

Does PCR Cycle Threshold for SARS-CoV-2 have Association with ABO Blood Group Types?

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ABSTRACT

Introduction: Corona Virus Disease-19 (COVID-19) caused by the Severe Acute Respiratory Syndrome-Corona Virus-2 (SARS-CoV-2) has led to a global pandemic. Currently, the standard method for the diagnosis of COVID-19 is the detection of SARS-CoV-2 by Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR). Lower Cycle threshold (Ct) value of PCR is found to be associated with increased probability of progression to severe disease and infectivity. Blood group A individuals have been found to be more susceptible to infection by SARS-CoV-2 while that of O blood group have lower risk of infection. There has been growing scientific interest to obtain data which may characterise the susceptibility to COVID-19 infection and determine the risk factors which may be associated with progression and severity of the disease.

Aim: To study the association of RT-qPCR Ct values for SARS-CoV-2 with ABO blood group types.

Materials and Methods: A prospective observational study was conducted at tertiary care centre in western Maharashtra, India where in a total of 116 symptomatic admitted patients between mid-July to mid-August 2020 and was found to be

positive for SARS-CoV-2 by RT-qPCR were enrolled in the study. Ct values for pan-Sarbecovirus E-gene and SARS-CoV-2 specific RdRP gene were recorded for each patient. In addition, all the patients were typed for ABO blood grouping. Distribution of different ABO blood group types in SARS-CoV-2 positive patients were analysed and was compared with blood groups in 882 non-COVID-19 blood donors comprising control group. Ct values for E and RdRP gene were also analysed in respect to different age group, gender and blood group types. Blood group records of voluntary healthy blood donors reporting at our centre between September to November 2019 were obtained.

Results: Of the 116 patients, 33 (28.45%) comprised of type A, 34 (29.31%) of type B, 41 (35.34%) were of type O and remaining 8 (6.90%) had AB blood group type. Compared to the control group, no significant difference was noted in the distribution of ABO blood types in SARS-CoV-2 positive patients. Also, no statistical significance was found in the Ct values with respect to age, gender and different ABO blood group types.

Conclusion: RT-qPCR Ct values for SARS-CoV-2 infection have no association with ABO blood group types.

Keywords: Corona virus disease-19, Reverse transcriptase quantitative polymerase chain reaction, Viral load

INTRODUCTION

A cluster of cases with pneumonia with unknown cause were noticed in Wuhan, China in December 2019. In January 2020, World Health Organisation (WHO) officially announced a new virus initially named as the 2019-novel coronavirus (2019-nCoV) and later changed to SARS-CoV-2 as the cause of disease. Later on, in February 2020, WHO officially named the disease as COVID-19. Soon the disease spread globally and in March 2020, WHO declared the coronavirus outbreak a pandemic [1]. As of 14 August 2020, globally a total of 20,687,815 confirmed cases including 750,400 deaths has been reported with 2,461,190 cases including 48,040 deaths alone in India [2]. This has led to the shift in focus of attention of the healthcare workers, scientists and researchers worldwide towards this disease.

RT-qPCR test from respiratory samples (nasopharynx/oropharynx) is currently the standard method for the diagnosis of SARS-CoV-2 infection. This test is semiquantitative in nature and the Ct value is a surrogate marker for a measure of the viral load in the sample [3]. Ct values denote the number of amplification cycles required for the target gene to achieve or exceed a specified threshold level. Ct value is inversely proportional to the viral load and several studies have reported association of lower Ct value with increased probability of progression to severe disease, infectivity and greater transmissibility [4-7].

The antigenic determinants of ABO blood groups have been reported in past to be associated with certain viral infections such as rotavirus, noroviruses (NoVs), dengue virus, Norwalk

virus and hepatitis B virus [8-11]. During this ongoing pandemic, researchers have demonstrated linkage of ABO blood group type with COVID-19 infection. Cheng Y et al., showed that individuals with O blood group as compared to non-O blood group ones were less susceptible to infection with SARS-CoV-2 [12]. Zhao J et al., based on the comparison of ABO blood groups of controls from the general population with SARS-CoV-2 infected patients reported a significantly higher risk of the infection in individuals with blood group A and lower risk in blood group O individuals [13]. Similar finding has been reported by Zietz M and Tatonetti NP in a cohort of 7770 SARS-CoV-2 positive cases [14].

Keeping in view the association of susceptibility of SARS-CoV-2 infection with ABO blood group types and Ct value with that of disease severity and infectivity rate, we aimed to assess the relationship between RT-qPCR Ct value for SARS-CoV-2 and ABO blood group type and to determine any blood type dependent difference in viral load. To the best of our knowledge, this is the first study to focus on such an association.

MATERIALS AND METHODS

A prospective and observational study was carried out at ICMR approved COVID-19 diagnostic facility of Western Maharashtra, India. Convenient sampling was used wherein all consecutive patients admitted to our tertiary care centre between mid-July to mid-August 2020 were recruited for COVID-19 testing and blood grouping. Only patients who were found to be positive for SARS-

CoV-2 by RT-qPCR were included in the study. Asymptomatic, non-admitted COVID-19 positive patients were excluded from the study. Samples obtained from all the consecutive admitted patients with COVID-19 helped us to prevent selection bias. This was a pilot study as there is no data on prior studies in Indian settings. The study was approved by the ethical Committee of the institution (IEC/2020/289) and written informed consent was obtained from all the patients.

Nasopharyngeal/Oropharyngeal swabs were collected in Viral Transport Medium (VTM) and were tested for SARS-CoV-2 within 24 hours of collection using commercially available TRUPCR SARS-CoV-2 RT qPCR kit version 2.0 (Cat No. 3B304, Kilpest India Ltd, Bhopal, India) on Light Cycler 480 II (Roche) platform (96 well plates). The samples were reported positive based on the detection of pan-Sarbecovirus E-gene sequence and SARS-CoV-2 specific RdRP gene sequence in accordance with the manufacturer instructions. Ct values for both E and RdRP genes obtained at the time of admission including age and gender of all the patients tested positive for SARS-CoV-2 were recorded. In addition, whole blood venous sample in EDTA vacutainer of all admitted 116 patients were obtained for ABO blood group typing. Records of blood groups of voluntary blood donors aged between 18 to 60 years at our hospital were obtained as control group to reflect the distribution of blood group types in the general population. These records were obtained from September 2019 to November 2019 to avoid inclusion of asymptomatic SARS-CoV-2 positivity. This control group was necessary to study any significant difference in the distribution of blood groups between SARS-CoV-2 positive and negative groups.

STATISTICAL ANALYSIS

The data were expressed as mean and Standard Deviation (SD) of Ct values and were subjected to Welch's t-test for evaluation of statistical significance of association of Ct value of E and RdRP gene with different blood group types, age groups and gender. Fisher's-exact test was used for comparison of ABO blood group distribution in SARS-CoV-2 positive patients and Control group. The statistical significance level was set at $p < 0.05$. All statistical computations were performed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA).

RESULTS

Distribution of ABO Blood Group in SARS-Cov-2 Positive Patients

Of the 116 patients positive for SARS-CoV-2 enrolled in the study, ABO blood group typing revealed that 33 (28.45%) had type A, 34 (29.31%) had type B, 41 (35.34%) were of type O and remaining 08 (6.90%) had AB blood group type. This distribution in COVID-19 patients was compared to control group comprising of 882 non-COVID-19 healthy blood donors. In the control group, type B was the most common followed by type O while type AB was the least common [Table/Fig-1]. The SARS-CoV-2 positive patient group comprised of higher percentages of individuals with type O and was the most common type followed by type B blood group. However, no significant difference was noted in the distribution of ABO blood types between the two groups i.e., SARS-CoV-2 positive and control [Table/Fig-1].

	SARS-CoV-2 +ve	Control group	p-value
Total (n)	116	882	
ABO blood group			
A	33 (28.45%)	256 (29.02%)	0.9841
B	34 (29.31%)	266 (30.16%)	0.9365
AB	08 (6.90%)	98 (11.11%)	0.2206
O	41 (35.34%)	262 (29.70%)	0.2566

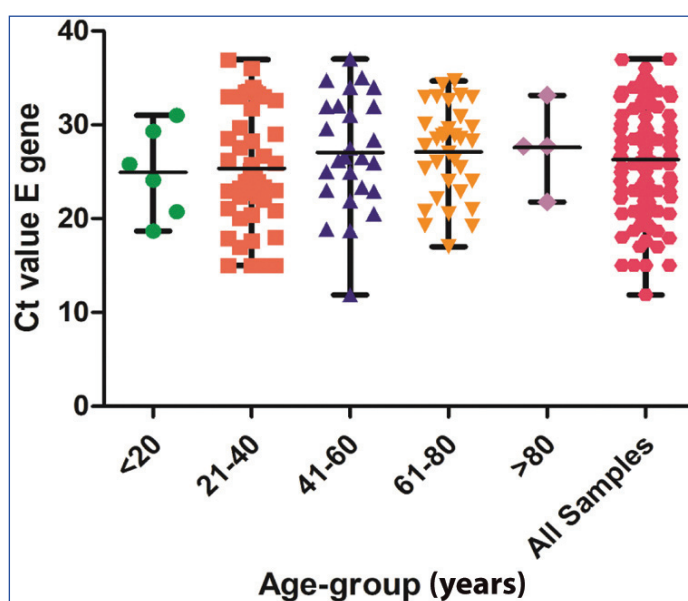
[Table/Fig-1]: Comparison of ABO blood group distribution in SARS-CoV-2 positive patients and Control group. Fisher's-exact test was used to calculate p-value (significant level $p < 0.05$)

Cycle Threshold (Ct) Values with Respect to Age, Gender and ABO Blood Group Types in SARS-CoV-2 Positive Patients

The study cohort comprised of 82 males and 34 females and was categorised into different age groups as shown in [Table/Fig-2]. The mean and SD of Ct values for E and RdRP gene in different age group, gender and ABO blood group types are shown in [Table/Fig-2]. Scatter dot plot of Ct values for both E gene and RdRP gene in different age group are shown in [Table/Fig-3,4], respectively while for that in different blood group types are plotted in [Table/Fig-5,6]. No statistical significance was noted in the Ct values with respect to age, gender and ABO blood group types either within or between the groups.

Characteristic	No. of samples	E gene			RdRP gene		
		Mean	SD	p-value	Mean	SD	p-value
All samples	116	26.29	5.65	-	28.20	5.51	-
Age (years)							
≤20	06	24.95	4.77	0.7702	26.11	4.29	0.6076
21-40	47	25.34	6.15	0.4641	27.44	5.96	0.4952
41-60	26	27.06	6.03	0.6290	29.08	5.67	0.8061
61-80	33	27.11	4.83	0.3081	28.72	4.69	0.2961
>80	04	27.59	4.64	0.8608	30.39	7.19	0.3398
Gender							
Males	82	26.05	5.74	0.8499	27.84	5.46	0.9446
Females	34	26.85	5.49	0.9066	29.09	5.60	0.8661
ABO blood group							
A	33	25.36	5.67	0.9373	27.54	5.13	0.6582
B	34	25.59	5.36	0.7508	27.40	5.02	0.5449
AB	08	28.68	3.90	0.2980	30.06	4.60	0.6489
O	41	27.15	6.06	0.5630	29.04	6.28	0.2861

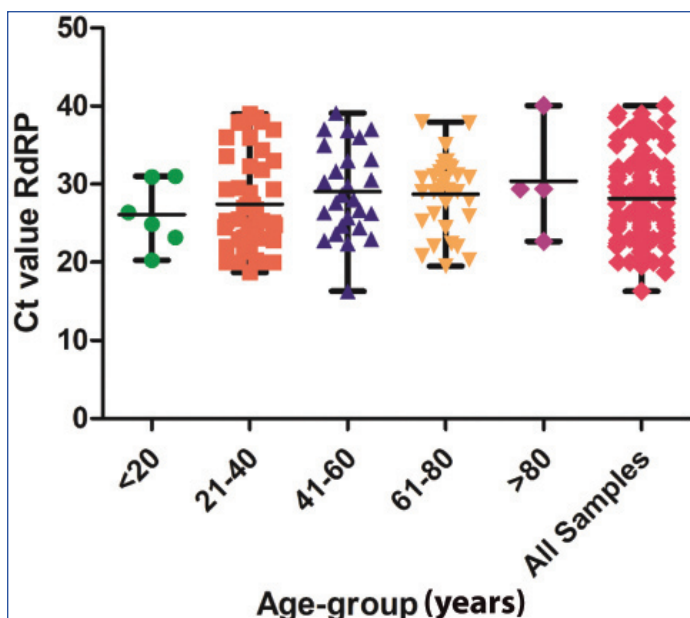
[Table/Fig-2]: Cycle threshold (Ct) values of E gene and RdRP gene in SARS-CoV-2 positive patients and its correlation in different age group, gender and ABO blood group types. SD: Standard deviation p-values are based on a comparison of mean CT values of each age group, gender and ABO blood group type vs the mean of all Ct values for E gene and RdRP gene using Welch's t-test



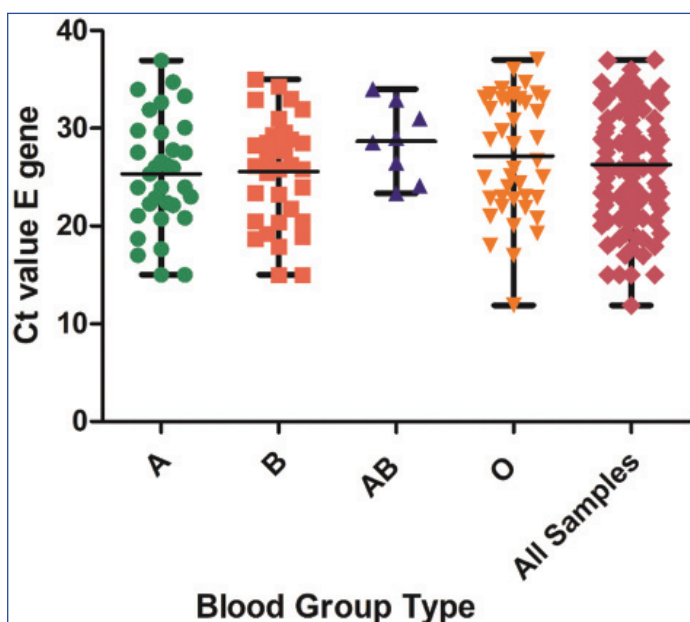
[Table/Fig-3]: Scatter dot plot with line at mean and range showing comparison of Cycle Threshold (Ct) of E gene by age group.

DISCUSSION

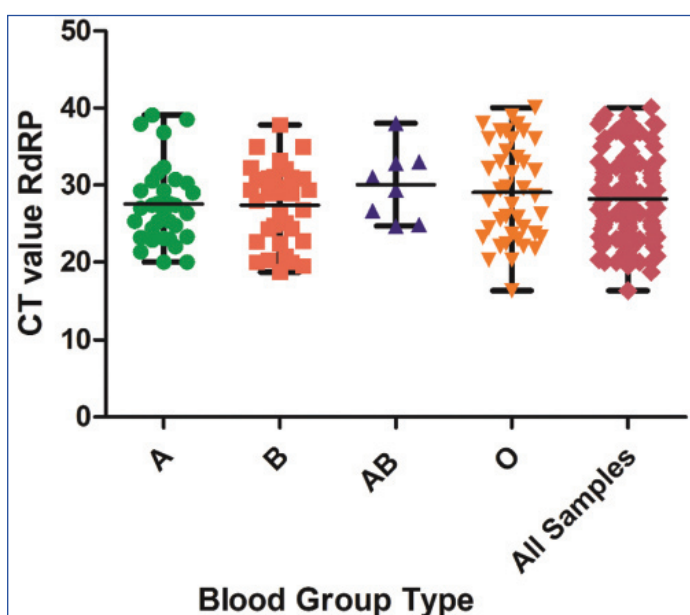
Blood group antigens are well known to modify the immune response to infection and based on the differences in blood group antigen expression, individuals may be susceptible or protected against certain infections [15]. Few studies mostly originating from China, have demonstrated that patients with blood group A were more



[Table/Fig-4]: Scatter dot plot with line at mean and range showing comparison of Cycle Threshold (Ct) of RdRP gene by age group.



[Table/Fig-5]: Scatter dot plot with line at mean and range showing comparison of Cycle Threshold (Ct) of E gene by ABO blood group type.



[Table/Fig-6]: Scatter dot plot with line at mean and range showing comparison of Cycle Threshold (Ct) of RdRP gene by ABO blood group type.

susceptible to SARS-CoV-2 infection, whereas patients with blood group O relatively had a lower risk [13,14,16]. Coronavirus is an enveloped virus that targets host cells via viral adhesion glycoproteins. The SARS-CoV spike (S) protein is such a viral adhesion glycoprotein. Guillon P et al., have demonstrated that the natural human anti-A antibodies might block the interaction between SARS-CoV spike protein and its receptor angiotensin-converting enzyme 2, thus providing protection. This could well be explained by individuals with blood group A with absence of naturally occurring anti-A antibodies tend to be more susceptible to SARS-CoV infection [17].

Several studies on COVID-19 have demonstrated that lower Ct values were associated with more severe disease and infectivity [4-7]. This is in conformity with earlier studies published on Ct values in other respiratory infections such as Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Respiratory Syncytial virus [18,19]. The association of susceptibility of SARS-CoV-2 infection with ABO blood group types and Ct value with that of disease severity and infectivity rate led us to assess the relationship between RT-qPCR Ct value for SARS-CoV-2 and ABO blood group type and to determine any blood type dependent difference in viral load.

Present study did not showed any association of ABO blood group type with susceptibility to infection by SARS-CoV-2. It also did not reveal any significant difference in Ct values for pan-Sarbecovirus E and SARS-CoV-2 specific RdRP gene in different blood group types. These results were in variance with those reported earlier [4-7,13,14,16]. This variance in the results may be explained based on the differences in the ethnicity of the population studied. There is a definite racial element to ABO blood typing [20,21]. Present study certainly raises a pertinent question on the effects of ethnicity on COVID-19 susceptibility and severity. Moreover, the time from onset of symptoms to sampling varied between patients which could have affected the Ct values.

Unlike the study by Buchan BW et al., where they showed significant correlation of Ct value with age between 80-89 years, present study did not show any significance with different age group. This difference may be attributed to very small number of patients in this group i.e., 4 patients comprising just 3.45% of the total cohort. However, present study result of Ct values showing no statistical significance with gender difference corroborated with that of Buchan BW et al., [22].

Limitation(s)

The main limitation in this study was the small sample size. Moreover, there was a lead time bias as the time from onset of symptoms to sampling differed between patients. The Ct value of SARS-CoV-2 is known to vary during the course of infection and tends to be higher in early symptomatic stage. Additionally, Berksonian bias cannot be ruled out as our study population includes hospitalised patients. However, present centre being a multi-speciality government hospital, people from different strata and socio-economic status were screened for SARS-CoV-2 infection before admission, during current pandemic.

CONCLUSION(S)

Present study did not find any association of Ct values for SARS-CoV-2 with ABO blood group types. However, the knowledge on COVID-19 is constantly evolving and a wide range in response and course of COVID-19 disease has been noted in different ethnic population. Since there is no published data from India on association of ABO blood group types with susceptibility to SARS-CoV-2 infection and its correlation with Ct values. Therefore, further large-scale studies based on Indian population are needed for more definitive results.

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REFERENCES

- [1] Wiersinga WJ, Rhodes A, Cheng AC, Peacock SJ, Prescott HC. Pathophysiology, transmission, diagnosis, and treatment of Coronavirus Disease 2019 (COVID-19): A Review. *JAMA*. 2020;324(8):782-93.
- [2] WHO. Coronavirus disease (COVID-2019) situation reports. 2020. <https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situationreports>. Accessed 14 Aug 2020.
- [3] Tom MR, Mina MJ. To interpret the SARS-CoV-2 test, consider the cycle threshold value. *Clin Infect Dis*. 2020:ciaa619.
- [4] Liu Y, Yan LM, Wan L, Xiang T, Li A, Liu JM, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*. 2020;20:656-57.
- [5] Liu Y, Liao W, Wan L, Xiang T, Zhang W. Correlation between relative nasopharyngeal virus RNA load and lymphocyte count disease severity in patients with COVID-19. *Viral Immunol* [Internet]. 2020 Apr 10 [cited 2020 Aug 17]. Available from: <https://www.libertpub.com> DOI: 10.1089/vim.2020.0062.00 [Epub ahead of print].
- [6] Liu YY, Yang Y, Zhang C, Huang F, Wang F, Yuan J, et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. *Sci China Life Sci*. 2020;63:364-74.
- [7] Rao SN, Manissero D, Steele VR, Pareja J. A narrative systematic review of the clinical utility of cycle threshold values in the context of COVID-19. *Infect Dis Ther*. 2020;9:573-86.
- [8] Kalayanarooj S, Gibbons RV, Vaughn D, Green S, Nisalak A, Jarman RG, et al. Blood group AB is associated with increased risk for severe dengue disease in secondary infections. *J Infect Dis*. 2007;195(7):1014-17.
- [9] Hennessy EP, Green AD, Connor MP, Darby R, MacDonald P. Norwalk virus infection and disease is associated with ABO histo-blood group type. *J Infect Dis*. 2003;188(1):176-77.
- [10] Kazi AM, Cortese MM, Yu Y, Lopman B, Morrow AL, Fleming JA, et al. Secretor and salivary ABO blood group antigen status predict rotavirus vaccine take in infants. *J Infect Dis*. 2017;215(5):786-89.
- [11] Liu J, Zhang S, Liu M, Wang Q, Shen H, Zhang Y. Distribution of ABO/Rh blood groups and their association with hepatitis B virus infection in 3.8 million Chinese adults: A population-based cross-sectional study. *J Viral Hepat*. 2018;25(4):401-11.
- [12] Cheng Y, Cheng G, Chui CH, Lau FY, Chan PKS, Ng MHL, et al. ABO blood group and susceptibility to severe acute respiratory syndrome. *JAMA*. 2005;293(12):1450-51.
- [13] Zhao J, Yang Y, Huang HP, Li D, Gu D, Lu X, et al. Relationship between the ABO blood group and the COVID-19 susceptibility. *medRxiv* [Internet]. 2020 Mar 27 [cited 2020 Aug 17]. Available from: <https://www.medrxiv.org/content/10.1101/2020.03.11.20031096v2>. [Epub ahead of print].
- [14] Zietz M, Tatonetti NP. Testing the association between blood type and COVID-19 infection, intubation, and death. *medRxiv* [Internet]. 2020 Apr 11 [cited 2020 Aug 17]. Available from: <https://www.medrxiv.org/content/10.1101/2020.04.08.20058073v2>. [Epub ahead of print].
- [15] Cooling L. Blood groups in infection and host susceptibility. *Clin Microbiol Rev*. 2015;28(3):801-70.
- [16] Wu Y, Feng Z, Li P, Yu Q. Relationship between ABO blood group distribution and clinical characteristics in patients with COVID-19. *Clinica Chimica Acta*. 2020;509:220-23.
- [17] Guillon P, Clément M, Sébille V, Rivain JG, Chou CF, Ruvoën-Clouet N, et al. Inhibition of the interaction between the SARS-CoV spike protein and its cellular receptor by anti-histo-blood group antibodies. *Glycobiology*. 2008;18(12):1085-93.
- [18] Feikin DR, Alraddadi B, Qutub M, Shabouni O, Curms A, Oboho IK, et al. Association of higher MERS-CoV virus load with severe disease and death, Saudi Arabia, 2014. *Emerg Infect Dis*. 2015;21:2029-35.
- [19] Wishaupt JO, Ploeg TV, Smeets LC, Groot R, Versteegh FG, Hartwig NG. Pitfalls in interpretation of CT-values of RT-PCR in children with acute respiratory tract infections. *J Clin Virol*. 2017;90:01-06.
- [20] Liu J, Zhang S, Wang Q, Shen H, Zhang Y, Liu M. Frequencies and ethnic distribution of ABO and RhD blood groups in China: A population-based cross-sectional study. *BMJ Open*. 2017;7(12):e018476.
- [21] Garratty G, Glynn SA, McEntire R. ABO and Rh (D) phenotype frequencies of different racial/ethnic groups in the United States. *Transfusion*. 2004;44(5):703-06.
- [22] Buchan BW, Hoff JS, Gmehlin CG, Perez A, Faron ML, Price LSM, et al. Distribution of SARS-CoV-2 PCR cycle threshold values provide practical insight into overall and target-specific sensitivity among symptomatic patients. *Am J Clin Pathol*. 2020;154(4):479-85.

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