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The performance of the SARS-CoV-2 RT-PCR test as a tool for detecting SARS-CoV-2 infection in the population

Dear Editor,

Worldwide, detection and monitoring of SARS CoV-2 infection continues to be based on results of the real-time reverse-transcription polymerase chain reaction (RT-PCR) test. A recent scoping review in this journal reported that assessment of the diagnostic accuracy of the RT-PCR test for SARS-CoV-2 has been less than perfect [1]. We analysed real-world data from a large laboratory in the city of Münster (population 313,000), Germany, derived from a single fully automated high throughput RT-PCR platform (cobas SARS-CoV-2 RT-PCR system, Roche Diagnostics) utilizing the same two gene targets for the entire study period (weeks 10-49, 2020). This laboratory performed about 80% of all SARS-CoV-2 RT-PCR tests in the Münster region during this time. We explored changes in the percentage of positive RT-PCR tests (positive rate) over time. In addition, we assessed the influence of covariates such as age, sex, calendar time, and symptoms at the time of first RT-PCR test on the distribution of cycle threshold (Ct) values.

Nearly all swab specimens were tested within 24 hours of collection. The tests and their interpretation were carried out in accordance with the Roche cobas SARS-CoV-2 emergency use authorization (EUA) protocol, the specific targets of the test being the open reading frame (ORF) 1ab and the pan-Sarbecovirus E genes. The limit of detection, defined as the concentration of analyte that will be detected in 95% of replicate tests was 0.007 median tissue culture infectious doses (TCID50) per ml for target 1 and 0.004 TCID50/ml for target 2, corresponding to Ct values of approximately 33 and 36, respectively (cobas® SARS-CoV-2 package insert, version 1.0).

RT-PCR tests that had not crossed the positivity threshold after the 40th cycle were reported as “negative”. The Ct value is inversely proportional to the initial amount of target nucleic acid and is thus a relative indicator of the concentration of viral particles in the clinical specimen. An increase in Ct value of three points indicates that the initial amount of viral particles was smaller by a factor of about ten.

We categorized our population-based Ct values according to the recommendations of the UK Office for National Statistics (ONS) COVID-19 household survey as < 25 and ≥ 25 [2]. Since there has been some discussion regarding this Ct-threshold [3-5], we performed a second categorization using a cutoff of < 30 versus ≥ 30. For a small subset of 58 people, sufficient clinical information was available to allow classification as symptomatic or asymptomatic.

Of 162,457 tested individuals, 4,164 (2.6%) had a positive RT-PCR test. The positive rate was lower among children aged 0-9 years (2.2%) and among adults aged 70 or more (1.6%), compared to the intermediate group aged 10-69 years (2.8%). The positive rate was strongly linked to the national SARS-CoV-2 test strategy. During the first and third phase of national testing, predominantly symptomatic people were tested. During these phases, the positive rates were higher than during the intermittent second phase corresponding to the summer season, when predominantly asymptomatic individuals were tested. The positive rate during the third phase was considerably higher than during the first phase. During the peak of testing asymptomatic individuals, only 0.4% tested positive with a mean Ct value of 28.8. Higher mean Ct values were observed among children aged 0-9 years (28.6) and adults above 70 years (27.0). Only 40.6% of positive tests showed Ct values below the threshold of 25, indicating a likelihood of the person being infectious (**Table 1**). In the small group of individuals for whom clinical information was available, symptomatic subjects had a markedly lower mean Ct value of 25.5 compared to asymptomatic subjects, who showed a mean Ct value of 29.6 (**Figure 1**).

Most positive tests in our sample showed Ct values of 25 or higher, indicating a low viral load. Ct values were on average lower in symptomatic than in asymptomatic individuals. Our results are similar to the observations made in the ONS Survey with consistently low positive rates (0.06%) during the summer months,

Table 1
Characteristics of people who underwent PCR testing in the region of Münster, North Rhine-Westphalia, Germany, March 26 - December 6, 2020

	Number of tests[1)]	Positive tests		Mean Ct value among positive tests[2)]		Percentage of positive tests with Ct values[2)]	
	N	N	%	Mean	SD	< 25	<30
All	162,457	4164	2.6	26.5	5.2	40.6	69.6
Men	70,043	1981	2.8	26.4	5.3	42.0	69.6
Women	92,113	2165	2.4	26.6	5.1	39.4	69.5
Unknown	301	18	6.0	27.4	5.2	38.9	66.7
Swab site							
Nose & throat	8637	222	2.6	25.9	5.4	43.0	72.9
Throat	7059	151	2.1	26.2	4.5	41.7	77.2
Unspecified/other	146,761	3791	2.6	26.6	5.2	40.4	69.1
Age group							
0-9	9978	222	2.2	28.6	4.7	21.1	56.5
10-19	15,200	536	3.5	26.8	4.9	38.2	71.4
20-29	21,613	745	3.5	26.4	5.1	41.6	69.4
30-39	21,830	572	2.6	26.3	5.1	42.7	72.3
40-49	21,373	600	2.8	26.3	5.4	43.8	69.1
50-59	25,367	665	2.6	26.0	5.3	44.4	72.9
60-69	17,460	351	2.0	26.0	5.1	46.0	73.5
70-79	12,155	214	1.8	27.1	5.2	35.3	65.8
80-89	13,196	185	1.4	26.8	5.2	37.4	64.5
90-99	3699	55	1.5	27.0	5.4	37.0	63.0
100+	29	1					
unknown	557	18	3.2	31.3	4.9	11.8	29.4
Calendar week							
10-19	12,985	305	2.4	28.7	5.1	22.1	46.8
20-44	132,488	2418	1.8	26.5	5.2	40.5	69.6
45-49	16,984	1441	8.5	26.4	5.1	41.8	70.7
Specific phases of the pandemic[3)]							
Peak 1st wave	2190	36	1.6	27.8	5.4	26.5	55.9
Traveler return	16,874	68	0.4	28.8	5.5	26.9	55.2
Peak 2nd wave	4022	367	9.1	26.6	5.1	39.5	69.8

Legend table: SD = standard deviation
[1)] only persons with tests that were clearly either positive or negative were included
[2)] among 4164 people tested positive, the Ct value was available for 3810 people (91.5%); Ct values were not retrievable for positive tests during the calendar weeks 12-13 and 16-25 in 2020
[3)] Peak of 1st wave in weeks 12-13 (16.-29.3.2020); proxy weeks 13-14; unselective testing in weeks 33-34 (peak of tests for traveler return); peak of 2nd wave in weeks 50-51 (7.-20.12.2020), proxy weeks 48-49

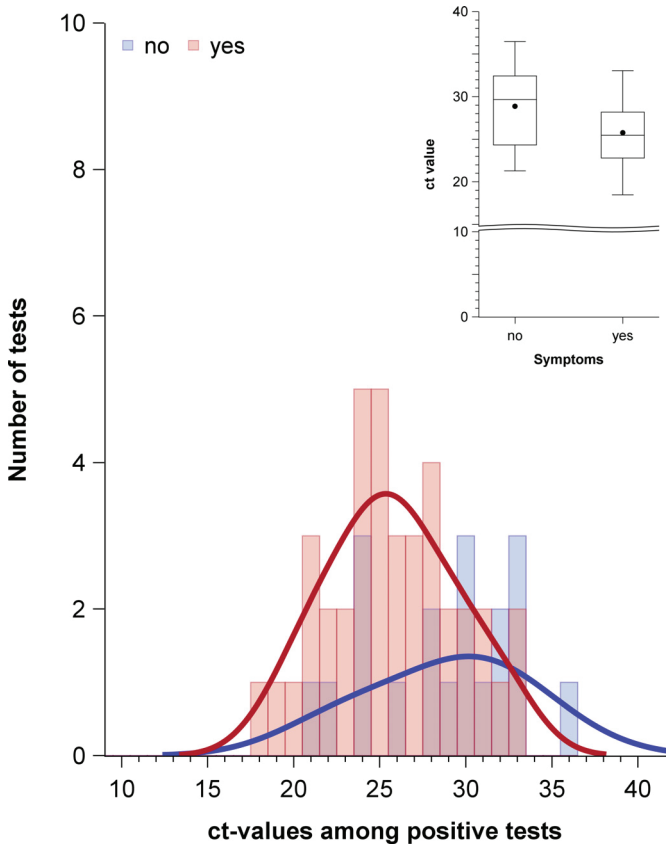

Figure 1. Ct value distribution among symptomatic and asymptomatic individuals´with positive tests in the region of Münster, North Rhine-Westphalia, Germany, 2020
Legend: "no" means "no symptoms", "yes" means "symptoms"; dots in the box plot indicate mean values and horizontal lines in the boxes indicate median values. Asymptomatic individuals : n=19, median 29.6, mean 28.8, SD 4.3; symptomatic individuals: n=39 median 25.5, mean 25.8, SD 3.7

followed by a rise to more than 1% by the end of October 2020. A substantial proportion (45%-68%) of test positive individuals in the UK did not report symptoms at the time of their positive PCR test [6].

In light of our findings that more than half of individuals with positive PCR test results are unlikely to have been infectious, RT-PCR test positivity should not be taken as an accurate measure of infectious SARS-CoV-2 incidence. Our results confirm the findings of others that the routine use of "positive" RT-PCR test results as the gold standard for assessing and controlling infectiousness fails to reflect the fact "that 50-75% of the time an individual is PCR positive, they are likely to be post-infectious" [7].

Asymptomatic individuals with positive RT-PCR test results have higher Ct values and a lower probability of being infectious than symptomatic individuals with positive results. Although Ct values have been shown to be inversely associated with viral load and infectivity, there is no international standardization across laboratories, rendering problematic the interpretation of RT-PCR tests when used as a tool for mass screening.

Declaration of Competing Interest

Paul Cullen has received speaker's fees from Roche Diagnostics. None of the other authors declares any conflict of interest.

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