children may have greater NP viral
88 loads than older children and adults (10). Culture-competent SARS-CoV-2 can be isolated from
89 children of all ages with symptomatic COVID-19 (11). While these data highlight the potential
90 for children of any age to transmit SARS-CoV-2, the differences in upper respiratory viral loads
91 among children with and without symptoms of COVID-19 are unknown. In contrast, multiple
92 studies have demonstrated high viral loads in asymptomatic adults, and the ability of
93 asymptomatic adults to transmit SARS-CoV-2 has been well described (12, 13).

94

95 Identifying the role of children without COVID-19 symptoms in the transmission of SARS-CoV-
96 2 is a critical unanswered question. Hospitals have established large-scale screening programs
97 requiring testing of asymptomatic children prior to surgery, aerosol-generating procedures,
98 and/or hospital admission. In the community, the role of asymptomatic children in transmission
99 of SARS-CoV-2 impacts decisions about the safety of reopening day cares and returning to
100 classroom instruction in schools, as well as informing decisions regarding strategies for post-
101 exposure SARS-CoV-2 testing in asymptomatic children as a method for interrupting ongoing
102 transmission.

103

104 To further clarify the viral burden in children infected with SARS-CoV-2, we performed a
105 collaborative study of asymptomatic and symptomatic children tested by nine children's
106 hospitals in the United States and Canada. Our objectives were to delineate the distribution of
107 SARS-CoV-2 viral loads in upper respiratory samples from asymptomatic and symptomatic
108 children diagnosed through hospital testing programs and to determine whether viral load
109 distributions are consistent across age categories, SARS-CoV-2 assays, and institutions, all of
110 whom were experiencing different stages of COVID-19 community activity.

111

112 **Materials and Methods**

113 Study Population

114 Charts for patients (ages 0-17 years [y]) testing positive on SARS-CoV-2 assays in use for
115 clinical testing at each institution were retrospectively reviewed (blinded to Ct values, which
116 were not reported clinically).

117

118 Symptomatic patients had two or more symptoms consistent with COVID-19 (cough,
119 fever/chills, shortness of breath, sore throat, abdominal pain, diarrhea, fatigue, myalgias, new
120 loss of taste or smell, headache, congestion/rhinorrhea, nausea/vomiting, rash, or conjunctivitis)
121 at the time of testing, and were tested due to clinical suspicion of COVID-19. Asymptomatic
122 patients had no symptoms of COVID-19 (as defined above), or any clinical suspicion of COVID-
123 19 (other than potential contact status), at time of test. The primary reason for testing was coded
124 as either surveillance (contact tracing or broad community surveillance), pre-operative/aerosol
125 generating procedure (pre-op/AGP), or hospital admission screening (pre-admission). Only the
126 first positive test for each patient was included. Within each institution, each asymptomatic
127 patient was matched with up to two symptomatic patients by age bracket (0-4y, 5-9y, 10-13y, 14-
128 17y) and date of testing (as close as possible, but within 30 days).

129
130 At each institution, all asymptomatic and symptomatic patients compared were required to have
131 been tested with the same sample type [either NP or oropharyngeal (OP)].
132 Clinical data collected for each patient at the time of testing included the following: age, sex,
133 race, ethnicity, immunocompromise, diabetes. For symptomatic patients, the presence/absence of
134 symptoms from the list above were scored. For asymptomatic patients, data were also collected
135 on known contacts and their timing prior to the test date (≤ 2 weeks, >2 weeks, or unknown) and
136 any development of new symptoms of COVID-19 within 5D after the positive test.

137 138 Ct values and viral load estimates

139 Molecular assay used and Ct values were recorded; if the assay had more than one target, the Ct
140 values for the sample were averaged, and if only one target was positive, that single Ct was used.

141 Each institution calculated the median/interquartile range (IQR) for Ct values for all positive
142 symptomatic pediatric patients (0-17y) (or a representative subset) tested with that assay at that
143 institution over the study period (i.e. institutional symptomatic median), for each assay used at
144 that institution. For each assay used, each institution also provided a conversion between Ct
145 value (which is inversely related to the amount of nucleic acid target in a sample) and estimated
146 viral load (copies/mL of original patient sample) based on data from internal validation studies,
147 the manufacturer, or package inserts.

148

149 Statistical analysis

150 To address variation in Ct data due to the use of multiple assays by the nine institutions, we
151 calculated adjusted Ct values using a centering technique. With this technique, the adjusted Ct
152 values were the difference between individual Ct values and the institutional symptomatic
153 median (defined above) for each respective assay. These observations are reported as adjusted
154 Ct values.

155 Continuous variables were summarized using medians and IQR; categorical variables were
156 summarized using counts and percentages. Due to non-normality of data, non-parametric
157 Wilcoxon and Fisher's exact test were used to assess for significance of differences in
158 continuous and 2x2 tables as applicable. Logistic regression analysis was used to compare
159 dichotomous outcomes and to generate odds ratios. Tests were 2-sided and a p-value<0.05 was
160 considered statistically significant. SAS (version 9.4, SAS Institute, Cary, NC) software was
161 used.

162

163 Each institution independently obtained IRB approval for chart review with waiver of informed
164 consent; only fully deidentified data were analyzed.

165

166 **Results**

167 Study periods for the nine institutions covered from March to July, 2020 (Table S1). Age
168 distributions and other demographic and comorbidity data for the combined asymptomatic (n =
169 339) and symptomatic (n = 478) populations are listed in Table 1. Patients contributed by each
170 institution are summarized in Table S2. Distribution of symptoms in the symptomatic children
171 are presented in Table S3.

172

173 Because Ct values are assay-dependent and the goal was to analyze aggregate Ct data from
174 multiple assays and institutions, Ct values for each assay were adjusted by centering each value
175 around the institutional symptomatic median (Methods, Table S1). Each institution also provided
176 a viral load estimate (copies/mL sample) for each Ct value (Methods, Table S1).

177

178 Adjusted Ct values and estimated viral loads for asymptomatic versus symptomatic children in
179 all age brackets were compared (Fig 1). The median adjusted Ct value in asymptomatic children
180 was 8.6 (IQR 2.5 to 12.2) compared to -1.7 (IQR -6.0 to 4.8) in symptomatic children ($p <$
181 0.0001), a difference of 10.3 Ct (Fig 1A). We observed similar results when comparing median
182 estimated viral loads in asymptomatic children [2.0×10^3 copies/mL (IQR 1.62×10^2 to 1.7×10^5)
183 versus symptomatic children [1.3×10^7 copies/mL (IQR 5.6×10^4 to 3.8×10^8)] ($p < 0.0001$) (Fig
184 1B). Differences of similar magnitude were observed in each of the four age brackets ($p < 0.0001$
185 for each age bracket, for both adjusted Ct and viral load, Figure 2A,B; Table S4), though

186 interestingly the adjusted Ct difference narrowed with increasing age (11.95 Ct for ages 0-4;
187 10.32, ages 5-9; 9.78, ages 10-13; 8.49, ages 14-17), correlating with progressively decreasing
188 median viral burden in the symptomatic group within each age bracket (Fig 2B, Table S4).
189 These differences were consistent across all institutions (Figure S1, Figure S2) and were not
190 affected by sex or ethnicity (Table S4).

191

192 To understand whether there were any patient factors that could help predict the asymptomatic
193 children with the lowest Ct values/highest viral loads, odds ratios were calculated to assess the
194 estimated risk of having a Ct value in the lowest quartile (or viral load in the highest quartile)
195 within the asymptomatic Ct value distribution.

196

197 Asymptomatic children with diabetes (OR 6.5, $p = 0.01$), recent contact with a COVID-19 case
198 (OR 2.3, $p = 0.02$), and testing for surveillance (OR 2.7, $p = 0.005$) had higher estimated risk of
199 having a Ct value in the lowest quartile than children without, while immunocompromise had no
200 effect (Table 2). Sex, race, and ethnicity also had no effect (Table 2). Similar results were
201 obtained for the same analyses using estimated viral loads (Table 2). Comparisons of median
202 adjusted Ct values and viral loads for asymptomatic patients with and without these risk factors
203 are in Table S5.

204

205 Figure 3 compares adjusted Ct values (3A) and estimated viral loads (3B) in asymptomatic
206 children by test indication (surveillance, pre-op/AGP, and pre-admission), versus symptomatic
207 children; Table S5 shows median adjusted Ct values and viral loads in those three groups.
208 Asymptomatic children tested for surveillance had significantly lower median adjusted Ct

209 values/higher estimated viral loads than those tested for pre-op/AGP or pre-admission, and
210 significantly higher adjusted Ct values/lower estimated viral loads than symptomatic patients
211 (Fig 3). Figures S3 and S4 show the patients with immunocompromise and diabetes,
212 respectively, highlighted within the adjusted Ct distributions for the asymptomatic and
213 symptomatic populations; Figures S5, S6, and S7 show the patients with known contacts, recent
214 contacts, and those tested for surveillance, respectively, highlighted within the asymptomatic
215 group.
216 Pre-symptomatic children (those who developed symptoms consistent with COVID-19 within 5
217 days following the test) trended towards higher median viral loads [7.7×10^4 (1.1×10^2 - $2.4 \times$
218 10^6), $n = 14$] than non-pre-symptomatic children [1.4×10^3 (1.3×10^2 - 7.3×10^4), $n = 172$],
219 though this difference was not significant ($p = 0.30$) (Table S5).

221 Discussion

222 Our study explores the distribution of upper respiratory viral loads in asymptomatic children
223 identified as infected with SARS-CoV-2 by hospital testing programs. By combining results
224 from nine institutions testing pediatric patients, we assembled a robust dataset across all age
225 brackets for extensive analysis. We have demonstrated that Ct values were significantly higher,
226 and estimated viral loads significantly lower, in asymptomatic children of all ages compared to
227 symptomatic children matched by age bracket and test collection date range. These differences
228 in viral burden were consistent across all nine collaborating institutions, each of which was
229 experiencing a different stage of the pandemic over the study period and using a different panel
230 of SARS-CoV-2 assays for patient testing, increasing the generalizability of our findings.

231

232 While asymptomatic and symptomatic children in this study clearly had different viral load
233 distributions, there was overlap between these distributions in all age brackets, raising the key
234 question of whether there were certain risk factors that could help to identify outliers in the
235 asymptomatic population with the lowest quartile of Ct values/highest quartile of viral loads.
236 Our analysis demonstrated that asymptomatic children with diabetes and/or recent contact with a
237 COVID-19 case, as well as those tested for surveillance purposes (rather than for pre-procedure
238 or pre-admission purposes), had significantly higher estimated risk of being in the quartile with
239 the highest viral burden. Despite small numbers of diabetic patients in our study, the finding that
240 diabetic children were more at risk of having high viral loads requires further dedicated
241 investigation, as it is consistent with studies in adults that have demonstrated more severe disease
242 and poorer prognosis in patients with diabetes (14, 15). The asymptomatic population with
243 known/recent COVID-19 contact overlapped with the population tested for surveillance
244 purposes, though not perfectly (as some pre-procedure or pre-admission patients had contacts).
245 Our data suggest that timing of infection impacted the viral load distribution among
246 asymptomatic children in our study, with patients more likely to have recent infections (i.e.,
247 recent contacts) showing higher viral loads than those potentially more likely to have remote
248 infections (those tested per pre-procedure/pre-admission protocol).
249
250 Our finding of lower viral loads in the asymptomatic children in our study raises the question of
251 what this might mean regarding their potential for disease transmission. There is evidence in the
252 literature that asymptomatic individuals can spread infection, but these data are almost
253 exclusively in adults. The prevalence of asymptomatic infection among different cohorts of
254 infected adults has been estimated to range from 18-75% (16-24); cases of transmission from

255 asymptomatic adults have been reported (25-28), and viable virus may be recovered in culture
256 from samples collected from asymptomatic individuals (18, 29).
257
258 Correlation of viral load with ability to recover virus in culture is challenging, though several
259 investigators have reported difficulty in isolating virus when viral loads measured in patient
260 samples are below approximately 1×10^5 copies/mL (9, 24, 30, 31). However, virus has been
261 recovered from samples with RNA levels as low as 1.2×10^4 copies/mL (11). It is worth noting
262 that although isolation of virus in culture has been used as a surrogate for infectivity, inability to
263 recover replicating virus in culture does not necessarily preclude transmissibility (32).
264
265 Importantly, prior work examining whether the amount of viral RNA in respiratory secretions
266 differs between symptomatic and asymptomatic individuals has generally involved well-defined
267 cohorts of adults, notably where exposure of the individuals within a given cohort likely
268 occurred recently and, in many studies, at about the same time. In general, using either Ct values
269 or conversions to viral loads, these studies have found roughly equivalent RNA levels between
270 asymptomatic and symptomatic individuals (16-18, 21, 22, 33, 34). Given that asymptomatic
271 patients with a recent known COVID-19 contact were more likely to have higher viral loads in
272 our study, one hypothesis is that the lower median viral loads in the pre-procedure/pre-admission
273 testing groups reflect that more of those children had remote infection. This suggestion is
274 supported by a recent study of children who were all close contacts of people with SARS-CoV-
275 2-infection that found similar viral loads on NP swabs from children with and without symptoms
276 (though all reported viral loads were relatively low) (35). Unfortunately, there are minimal
277 published data describing results of testing asymptomatic populations with a wider range of

278 potential exposure timing. One study which investigated asymptomatic adult healthcare workers
279 who were identified as infected through a screening program found higher Ct values (and
280 therefore lower viral loads) in those individuals as compared with adults with symptomatic
281 infection (36).

282

283 Additional data in children are limited to very small studies with conflicting results about the
284 comparability of SARS-CoV-2 RNA levels between symptomatic and asymptomatic children
285 (37, 38). More generally, symptomatic children appear to have RNA levels comparable to or
286 higher than adults (9-11) and unlike reports in adults (39), RNA levels in children do not appear
287 to correlate with severity of illness (40).

288

289 Our study has some important limitations. As above, given that our asymptomatic population
290 may be biased towards lower viral loads due to a higher frequency of remote infections picked
291 up on screening testing, it may not fully represent the distribution of viral loads in *recently*
292 *infected* asymptomatic children. We note that the 14 pre-symptomatic children in our study had
293 a slightly higher median viral load [7.7×10^4 (1.1×10^2 - 2.4×10^6)] than those who did not
294 develop symptoms, but the viral loads in these pre-symptomatic children were still relatively
295 low. Many patients in our study did not have data available regarding contacts or subsequent
296 symptoms, and data from a larger cohort of pre-symptomatic children (perhaps from dedicated
297 contact tracing programs) will be necessary to fully elucidate the range of viral burden in these
298 children; in particular, it will be critical to define the peak viral load in asymptomatic and pre-
299 symptomatic children to clarify diagnostic test options in this population. We note that even in
300 the asymptomatic surveillance sub-cohort with highest viral loads, median viral loads were still

301 significantly lower than in the symptomatic cohort. 75% of these asymptomatic subjects had
302 viral loads less than 1.2×10^7 copies/mL (and for recent contacts, 75% had less than 1.8×10^6),
303 which has implications for assay selection if the goal is to capture all positive patients under the
304 assumption that patients with any viral load can potentially transmit; in the pre-procedure and
305 pre-admission groups, almost all viral loads are likely below the limits of detection of available
306 rapid antigen tests (estimated at 1×10^6 copies/mL based on information in package inserts).
307 Additional studies will also be necessary to determine the extent to which individuals of any
308 age are able to transmit infection at low viral loads.

309 We do not believe that stage of outbreak impacted our findings because we included patients
310 from centers across the country, and we matched symptomatic and asymptomatic patients by
311 time of testing; similar results were observed at each institution. We may have slightly biased our
312 symptomatic population towards more severe disease by requiring that each patient have a
313 minimum of 2 symptoms of COVID, but we did this in order to maximize the likelihood that
314 symptoms were truly caused by SARS-CoV-2.

315

316 Our methods of combining and comparing data across institutions also have limitations. Our
317 conversion of Ct to viral load for each assay was done based either on standard curves performed
318 by the laboratory or the manufacturer, or on data in package inserts. We normalized Ct values
319 from each assay to median values for all symptomatic patients (0-17y) from that institution tested
320 by that assay to be able to make an optimal comparison across institutions and assays.

321 Importantly, we compared asymptomatic to symptomatic cohorts both by adjusted Ct value and
322 by estimated viral load and obtained similar results, indicating that these limitations were
323 effectively mitigated. Finally, we note that these limitations apply equally to both asymptomatic

324 and symptomatic cohorts from each institution and thus should not affect the comparison of
325 those cohorts.

326

327 Conclusions

328 Our findings that viral loads in asymptomatic children diagnosed with SARS-CoV-2 infection by
329 hospital testing programs are significantly lower than those in symptomatic children may provide
330 some level of reassurance about returning to daycare and school with proper safety measures
331 (masks, hygiene, distancing, and ventilation) in place and rigorous exclusion of symptomatic
332 children from the school setting. However, the observation that all age brackets of asymptomatic
333 kids include outliers with low Ct values/high viral load—and the imperfect ability to predict who
334 these outliers will be—indicates that safety precautions for daycares and schools are indeed
335 necessary. Our data underscore that timing of diagnostic testing relative to initial infection
336 impacts viral burden, and that peak viral loads in asymptomatic children remain to be defined in
337 future studies. Regardless, the lower viral loads in the asymptomatic children in our study
338 should raise caution about using low sensitivity tests for asymptomatic screening programs in
339 pediatric populations.

340

341 **Acknowledgments**

342 This research did not have any dedicated funding.

343

344 R.Y., J.D.B., C.A.B., H.W., T.J.S., S.J., S.D., R.C.J., S.C.K., A.C., J.B., X.Z., E.M., M.L., L.A.,
345 C. S., K.G., A.H-L, D.W., and N.R.P. have no conflicts of interest to report.

346

347 Other authors declare the following potential conflicts of interest: L.K.K. has received research
348 support from Merck, unrelated to this study. W.J.M. has the following engagements, all
349 unrelated to this study: local PI on trials from Ansun BioPharma, Astellas Pharma, AstraZeneca,
350 Abbott Laboratories, Janssen Pharmaceuticals, Karius, Merck, Melinta Therapeutics, Roche,
351 and Tetrphase Pharmaceuticals; and consultant for Seqirus. B.A.P. received an honorarium
352 from Quidel, unrelated to this study.

353

354 N.R.P. and L.K.K. had full access to all the data in the study and take responsibility for the
355 integrity of the data and the accuracy of the data analysis.

356

357 We wish to acknowledge all of the laboratory staff who contributed to generating the clinical
358 COVID-19 testing data analyzed in this manuscript.

359

360

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Figure Legends

Figure 1

Comparison of adjusted Ct values (A) and estimated viral loads (B) for asymptomatic (n = 339) versus symptomatic (n = 478) children. The bottom and top edges of the boxes for each cohort indicate the interquartile range (IQR), the horizontal line bisecting the box indicates the median value, and the whiskers represent values 1.5 times the IQR. P values for comparison of the respective medians are shown.

Figure 2

Comparison of adjusted Ct values (A) and estimated viral loads (B) for asymptomatic versus symptomatic children, separated by age brackets (n = 118 vs. 197 ages 0-4; 79 vs 97 ages 5-9; 69 vs 75 ages 10-13; 73 vs 109 ages 14-17). The bottom and top edges of the boxes for each cohort indicate the interquartile range (IQR), the horizontal line bisecting the box indicates the median value, and the whiskers represent values 1.5 times the IQR.

Figure 3

Comparison of adjusted Ct values (A) and estimated viral loads (B) for asymptomatic children tested for three different indications (surveillance, pre-op/aerosol-generating procedure, and pre-admission) versus symptomatic children. The bottom and top edges of the boxes for each cohort indicate the interquartile range (IQR), the horizontal line bisecting the box indicates the median value, and the whiskers represent values 1.5 times the IQR. The P values for the comparison of the medians of the surveillance and pre-procedure groups are shown.

563 **Table 1: Study participants and demographics**

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Variable	Asymptomatic (n=339)	Symptomatic (n=478)	P
Sex			
Male	178 (52.5%)	248 (51.9%)	0.887
Female	161 (47.5%)	230 (48.1%)	
Age Bracket			
0-4	118 (34.8%)	197 (41.2%)	0.136
5-9	79 (23.3%)	97 (20.3%)	
10-13	69 (20.4%)	75 (15.7%)	
14-17	73 (21.5%)	109 (22.8%)	
Ethnicity			
Hispanic/Latino	169 (49.9%)	285 (59.6%)	0.002
Non-Hispanic/Latino	132 (38.9%)	131 (27.4%)	
Not specified	38 (11.2%)	62 (13.0%)	
Immunocompromise			
Yes	35 (10.3%)	16 (3.3%)	<0.001
No	304 (89.7%)	462 (96.7%)	
Diabetes			
Yes	9 (2.7%)	10 (2.1%)	0.642
No	330 (97.3%)	468 (97.9%)	
Race			0.002
Asian	16 (5.0%)	11 (2.4%)	
Black or African American	58 (18.1%)	70 (15.2%)	
White or Caucasian	135 (42.1%)	161 (34.8%)	
Other ^a	112 (34.9%)	220 (47.6%)	

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- a. "Other" reflects the response of patients that did not wish to select one of the other race categories, based on chart review. Includes American Indian/Alaska Native (n = 1), Native Hawaiian/Other Pacific Islander (n =1).

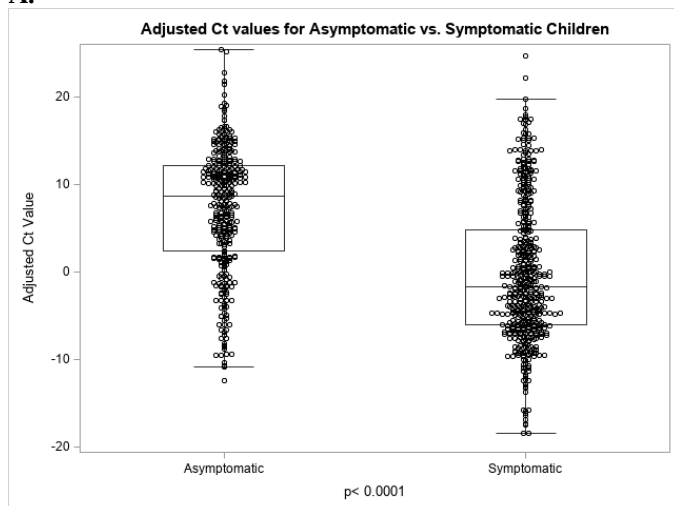
570 **Table 2: Estimated Risk for Being in the Lowest Quartile of Adjusted Ct Values (adjusted**
 571 **Ct <2.47, n = 84) or Highest Quartile of Estimated Viral Loads (Copies/mL $\geq$ 1.700E+05,**
 572 **n = 86) in the Asymptomatic Population**
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Explanatory factors (n)	OR (95% CI) Adjusted Ct Value	P	OR (95% CI) Estimated Viral Load	P
Sex (339) male (178) vs female (161)	1.218 (0.743, 1.995)	0.4342	1.075 (0.659, 1.754)	0.7726
Race (339)		0.5816		0.4142
Asian (16) vs White or Caucasian (135)	1.855 (0.626, 5.4920)	0.2643	2.503 (0.864, 7.258)	0.0911
Black or African American (58) vs White or Caucasian (135)	0.893 (0.430, 1.855)	0.7615	1.123 (0.553, 2.282)	0.7488
Other ^a (112) vs White or Caucasian (135)	1.080 (0.607, 1.923)	0.7938	1.125 (0.630, 2.008)	0.6913
Ethnicity (339) Hispanic/Latino (169) vs Non- Hispanic/Latino (132)	1.272 (0.749, 2.159)	0.3740	1.218 (0.720, 2.062)	0.4616
Immunocompromise (339) yes (35) vs no (304)	0.737 (0.310, 1.755)	0.4908	0.712 (0.299, 1.695)	0.4428
Diabetes (339) yes (9) vs no (330)	6.459 (1.579, 26.427)	0.0095	6.248 (1.528, 25.556)	0.0108
Known contact with COVID- 19 Case (235) yes (64) vs no (171)	1.968 (1.035, 3.743)	0.0390	2.190 (1.154, 4.157)	0.0164
Timing of known COVID-19 contact (57) $\leq$ 2 weeks (48), $>$ 2 weeks (9)	2.015 (0.993, 4.089)	0.0525	4.387 (0.505, 38.093)	0.1800
Recent contact ($\leq$ 2 weeks) (48) vs. no known contact (171)	2.293 (1.135, 4.632)	0.0207	2.293 (1.135, 4.632)	0.0207
Reason for testing (339)		0.0104		0.0046
Surveillance (39) vs Pre-op/AGP (245)	2.702 (1.349, 5.411)	0.0050	2.702 (1.349, 5.411)	0.0050
Surveillance (39) vs. Pre- admission (55)	3.949 (1.521, 10.257)	0.0024	4.381 (1.691, 11.353)	0.0024
Pre-admission (55) vs Pre- op/AGP (245)	1.585 (0.732, 3.433)	0.2200	1.621 (0.749, 3.509)	0.2200
Surveillance (39) vs. Pre- op/AGP or Pre-admission (300)	2.687 (1.350, 5.351)	0.0049	2.925 (1.474, 5.804)	0.0021
Symptoms in 5D after test Yes (14) vs. no (172)	2.396 (0.786, 7.309)	0.1245	2.558 (0.837, 7.816)	0.0994

574 a. "Other" reflects the response of patients that did not wish to select one of the other race categories, based
 575 on chart review.

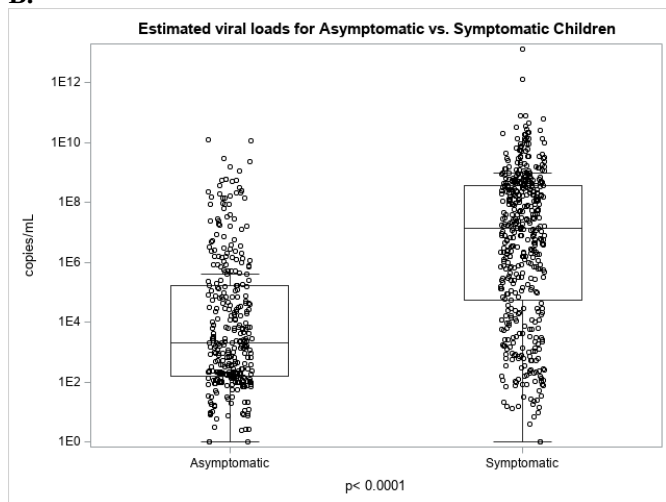
576 Abbreviations: Ct, cycle threshold; OR, odds ratio; Pre-op/AGP, pre-operative/aerosol-generating
 577 procedure; D, days

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579 **Figure 1**580 **A.**

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B.

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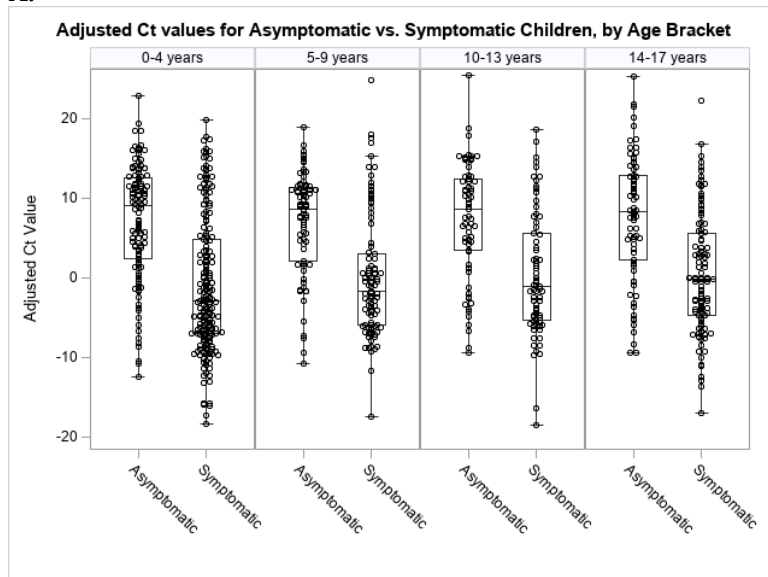
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Ct, cycle threshold value

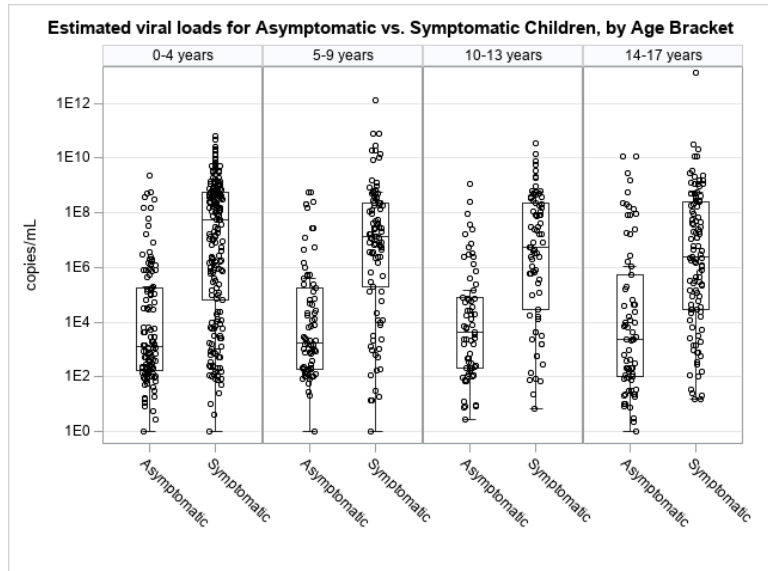
587 **Figure 2**

588 **A.**



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590 **B.**



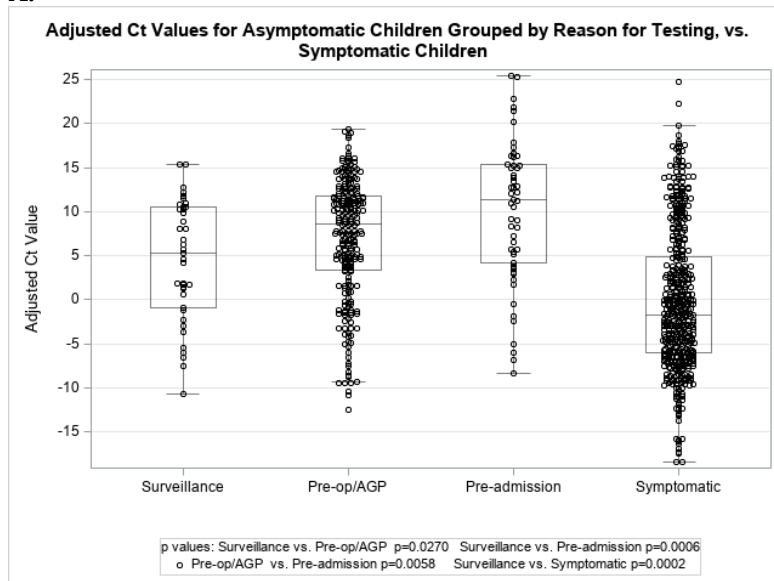
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592 Ct, cycle threshold value

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594 **Figure 3**

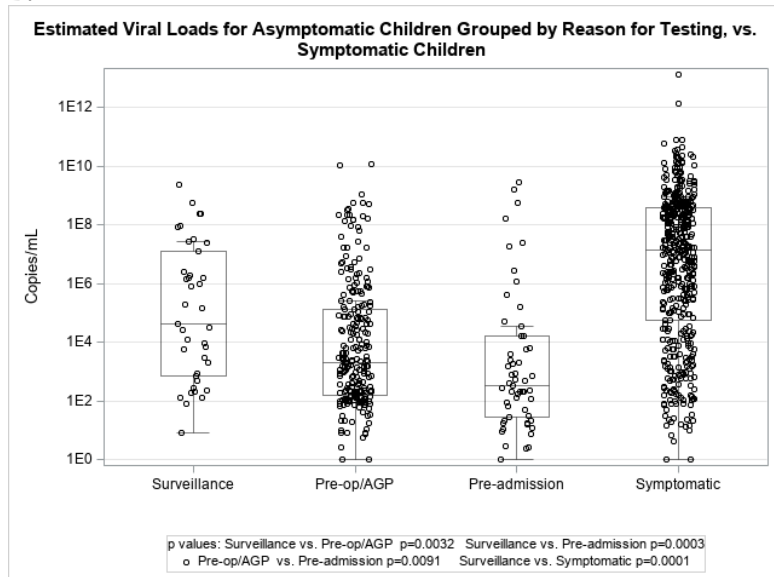
595 **A.**



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Ct, cycle threshold value

Supplementary Tables

Table S1: Assays used, symptomatic medians, conversion of cycle threshold (Ct) to estimated viral load (VL), and study period at each institution

Hospital	Assays	Symptomatic median (IQR)	Data used to generate equation for conversion of Ct to estimated VL	Study period
BCH	Hologic Panther Fusion	27.2 (19.3-36)	Manufacturer data (linearity experiment)	4/9/20-7/30/20
CHLA	CDC RT-PCR	23.68 (17.69-25.88)	Internal data (standard curve)	3/12/20-7/28/20
	Thermo Fisher TaqPath COVID-19 RT-PCR	24.01 (16.74-24.14)	Internal data (standard curve)	6/1/20-7/28/20
	Diasorin Simplexa COVID-19	17.98 (13.91-21.11)	Internal data (standard curve)	4/2/20-7/28/20
	Cepheid Xpert Xpress SARS-CoV-2	29.65 (18.03-27.75)	Internal data (standard curve)	4/16/20-7/28/20
	BioGX SARS-CoV-2 BDMAX	19.45 (10.85-20.70)	Internal data (standard curve)	4/25/20-7/28/20
CHWI	LDT	28.2 (18.35-38.05)	Internal data (standard curve)	4/1/20-7/26/20
	Diasorin Simplexa COVID-19	21.45 (12.45-30.45)	Internal data (standard curve)	4/1/20-7/26/20
Lurie	Abbott RealTime SARS-CoV-2	11.08 (4.86-17.30)	Internal data (quantified run control Ct vs sample Ct)	3/23/20-7/10/20
Toronto	Altona RealStar SARS-CoV-2 RT-PCR	33.62 (25.12-42.12)	Internal data (standard curve)	3/13/20-7/13/20
	Seegene	27.40 (17.17-37.63)	Internal data (standard curve)	3/13/20-7/13/20
WU	Cepheid Xpert Xpress SARS-CoV-2	19.18 (14.53-23.83)	Back calculate from LOD stated in IFU	4/14/20-7/5/20
	Roche Cobas SARS-CoV-2	23.02 (17.10-28.95)	Back calculate from LOD stated in IFU	5/1/20-7/7/20
	Quidel Lyra SARS-CoV-2 (after Ct adjustment)	21.99 (21.07-22.92)	Back calculate from LOD stated in IFU	4/6/20-6/22/20
CHCO	CDC RT-PCR	24.75 (16.65-32.85)	CHLA CDC equation	3/24/20-7/11/20
	Cepheid Xpert Xpress SARS-CoV-2	25.27 (17.07-33.47)	Back calculate from LOD stated in IFU	3/24/20-7/11/20
TCH	Altona RealStar SARS-CoV-2 RT-PCR	18.29 (13.18-23.40)	Internal data (standard curve)	4/17/20-7/16/20
	Cepheid Xpert Xpress SARS-CoV-2	29.7 (24.3-35.1)	CHLA Xpert equation	6/17/20-7/20/20
CHOA	Diasorin Simplexa COVID-19	21.65 (16.5-26.7)	CHLA Diasorin equation	4/27/20-7/14/20

Abbreviations: IQR, interquartile range; Ct, cycle threshold; VL, viral load; LDT, Laboratory-Developed Test; LOD, limit of detection; IFU, instructions for use (package insert); BCH, Boston Children's Hospital; CHLA, Children's Hospital Los Angeles; CHWI, Children's Hospital of Wisconsin; Lurie, Ann & Robert H. Lurie Children's Hospital of Chicago; Toronto, Hospital for Sick Kids; WU, Washington University School of Medicine; CHCO, Children's Hospital Colorado; TCH, Texas Children's Hospital; CHOA, Children's Healthcare of Atlanta

Table S2: Median adjusted Ct values and estimated viral loads for asymptomatic vs symptomatic populations, by institution

Hospital	Adjusted Ct values			Estimated viral loads (copies/mL)		
	ASx (IQR) n	Sx (IQR) n	P	ASx (IQR) n	Sx (IQR) n	P
BCH	10.9 (3.3, 11.0) 25	n/a	n/a	1.87E+02 (1.75E+02, 3.59E+04) 25	n/a	n/a
CHLA	6.6 (1.6, 11.8) 67	-4.4 (-7.1, -0.2) 114	<.0001	2.63E+04 (7.47E+02, 1.07E+06) 67	4.40E+08 (1.32E+07, 2.15E+09) 114	<.0001
CHWI	7.0 (1.0, 9.9) 60	2.3 (-9.4, 10.0) 14	0.1404	1.10E+03 (1.29E+02, 2.10E+05) 60	2.75E+05 (9.66E+02, 1.30E+10) 14	0.0208
Lurie	11.7 (5.5, 15.0) 63	-0.8 (-5.0, 7.0) 126	<.0001	1.03E+03 (1.02E+02, 7.46E+04) 63	6.77E+06 (2.77E+04, 1.72E+08) 126	<.0001
Toronto	3.5 (1.4, 4.5) 7	-11.9 (-16.1, -2.5) 13	0.0030	1.30E+02 (1.70E+01, 1.40E+03) 7	1.60E+06 (1.000E+04, 1.300E+08) 13	0.0071
WU	11.6 (4.4, 18.7) 16	-1.0 (-4.0, 6.4) 31	0.0009	2.62E+03 (4.74E+02, 9.53E+05) 16	1.2E+07 (9.18E+04, 2.07E+08) 31	0.0016
CHCO	8.7 (5.1, 12.9) 34	-0.3 (-7.6, 9.2) 63	0.0001	5.25E+03 (2.31E+02, 5.17E+04) 34	1.84E+06 (6.17E+03, 1.16E+08) 63	0.0002
TCH	7.8 (-0.9, 13.3) 37	-0.5 (-4.6, 3.8) 57	0.0013	8.73E+02 (1.78E+01, 7.36E+05) 37	4.41E+06 (1.81E+04, 3.7E+08) 57	<.0001
CHOA	10.0 (3.7, 11.7) 30	0.0 (-5.2, 5.3) 60	0.0003	7.89E+02 (1.92E+02, 1.70E+05) 30	3.89E+06 (4.850E+04, 3.4E+08) 60	0.0003

Abbreviations: Ct, cycle threshold; IQR, interquartile range; ASx, asymptomatic; Sx, symptomatic; BCH, Boston Children's Hospital; CHLA, Children's Hospital Los Angeles; CHWI, Children's Hospital of Wisconsin; Lurie, Ann & Robert H. Lurie Children's Hospital of Chicago; Toronto, Hospital for Sick Kids; WU, Washington University School of Medicine; CHCO, Children's Hospital Colorado; TCH, Texas Children's Hospital; CHOA, Children's Healthcare of Atlanta

Table S3: Distribution of symptoms for symptomatic population, by age bracket

Symptom	Ages 0-4 (n=197)		Ages 5-9 (n= 97)		Ages 10-13 (n=75)		Ages 14-17 (n=109)		P
	n	%	n	%	n	%	n	%	
cough	113	57.4	49	50.5	38	50.7	72	66.1	0.086
Fever or chills	168	85.3	70	72.2	59	78.7	75	68.8	0.004
Shortness of breath	17	8.6	10	10.3	13	17.3	39	35.8	<0.0001
Sore throat	11	5.6	29	29.9	21	28.0	31	28.4	<0.0001
Abdominal pain	11	5.6	21	21.6	16	21.3	12	11.0	<0.0001
Diarrhea	34	17.3	15	15.5	10	13.3	26	23.9	0.265
Fatigue	18	9.1	11	11.3	10	13.3	26	23.9	0.006
Myalgias	4	2.0	6	6.2	15	20.0	31	28.4	<0.0001
New loss of taste or smell	1	0.5	4	4.1	11	14.7	29	26.6	<0.0001
Headache	4	2.0	20	20.6	24	32.0	44	40.4	<0.0001
Congestion or rhinorrhea	103	52.3	27	27.8	15	20.0	30	27.5	<0.0001
Nausea or vomiting	26	13.2	21	21.6	21	28.0	30	27.5	0.005
Rash	25	12.7	5	5.2	2	2.7	4	3.7	0.006
Conjunctivitis	8	4.1	2	2.1	0	0	4	3.7	0.301

Table S4: Comparison of median adjusted Ct and estimated viral load for asymptomatic vs. symptomatic patients for selected demographic variables

Adjusted Ct Value					
	Asymptomatic		Symptomatic		
	Median (IQR)	n	Median (IQR)	n	P
Sex					
male	8.64 (3.25, 11.75)	178	-1.12066 (-5.98, 5.18)	248	<0.0001
female	8.63 (1.74, 12.65)	161	-2.525 (-6.07, 4.67)	230	<0.0001
Age bracket					
0-4 years	9.02 (2.47, 12.61)	118	-2.93 (-7.02, 4.85)	197	<0.0001
5-9 years	8.57 (2.11, 11.4)	79	-1.75 (-5.96, 3.05)	97	<0.0001
10-13 years	8.68 (3.4, 12.45)	69	-1.10 (-5.35, 5.52)	75	<0.0001
14-17 years	8.25 (2.3, 12.9)	73	-0.24 (-4.67, 5.63)	109	<0.0001
Ethnicity					
Hispanic/Latino	8.78 (3.35, 12.53)	132	-0.67 (-5.59, 6.39)	131	<0.0001
Non-Hispanic/Latino	8.05 (1.74, 12.11)	169	-1.95 (-6.12, 3.8)	285	<0.0001
Immunocompromise					
yes	11 (3.3, 13.31)	35	-3.64316 (-9.4, 1.25)	16	0.0012
no	8.04 (2.0, 12.12)	304	-1.675 (-6.0, 4.85)	462	<0.0001
Diabetes					
yes	-0.53 (-3.65, 6.77)	9	0.95 (-3.42, 8.05)	10	0.548
no	8.82 (3.25, 12.2)	330	-1.75 (-6.08, 4.71)	468	<0.0001
Estimated Viral Load (Copies/mL)					
	Asymptomatic		Symptomatic		
	Median (IQR)	n	Median (IQR)	n	P
Sex					
male	1.67E+03 (1.75E+02, 1.66E+05)	178	8.06E+06 (3.22E+04, 3.51E+08)	248	<0.0001
female	2.21E+03 (1.42E+02, 2.50E+05)	161	2.58E+07 (6.80E+04, 4.38E+08)	230	<0.0001
Age bracket					
0-4 years	1.28E+03 (1.75E+02, 1.70E+05)	118	5.33E+07 (6.80E+04, 5.68E+08)	197	<0.0001
5-9 years	1.74E+03 (1.87E+02, 1.70E+05)	79	1.36E+07 (1.95E+05, 2.27E+08)	97	<0.0001
10-13 years	4.26E+03 (2.00E+02, 8.10E+04)	69	5.58E+06 (2.77E+04, 2.36E+08)	75	<0.0001

14-17 years	2.43E+03 (1.00E+02, 5.49E+05)	73	2.52E+06 (2.97E+04, 2.58E+08)	109	<0.0001
Ethnicity					
Hispanic/Latino	3.36E+04 (2.09E+02, 2.47E+05)	169	1.96E+07 (1.19E+05, 4.68E+08)	285	<0.0001
Non-Hispanic/Latino	1.27E+03 (1.18E+02, 7.65E+04)	132	3.72E+06 (1.25E+04, 1.87E+08)	131	<0.0001
Immunocompromise					
yes	6.90E+02 (1.63E+02, 4.76E+04)	35	1.55E+08 (1.21E+06, 4.63E+08)	16	<0.0001
no	2.34E+03 (1.57E+02, 1.95E+05)	304	1.33E+07 (4.14E+04, 3.80E+08)	462	<0.0001
Diabetes					
yes	1.64E+07 (2.60E+04, 8.70E+07)	9	2.94E+05 (4.11E+03, 1.88E+07)	10	0.4025
no	1.67E+03 (1.47E+02, 1.57E+05)	330	1.44E+07 (6.30E+04, 4.24E+08)	468	<0.0001

Abbreviations: Ct, cycle threshold; IQR, Interquartile range

Table S5

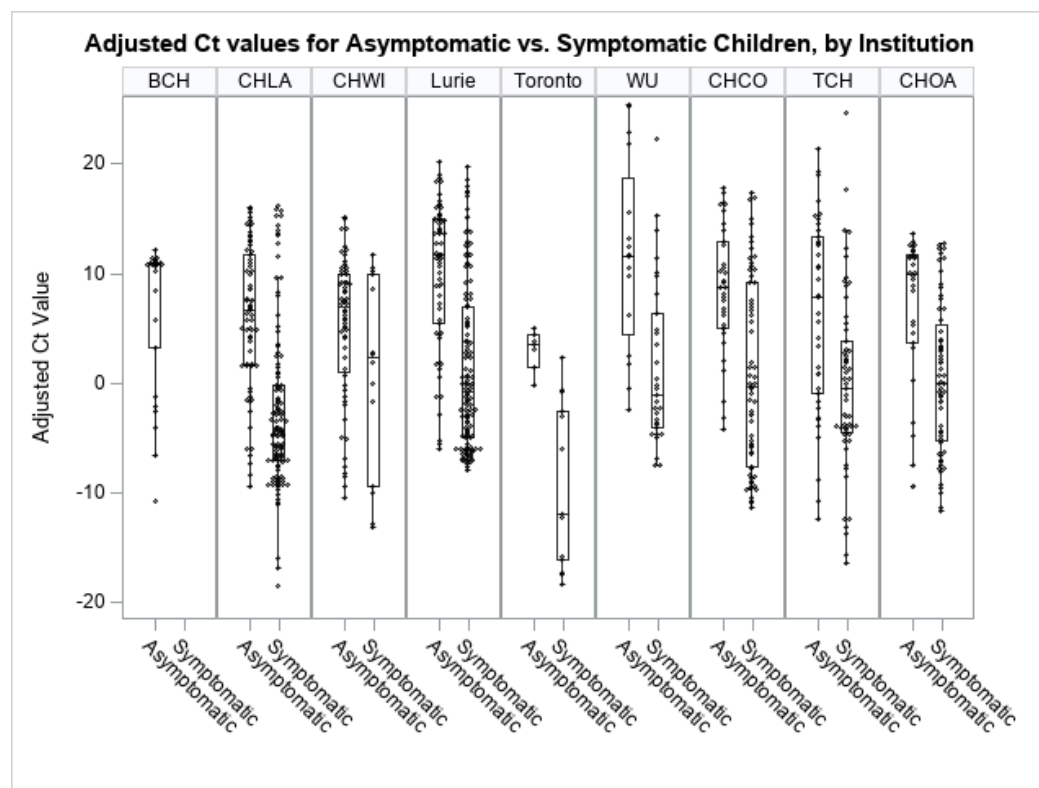
Comparison of median adjusted Ct and estimated viral load for asymptomatic patients with and without selected risk factors

	Median (IQR) Adjusted Ct Value	n	P	Median (IQR) Estimated Viral Load (copies/mL)	n	P
Contact with COVID-19 case						
yes	8.3 (1.5,11.8)	64	0.2653	6140 (366,1200000)	64	0.0062
no	9.2 (4.3,12.7)	171		1030 (102,35900)	171	
Timing of contact						
≤ 2 weeks prior to test	8.3 (1.2,10.9)	48	0.1063	6650 (366,1760000)	48	0.1542
>2 weeks prior to test	11.8 (5.6,13.7)	9		885 (187,1480)	9	
Reason for testing						
1. Surveillance	5.4 (-0.9,10.5)	39	0.0270 (1 vs 2); 0.0006 (1 vs 3)	41000 (748,12300000)	39	0.0032 (1 vs 2); 0.0003 (1 vs 3)
2. Pre-op/aerosol-generating procedure	8.6 (3.3,11.8)	245	0.0058 (2 vs 3)	2040 (163,132000)	245	0.0091 (2 vs 3)
3. Pre-admission	11.4 (4.2,15.3)	55		347 (28,16000)	55	
Recent contact compared to no contact						
contact ≤ 2 weeks prior to test	8.3 (1.2,10.9)	48	0.2211	6650 (366,1760000)	48	0.0069
no contact	9.2 (4.3,12.7)	171		1030 (102,35900)	171	
Symptoms post-test						
yes	3.5 (-0.9,10.9)	14	0.0745	76500 (106,2440000)	14	0.3008
no	9.1 (3.3,13.2)	172		1410 (125,73300)	172	

Abbreviations: Ct, cycle threshold; IQR, Interquartile range

Figure S1

Adjusted Ct value distributions, by hospital. Data from BCH were from asymptomatic patients only because asymptomatic patients were tested by OP swab and symptomatic patients by NP swab, precluding direct comparison.



Abbreviations: Ct, cycle threshold; BCH, Boston Children's Hospital; CHLA, Children's Hospital Los Angeles; CHWI, Children's Hospital of Wisconsin; Lurie, Ann & Robert H. Lurie Children's Hospital of Chicago; Toronto, Hospital for Sick Kids; WU, Washington University School of Medicine; CHCO, Children's Hospital Colorado; TCH, Texas Children's Hospital; CHOA, Children's Healthcare of Atlanta

Figure S2

Estimated viral load distributions in asymptomatic vs symptomatic patients, by hospital. Data from BCH were from asymptomatic patients only because asymptomatic patients were tested by OP swab and symptomatic patients by NP swab, precluding direct comparison.

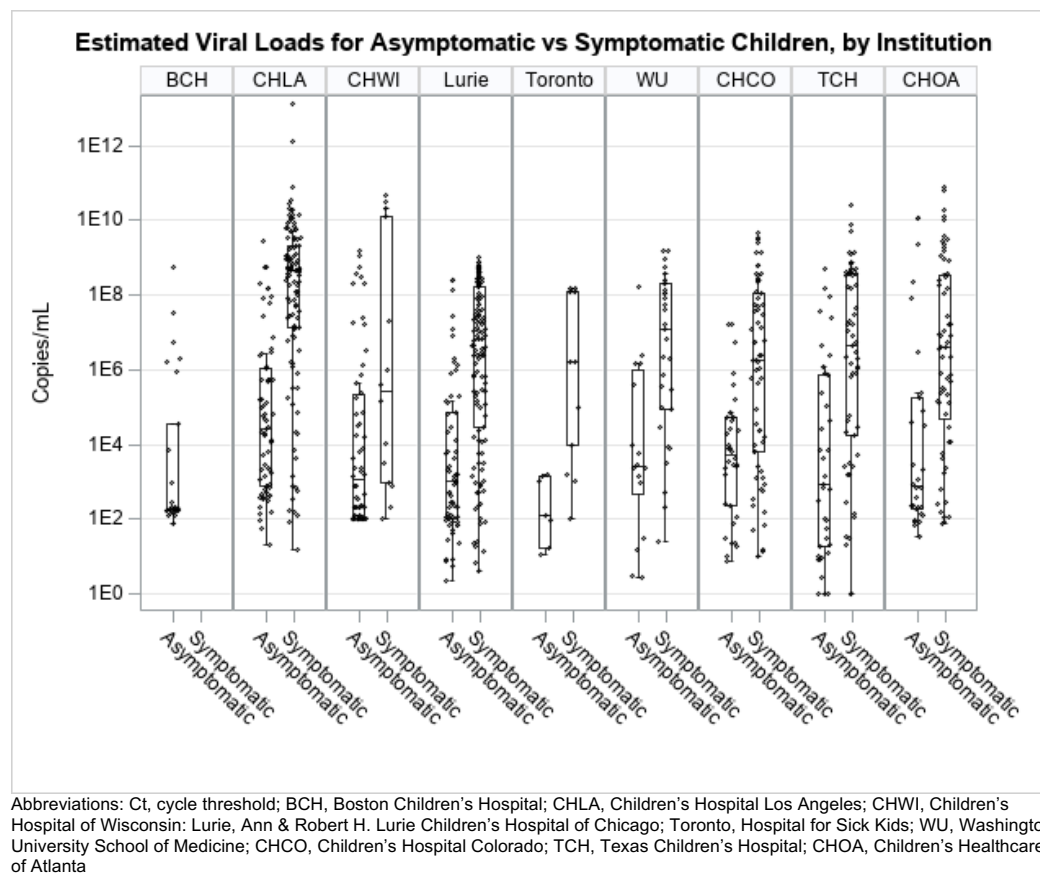


Figure S3

Distribution of adjusted Ct values for patients with immunocompromise (n = 35 asymptomatic, n= 16 symptomatic) within the full asymptomatic and symptomatic populations. Adjusted Ct values from patients with immunocompromise are indicated as black circles; Ct values from patients without immunocompromise are indicated as open diamonds.

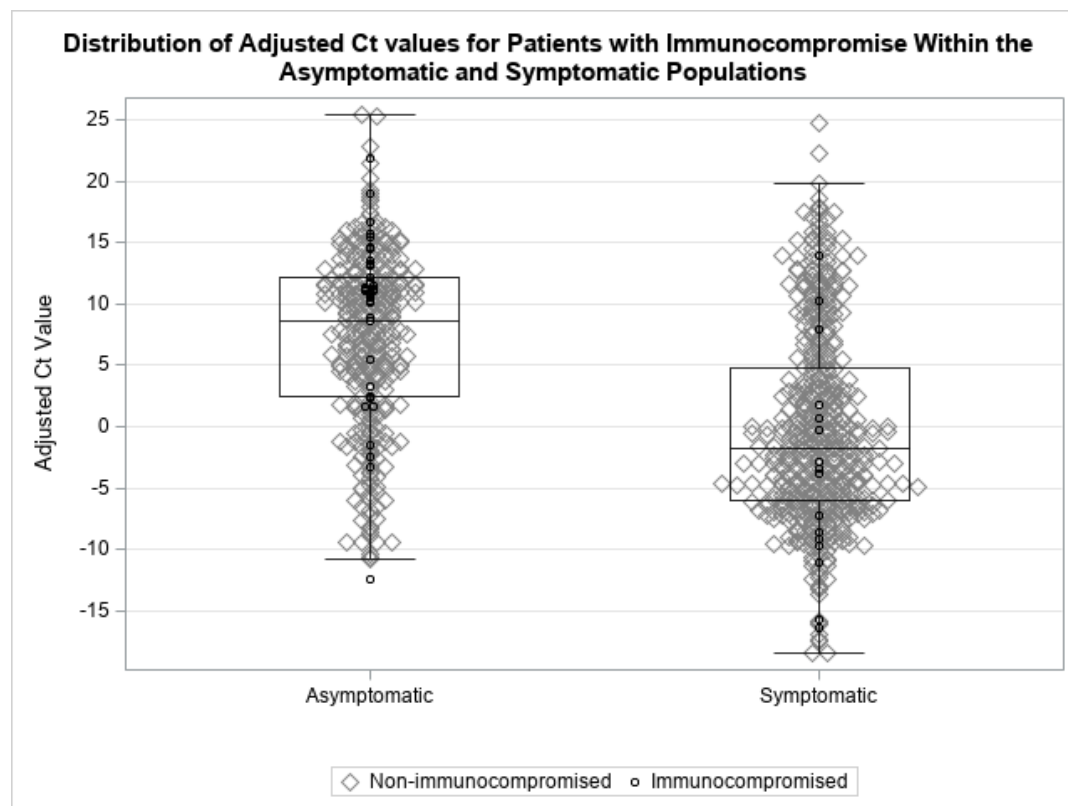


Figure S4

Distribution of adjusted Ct values for patients with diabetes (n = 9 asymptomatic, n = 10 symptomatic) within the full asymptomatic and symptomatic populations. Adjusted Ct values from patients with diabetes are indicated as black circles; Ct values from patients without diabetes are indicated as open diamonds.

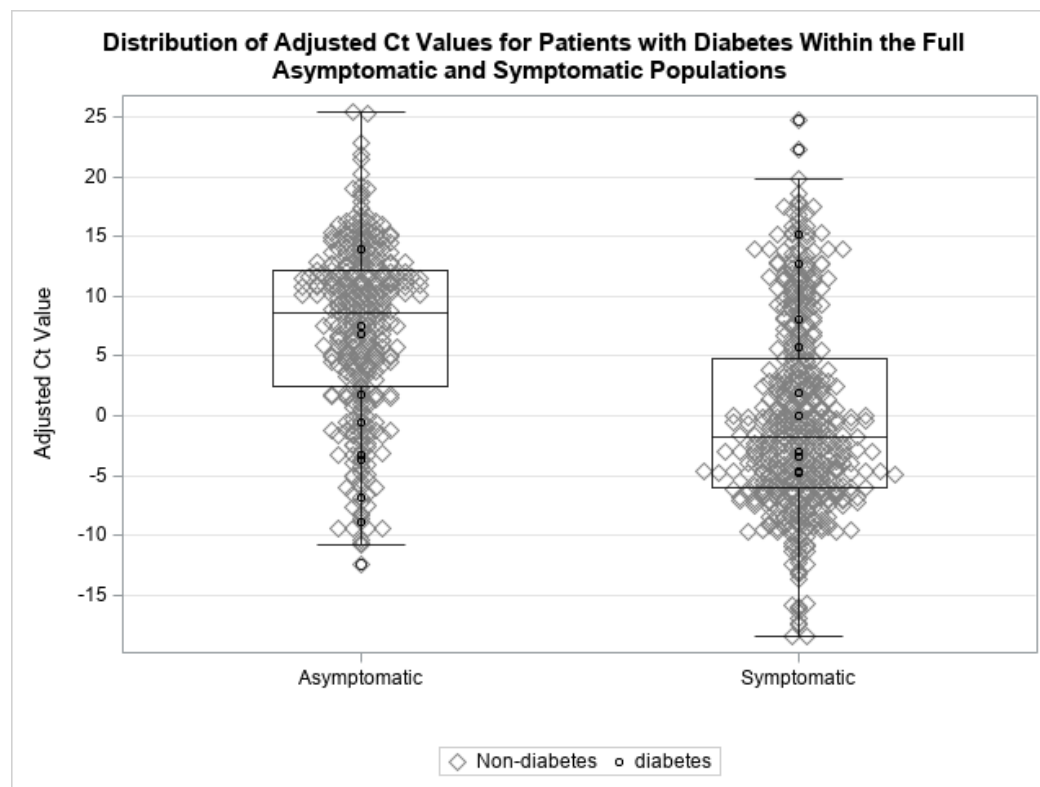


Figure S5

Distribution of adjusted Ct values for patients with known COVID-19 contact (n=64) within the full asymptomatic population, compared to the symptomatic population. Adjusted Ct values from asymptomatic patients with known COVID-19 contact are indicated as black circles; Ct values from asymptomatic patients without known contact and symptomatic patients are indicated as open diamonds.

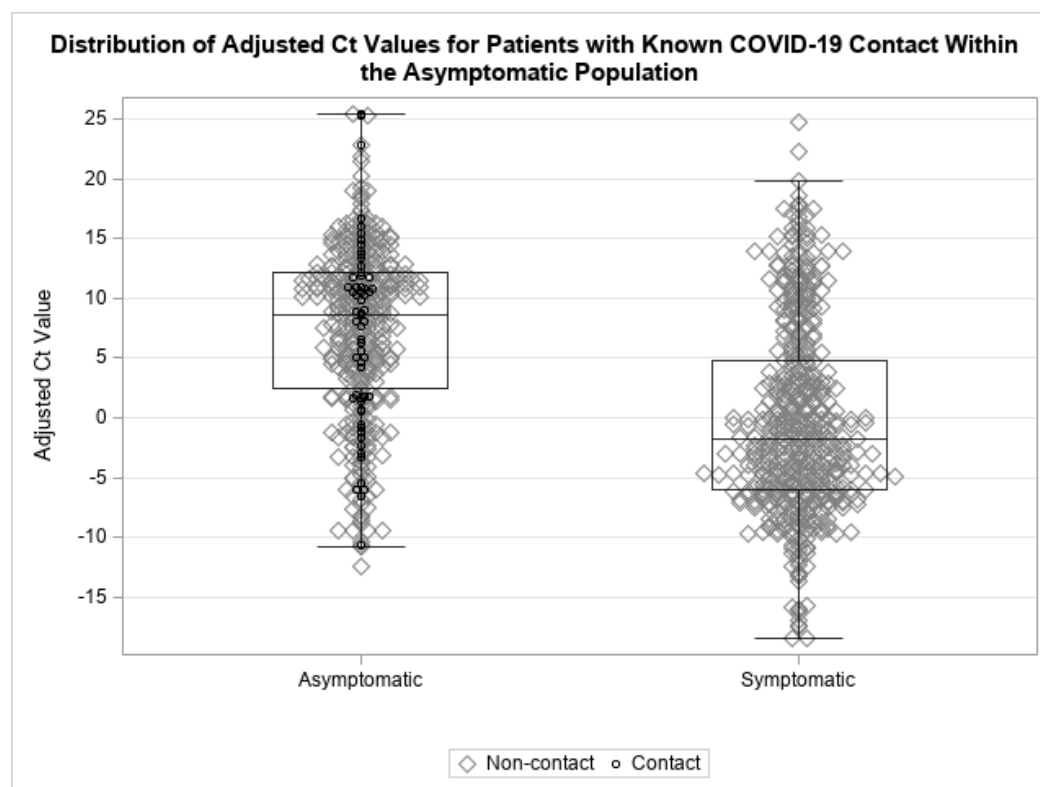


Figure S6

Distribution of adjusted Ct values for patients with known recent COVID-19 contact (≤ 2 weeks prior to test) ($n = 48$) within the full asymptomatic population, compared to the symptomatic population. Adjusted Ct values from asymptomatic patients with known recent contact are indicated as black circles; Ct values from asymptomatic patients without known recent contact and symptomatic patients are indicated as open diamonds.

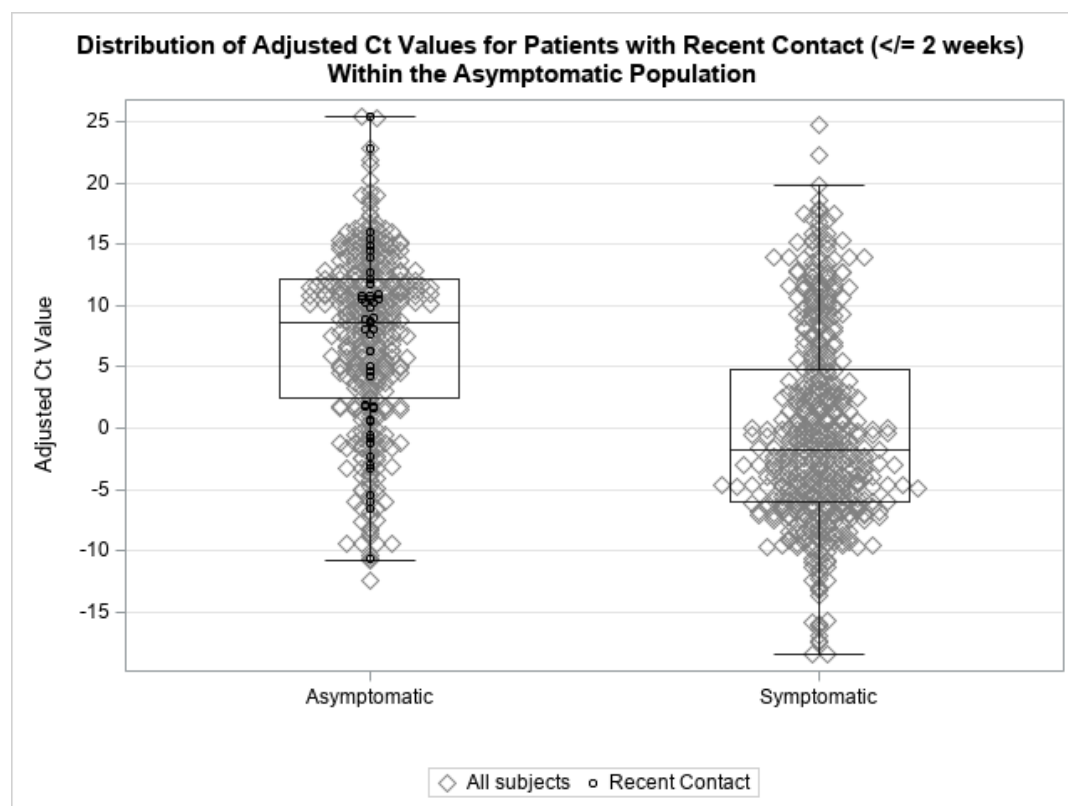


Figure S7

Distribution of adjusted Ct values for patients tested for surveillance within the full asymptomatic population, compared to the symptomatic population. Adjusted Ct values from asymptomatic patients tested for surveillance are indicated as black circles; Ct values from asymptomatic patients tested for other reasons and symptomatic patients are indicated as open diamonds.

