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Short communication

SARS-CoV-2 viral load assessment in respiratory samples

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ABSTRACT

Real-time reverse transcriptase polymerase chain reaction (rRTPCR) has been the main method for diagnosis of SARS-CoV-2 infection in the early stages of the COVID-19 pandemic. De-identified results from upper and lower respiratory samples submitted to a reference laboratory demonstrated a positivity rate of 14.9 % (4428 of 29,713 samples tested). Distribution of results by birth year cohort and specimen type suggested general consistency in mean, median and peak values but higher positivity rates in individuals born from 1964 to 1974. Female patients had a significantly lower positivity rate ($P < 0.0001$), although similar load mean and median values, compared to males. Overall, 15.3 % (676 of 4428 positive results) of positive results had viral loads greater than 8 log10 copies/mL, with occasional samples exceeding 10 log10 copies/mL. These results support quantitative assessment of SARS-CoV-2 viral load in patient testing and efforts to control viral transmission

In the early stages of the COVID-19 pandemic, molecular testing by real-time reverse transcriptase polymerase chain reaction (rRT-PCR) has been the main method for diagnosis of SARS-CoV-2 infection in symptomatic patients [1]. Qualitative assays have primarily been used for this purpose. Our reference laboratory developed and validated an assay that targets two regions of the N protein gene using standard rRT-PCR methods. Validation of upper and lower respiratory samples was focused on providing qualitative results. Positivity was defined as a cycle threshold (C_T) value $\leq$ 38.0. This cutoff was derived from the mean C_T value at the limit of detection plus approx. two standard deviations. Sensitivity of the assay, expressed as limit of detection, is 73 copies/mL. Although the intended use of the assay is to report qualitative results, linearity and dynamic range were assessed and demonstrated from 2.77 to 8.77 $\log_{10}$ copies/mL (slope = -3.32, y-intercept = 41.2, R^2 = 0.9998), with C_T values from 31.56 to 12.05 in the linear range of the assay. During review of laboratory results, we occasionally observed very low C_T values (i.e. < 10) from patient samples, suggesting extremely high concentrations of SARS-CoV-2 in respiratory specimens. This report characterizes the viral load of all positive samples in an unbiased reference laboratory caseload from the United States.

Using 29,713 de-identified rRT-PCR results, we described the viral load of respiratory samples tested during the first weeks of the COVID-19 pandemic in the United States. Overall 14.9 % (4428) of samples tested were positive for SARS-CoV-2. The distribution of positive samples by $\log_{10}$ copies/mL and C_T values is shown in Fig. 1. The overall viral load range of positive samples was 0.91–10.42 $\log_{10}$ copies/mL (C_T 6.16–37.92) with a mean of 5.85 $\log_{10}$ copies/mL and median of 6.05 $\log_{10}$ copies/mL. Samples with greater than 8 $\log_{10}$ copies/mL represented 15.3 % (676 of 4428) of results reported as positive. Samples with less than 3 $\log_{10}$ copies/mL represented 9.89 % (438 of 4428) of results reported as positive.

Assessment of viral load results by birth year of patients is shown in Table 1. Patients born from 1955 to 1994 had both the highest number and percentage of positive results, with peak values in patients born from 1960 to 1974. Mean and median viral load values did not vary markedly based on birth cohort, although it was noted that the highest maximum values (> 10 $\log_{10}$ copies/mL) were in patients born from 1995 to 2009. In Table 2, viral load results are shown by specimen type and patient gender. A majority of samples received (69.3 % or 20,603 of 29,713) did not have the specific specimen type identified. When specimen type was identified, NP swabs were the majority of samples tested (84.3 % or 7682 of 9110). Although more females were tested, the percentage of samples tested which were positive was significantly lower (Chi square test, $P < 0.0001$) compared to males while no significant differences were observed in viral load mean or median values.

Recent reports of C_T values and viral loads in SARS-CoV-2 infected patients have demonstrated shedding at high viral load levels in individual patients or small groups of patients. While some studies only report C_T values, which may vary between methods, these results provide a reasonable approximation of viral load. Lack of a common

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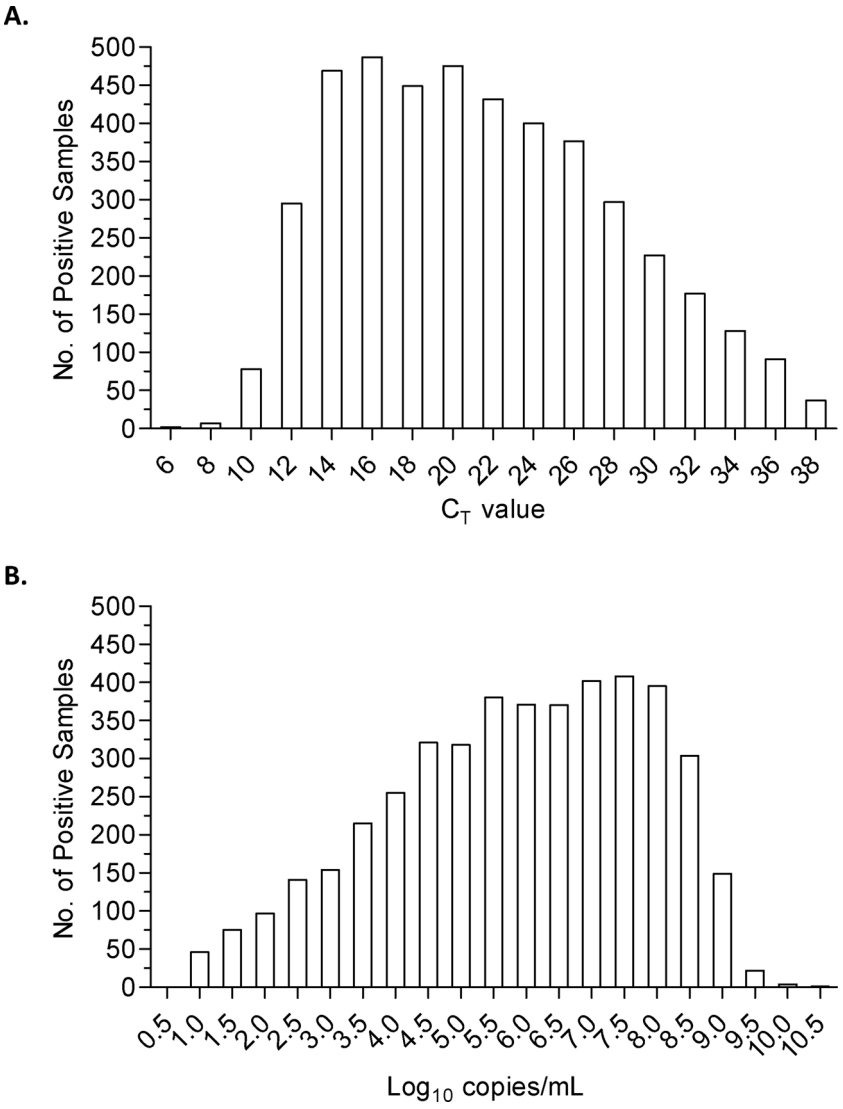

Fig. 1. Frequency distribution histograms of C_T (A) and Log_{10} copies/mL values (B) for SARS-CoV-2 viral loads in respiratory samples tested by a reference laboratory.

Table 1
SARS-CoV-2 viral load characteristics in respiratory samples by birth cohort.

				SARS-CoV-2 Log_{10} copies/mL			
Birth cohort	No. tested	No. Pos.	% of Pos. results	Average (SD)	Median	Minimum	Maximum
2010 - 2020	1197	15	0.34%	6.13 (2.07)	6.27	2.04	8.64
2000 - 2009	1157	81	1.83%	5.89 (2.29)	6.38	1.08	10.19
1995 - 1999	1935	248	5.60%	6.03 (2.03)	6.44	0.96	10.42
1990 - 1994	2647	388	8.76%	6.09 (1.97)	6.27	0.91	9.84
1985 - 1989	2994	405	9.15%	5.90 (2.06)	6.15	1.09	9.18
1980 - 1984	2862	380	8.58%	5.77 (2.01)	5.97	1.00	9.62
1975 - 1979	2601	416	9.39%	5.88 (1.94)	6.12	1.05	9.78
1970 - 1974	2548	450	10.16%	5.74 (1.95)	5.76	0.92	9.27
1965 - 1969	2497	463	10.46%	5.82 (1.88)	5.98	0.93	9.44
1960 - 1964	2447	453	10.23%	5.77 (2.00)	6.05	0.94	9.64
1955 - 1959	2180	359	8.11%	5.75 (1.94)	5.91	1.12	9.17
1950 - 1954	1625	264	5.96%	5.81 (1.96)	6.03	1.08	9.43
1945 - 1949	1224	201	4.54%	5.89 (1.94)	6.00	1.13	9.01
1940 - 1944	818	115	2.60%	5.77 (1.87)	5.75	1.44	9.20
1935 - 1939	446	83	1.87%	5.83 (2.06)	6.15	0.94	9.49
1930 - 1934	348	79	1.78%	5.68 (2.00)	6.04	1.20	8.64
1925 - 1929	134	25	0.56%	6.41 (1.77)	6.66	2.48	8.51
1920 - 1924	49	3	0.07%	6.67 (2.74)	7.82	3.54	8.64
1915 - 1919	4	0	0.00%	N.A.[a]	N.A.	N.A.	N.A.

[a] N.A., Not applicable.

Table 2
SARS-CoV-2 viral load characteristics in respiratory samples by specimen type and gender.

				SARS-CoV-2 Log_{10} copies/mL			
		No. tested	No. pos. (% pos)	Average (SD)	Median	Minimum	Maximum
Specimen type							
	BAL	130	8 (6.15 %)	5.37 (2.13)	5.06	2.65	8.90
	Nasal swab	677	61 (9.01 %)	5.52 (2.35)	5.99	1.38	9.18
	Nasal wash	42	5 (11.90 %)	5.23 (2.64)	5.74	2.36	8.52
	NP Swab	7682	711 (9.26 %)	5.66 (2.03)	5.78	0.93	9.49
	NP wash	63	5 (7.94 %)	6.09 (2.25)	6.90	2.97	8.59
	OP swab	191	8 (4.19 %)	4.52 (1.30)	4.47	2.69	6.53
	NP/OP swab	324	19 (5.86 %)	5.66 (1.65)	5.40	2.55	8.36
	Not specified	20,603	3611 (17.53 %)	5.90 (1.96)	6.13	0.91	10.42
Gender							
	Female	17,587	2153 (12.24 %)	5.88 (1.98)	6.09	0.91	10.42
	Male	11,766	2261 (19.22 %)	5.82 (1.97)	6.02	0.92	9.84
	Not specified	360	14 (3.89 %)	6.13 (2.20)	6.53	1.66	8.63

standard makes comparison of calculated viral load values between studies an approximation as well. A study of 82 SARS-CoV-2 infected patients showed viral loads of 4–8 log_{10} copies/mL in throat swabs and sputum from -1 to 15 days post-onset of clinical symptoms with occasional sputum samples having 10–11 log_{10} copies/mL [2]. A second study of 17 symptomatic patients reported C_T values as low as 13 in both throat and nasal swabs of one patient, with a majority samples having C_T values > 20 in the early clinical period [3]. Wang et al. [4] reported C_T values > 17 in similarly collected specimens. Viral load kinetics have also been studied with peak reported values of approx. 8 log_{10} copies/mL [5] and 9 log_{10} copies/mL [6], both in upper respiratory specimens. Initial and peak mean viral loads of 6.17 and 6.91 log_{10} copies/mL, respectively, have also been reported for 10 SARS-CoV-2 infected patients severe disease; values were 10-fold lower (initial viral load) and 30-fold lower (peak viral load) in 13 patients with mild disease by [7].

Viral loads in respiratory samples from patients infected with other respiratory coronaviruses have also been reported. In 14 patients infected with SARS-CoV-1, peak viral loads of approx. 5.5–8.5 log_{10}/mL were documented in nasopharyngeal aspirates [8]. For patients with severe MERS, peak viral loads in sputum or tracheal aspirates ranged from 6 to 9 log_{10} copies/mL while mild cases were at least 100-fold lower [9]. Viral load results for common respiratory coronaviruses such as strains NL63 and OC43 are generally 1000- to 10,000-fold lower compared to peak values for SARS-CoV-1, SARS-CoV-2 and MERS [10].

The limitations of this study are that clinical information was not available from the reference laboratory caseload and full de-identification of samples was required for research use. Most importantly, the proportion of samples tested from symptomatic patients versus asymptomatic individuals was not known, nor is it known when samples were collected relative to the time of symptom onset for those patients suffering from disease. Therefore the overall prevalence of positivity is minimally significant and the prevalence by birth year may well be skewed based on likelihood of sampling due to age or occupation. However, additional study of asymptomatic or symptomatic patient samples with defined clinical characteristics is warranted based on our data.

In summary, our results demonstrate that a significant number of patients have extremely high levels of virus (> 8 log_{10} copies/mL) in respiratory samples and thus may be responsible for a disproportionate level of transmission to contacts and contamination of environmental surfaces. Additionally, results demonstrated that samples with low viral loads (< 1000 copies/mL) are common, and individuals from which these samples were collected may represent a low risk for transmission. This study supports the concept that quantitative viral detection methods for SARS-CoV-2 diagnosis may provide significant benefits in efforts to control viral transmission, and perhaps even in the medical care of these patients.

Declaration of Competing Interest

The authors have no conflicts of interest to declare.

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