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Performance of UMELISA SARS-CoV-2 antigen and STANDARD F COVID-19 in the diagnosis of COVID-19 in Villa Clara, Cuba

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Abstract

A cross-sectional descriptive study was carried out with the objective of describing the performance of UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag FIA in the diagnosis of COVID-19, at the Provincial Laboratory of Microbiology and Sanitary Chemistry of Villa Clara, during the month of February 2022. 1056 patients who underwent Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) using the QIAcube HT genome extraction technique, UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag FIA were included. Sensitivity, specificity, positive and negative predictive value were calculated as efficacy indicators and Spearman's Rho coefficient for the correlation between variables. The result was that 29.3% were sick, of which 189 were female, with a median age of 46 years. 74.8% had symptoms, most often fever, cough and runny nose. UMELISA SARS-CoV-2 Antigen showed higher specificity with 97.45%; while STANDARD F COVID-19 Ag FIA had higher sensitivity with 99.35%. It was shown that there is a significant inverse correlation between the RT-PCR cycle threshold and the fluorescence values of both techniques studied (p=0.000). About a third of the patients studied were sick with symptoms, with a predominance of females. UMELISA SARS-CoV-2 Antigen turned out to be more useful in confirming the presence of the disease, while STANDARD F COVID-19 Ag FIA showed better performance in ruling out the presence of the disease.

Keywords: Performance; UMELISA SARS-Cov-2 Antigen; STANDARD F COVID-19 Ag FIA; RT; PCR; COVID-19

1. Introduction

The ongoing SARS-CoV-2 pandemic has been associated with a significant burden and unprecedented pressure on healthcare systems [1-3]. Therefore, the availability of accurate and rapid diagnostic tools for COVID-19 is essential for both active case monitoring and contact tracing strategies to reduce the circulation of the causative agent [1-4].

The gold standard for the diagnosis of SARS-CoV-2 infections is reverse transcription-polymerase chain reaction (RT-PCR), which is highly sensitive, but requires extensive laboratory infrastructure, expensive materials, and skilled personnel. These aspects limit the scalability and implementation of RT-PCR in many settings, especially those with low

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resources. Antigen detection rapid diagnostic tests (Ag-RDTs) provide results quickly and are easy to use, fulfilling the characteristics required for a public health testing tool [4-6].

Currently, there are different immunological tests that can detect SARS-CoV-2 nucleoprotein antigens. These assays are performed using different methodologies, such as enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), colloidal gold immunochromatography, and fluorescent immunoassays (FIA) [3-7].

Antigen tests for SARS-CoV-2 detect or can quantify nucleocapsid (N) or spike (S1 and S2) proteins of the virus. S1 appears to be the most variable antigen, making it a good candidate for differentiation among other coronaviruses. However, the S2 subunit shares similarity in antibody epitopes (region of an antigen recognized by an antibody) with S2 of the original SARS-CoV. Nucleoprotein (N) is the most abundant viral protein produced and shed during infection with SARS-CoV-2 [4-6].

Different studies have suggested the usefulness of these methods for pandemic control, by mass testing or testing to protect (high-risk settings, such as hospitals), release (contact testing), and enable (regular school or workplace testing) [2,5]. For the WHO Emergency Use List (EUL), Ag-RDTs are required to meet the targets of at least 80% sensitivity and 97% specificity for use in the diagnosis of SARS-CoV-2 [1,4,7,8,11].

In Cuba, as in the rest of the world, COVID-19 has generated a great impact on the country's economy, especially in the field of public health [9], being an important pillar the help provided by many countries, in terms of supplies, medical equipment and new diagnostics for SARS-CoV-2 infection. Our country has also worked since the beginning of the pandemic, developing PCR reagents, viral transport media and new diagnostic techniques [9,10].

Since the introduction of the UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag FIA diagnostic methods in Villa Clara, in July and September 2021 respectively, they have proven to be two important pillars for the rapid and timely diagnosis of COVID-19 positive patient contacts and in the epidemiological surveillance of areas of high transmission. The UMELISA SARS-CoV-2 Antigen (CIE, Cuba) is a heterogeneous sandwich enzyme-linked immunosorbent assay that employs the advantages of the high affinity reaction between Streptavidin and Biotin. This assay uses as solid phase ultramicroELISA plates coated with monoclonal and polyclonal antibodies specific to the SARS-CoV-2 protein (N). The STANDARD F COVID-19 Ag FIA kit (SD BIOSENSOR, Inc., Republic of Korea) is a test that allows rapid and qualitative detection of SARS CoV-2 nucleoproteins in nasopharyngeal exudate samples. It is based on immunofluorescence technology and uses europium-conjugated monoclonal antibodies to detect SARS-CoV-2 nucleoprotein antigens [4,5,6,12].

This study aims to describe the performance of UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag FIA for the diagnosis of COVID-19, at the Provincial Laboratory of Microbiology and Sanitary Chemistry of Villa Clara, during the month of February 2022.

2. Material and methods

2.1. Type of study and context

A cross-sectional descriptive analysis was carried out at the Provincial Laboratory of Microbiology and Sanitary Chemistry of Villa Clara, during February 2022.

2.2. Study population

A total of 1056 patients were included, from nasopharyngeal exudate samples taken in a universal transport medium (BTU) for viruses (BIOCEN, Cuba); RT-PCR with the QIAcube HT genome extraction technique, UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag were performed.

2.3. Data collection

The data required for the study were obtained from the patient registry of the Molecular Biology Laboratory and SUMA SARS-CoV-2 and epidemiological surveys. The procedures performed in each case for the application of the diagnostic methods are described below.

For real-time RT-PCR, the samples were processed using the QIAcube® HT genome extraction equipment (QIAGEN Group, Germany) after shaking with the Vortex shaker. This automatic extraction equipment processes from 24 to 96

samples in 2 hours and 10 minutes. The first step required extraction of viral RNA with the commercial QIAamp® 96 Virus QIAcube® HT Kit (QIAGEN Group, Germany) from 200 µL of sample, and the second step involved detection of the viral genome. Amplification was performed with the STAT-NAT®SARS-CoV-2 kit (SENTINEL DIAGNOSTICS, Italy) with probes amplifying three SARS-CoV-2 target genes; the RNA-dependent RNA polymerase (RdRp) gene, the envelope gene (E) and the nucleocapsid gene (N). Ribonuclease P (RNase P gene) was used as internal control (IC). The amplification and detection process were performed with the real-time RT-PCR platform: Rotor-Gene® Q MDx (QIAGEN Group, Germany). Samples with threshold value (Ct) of the SARS-CoV-2 RT-PCR cycle lower or equal to 33 were considered positive, according to the manufacturer's instructions. The expected run time of the process is about 2 hours.

For diagnosis with the UMELISA SARS-CoV-2 Antigen technique, samples were initially vortexed and diluted 1:2 with the R2 working solution (40 ul of sample + 40 ul of solution is recommended), using 96-well dilution plates with lids. The diluted samples were then shaken for 1 minute with the Shake option of the SUMA technology plate washer; they were incubated for 1 hour in a humid chamber at room temperature (20-250C). Subsequently, 10 ul were transferred to each well of the reaction plate, and the assay controls (2 positive and 4 negative) were added. The reaction plates were incubated for 1 hour at 37 0C in a humid chamber. Then, after washing to remove unbound sample components, biotylated protein N-specific antibodies were added and bound to the antibody-antigen complex formed on the solid phase. A new washing eliminated the biotinylated antibodies that did not react and remained in excess after incubation for 30 minutes at 37 0C in a humid chamber.

Next, the Streptavidin/Alkaline Phosphatase conjugate (binding to biotin molecules) was added and after another incubation step for 30 minutes at 37 0C in a humid chamber and washing, the fluorogenic substrate (4 Methylumbelliferyl phosphate) was added, which allowed detecting the presence of SARS-CoV-2 antigens in the sample by the intensity of the emitted fluorescence. They were incubated for 30 minutes at 20 - 25 0C in a humid chamber. Under these conditions, a fluorescence signal of the Positive Control ≥ 100 units and of the Negative Control between 4 and 12 units was guaranteed. Complying with these incubation conditions, it is common for the freshly reconstituted Positive Control to yield a value of 210 fluorescence units (FU), without constituting an erroneous execution of the technique. Therefore, this incubation time should not be shortened unnecessarily. The reading of the intensity of the fluorescence emitted in each determination was performed using a SUMA technology reader. The validation, interpretation of results and their printing were performed automatically by the SUMA reader with the UMELISA SARS-CoV-2 Antigen program. They can also be done manually by the operator following the instructions described below: the minimum conditions required to ensure the quality of the assay are as follows: at least one of the Positive Control (P1 or P2) duplicates must have a fluorescence value ≥ 100 units and at least two of the four Negative Control replicates must have a fluorescence between 4 and 12 units.

The STANDARD F COVID-19 Ag FIA test was performed according to the manufacturer's instructions: 1) the nasopharyngeal exudate sample was inserted into an extraction tube buffer and then shaken at least five times; 2) after removing the swab, 4 drops (approximately 100 µL) of plain mixture were added into the test device. When the sample came in contact with the strip, passive diffusion allowed the sample to react with the anti-SARS-CoV-2 antibodies immobilized on the membrane and generate a fluorescence signal. A control line is included in the strip to assess the correct migration of the sample. Interpretation of the result was performed after 30 min using the STANDARD F200 Analyzer (SD BIOSENSOR, Inc., Republic of Korea), which provided a COI (cut-off indices) value (Negative <1 and Positive ≥ 1).

2.4. Data processing

Data were brought to a file in Microsoft Excel program version 2016 and processed through SPSS version 22.0 and EPIDAT version 3.1 programs. Absolute (number of cases) and relative (percentages) frequencies were determined in the conformed frequency distributions, and measures of central tendency (median) were calculated for the variable age. To evaluate the performance of UMELISA SARS-CoV-2 Antigen and STANDARD F COVID-19 Ag FIA as diagnostic tests in relation to RT-PCR, as a reference test, the basic indicators were determined: sensitivity, specificity, positive and negative predictive value as indicators of efficacy, as well as their intervals, with a confidence level (CI) of 95%. Spearman's Rho coefficient was determined for the correlation between the variables, cycle threshold (Ct) of the RT-PCR and the fluorescence value (Fluor) of the UMELISA SARS-CoV-2, and the Ct of the RT-PCR and the cut-off index (COI) of the STANDARD F COVID-19.

3. Results

Table 1 shows the distribution of patients according to the RT-PCR results. Of a total of 1056 patients, 310 were found to have COVID-19, or 29.4%. Of the 310 patients confirmed as having COVID-19, 189 were female (61.0%), with a median age of 46 years.

Table 1 Status of patients according to RT-PCR results at the Provincial Laboratory of Microbiology and Sanitary Chemistry of Villa Clara, February 2022

Status of patients	Number	%
Diseased	310	29.4
Healthy	746	70.6
Total	1056	100

Source: Molecular Biology Laboratory patient registry

Of the total number of patients studied, 676 presented symptoms. Of the 310 patients, 232 presented symptoms, which constitutes 74.8% (Table 2).

Table 2 Distribution of patients according to symptomatology

Symptomatology		PCR		Total
		Diseases	Healthy	
Yes	Number	232	444	676
	%	74.8	59.5	64.0
No	Number	78	302	380
	%	25.2	40.5	36.0
	Total	310	746	1056
	%	100	100	100

Source: Epidemiological survey of patients

Among the main clinical manifestations reported by the ill patients, fever, cough and rhinorrhea predominated with 49.7%, 36.5% and 32.3%, respectively (Table 3). Of the 286 tests that were positive by UMELISA SARS-CoV-2 Antigen, 267 were truly sick and within the 770 negative tests, 727 were healthy (Table 4).

Corresponding to the results referred to above, the results of the main indicators to evaluate the performance of this diagnostic test were obtained. The percentage of patients with positive results to UMELISA SARS-CoV-2 within the total number of patients was 86.13 (sensitivity) and the percentage of patients with negative results to UMELISA SARS-CoV-2 within the total number of healthy patients was 97.45 (specificity). The percentage of patients among those who tested positive was 93.36 (positive predictive value) and the percentage of healthy patients among those who tested negative was 94.42 (negative predictive value) (Table 5).

Table 3 Clinical manifestations in patients suffering from COVID-19

Clinical manifestations	Number (n = 310)	%*
Fever	154	49,7
Tos	113	36,5
Rhinorrhea	100	32,3
Cephalea	72	23,2

General malaise	42	13,6
Sore throat	36	11,6
Nasal congestion	31	10
Arthralgias	21	6,8
Expectoration	19	6,1
Myalgias	10	3,2
Retrocular pain	6	1,9
Diarrhea	5	1,6
Dyspnea	4	1,3
Loss of taste	3	0,9
Loss of appetite	2	0,7
Odynophagia	2	0,7

*Percentage calculated in relation to n Source: Epidemiological survey of patients

Table 4 Results of patients tested with UMELISA SARS-CoV-2 Antigen and RT-PCR

UMELISA SARS-CoV-2 Antigen	**RT-PCR**		
	Deseases	**Healthy**	**Total**
Positive	267	19	286
Negative	43	727	770
Total	310	746	1056

Source: Molecular Biology Laboratory patient registry and SUMA SARS-CoV-2.

Table 5 Performance of UMELISA SARS-CoV-2 Antigen for the diagnosis of COVID-19

Indicators	**Value**	**Confidence interval (95 %)**	
		Lower limit	**Upper limit**
Sensibility	86.13	82.12	90.14
Specificity	97.45	96.26	98.65
Predictive value +	93.36	90.30	96.42
Predictive value -	94.42	92.73	96.10

Source: table 4.

Of the 340 tests that were positive for STANDARD F COVID-19 Ag FIA, 308 were truly diseased and of the 716 that were negative, 714 were healthy (Table 6).

Table 6 Result of patients tested with STANDARD F COVID-19 Ag FIA and RT-PCR

STANDARD F COVID-19 Ag FIA	**RT- PCR**		
	Sick	**Healthy**	**Total**
Positive	308	32	340
Negative	2	714	716

Total	310	746	1056

Source: Molecular Biology Laboratory patient registry and SUMA SARS-CoV-2.

STANDARD F COVID-19 Ag FIA showed a sensitivity level of 99.35% and specificity of 95.71% and positive and negative predictive values of 90.59% and 99.72%, respectively (Table 7).

Table 7 Performance of the STANDARD F COVID-19 Ag FIA for the diagnosis of COVID-19

Indicators	**Value**	**Confidence interval (95 %)**	
		Lower limit	**Upper limit**
Sensibility	99.35	98.30	100.00
Specificity	95.71	94.19	97.23
Predictive value +	90.59	87.34	93.84
Predictive value -	99.72	99.26	100.00

Source: table 6.

Table 8 shows the correlation between the cycle threshold (Ct) of the RT-PCR and the fluorescence value (Fluor) of UMELISA SARS-CoV-2 Antigen, as well as between the Ct of the RT-PCR and the cut-off index of STANDARD F COVID-19 Ag FIA. In both cases an inverse correlation with statistical significance (p=0.000) was observed; as Ct values increase, there is a decrease in the fluorescence values of UMELISA and COI values of STANDARD F COVID-19 Ag FIA.

Table 8 Correlation between the fluorescence value (Fluor) of UMELISA SARS-CoV-2 Antigen and cut-off index (COI) of STANDARD F COVID-19 Ag FIA with the cycle threshold (Ct) of RT-PCR

Variables	**Cycle Threshold (Ct) RT-PCR**	
	Rho de Spearman	**Significance ***
Fluor Value		
UMELISA SARS-CoV-2 Antigen	-0.227	0.000
COI Value		
STANDARD F COVID-19 Ag FIA	-0.228	0.000

* Correlation is significant at the 0.01 level (2-tailed); Source: Molecular Biology Laboratory patient registry and SUMA SARS-CoV-2.

4. Discussion

According to the results presented in Table 5, the UMELISA SARS-CoV-2 Antigen proved to be a more specific than sensitive diagnostic test, expressing fewer false positive results and indicating the presence of the disease within diagnosed patients, results that agree with those obtained by Zang et al. (2020) [3] and Liu et al. (2022) [6].

The results obtained in our study with the UMELISA SARS-CoV-2 Antigen agreed with the targets set by WHO for antigen detection tests [13,14], of at least 80% sensitivity and 97% specificity to be used in the diagnosis of SARS-CoV-2, which agrees with other authors in this regard [4,5,15]. The results showed similarity with those obtained by the manufacturer of the test and with the "Pedro Kourí" Institute of Tropical Medicine (IPK - reference laboratory of the country), according to information provided by the UMELISA SARS-CoV-2 Antigen of the Immunoassay Center, edition No. 1 of 2021.

Table 7 shows the results in relation to the STANDARD F COVID-19 Ag FIA, which proved to be a more sensitive than specific diagnostic test, expressing fewer false-negative results and indicating the absence of the disease in diagnosed patients [15-19].

In a study conducted in Milan, Italy, in 2020, where the rapid diagnostic test STANDARD F COVID-19 Ag FIA was evaluated in nasopharyngeal swab samples from symptomatic patients, they obtained a sensitivity of 45% and specificity of 100% [7]; not agreeing with our results; it should be noted that the population studied in this research

was smaller than ours. In Spain, in a study in symptomatic patients with a high viral load (Ct ≤ 30), they obtained a lower sensitivity (92.9%) and a higher specificity (99.6%) in relation to our results, highlighting that their studied population was much smaller (n=68) [12].

Table 8 presents the results regarding the correlation between the fluorescence value (Fluor) of UMELISA SARS-CoV-2 Antigen and cut-off index (COI) of STANDARD F COVID-19 Ag FIA with the cycle threshold (Ct) of RT-PCR. Interpreting diagnostic test values for COVID-19 is essential for clinicians, epidemiologists and microbiologists [20-25]. Ct is a semiquantitative value inversely related to the amount of RNA in the sample, so that a low Ct number is related to higher viral load and vice versa [4,5,12,15,21,26]. RT-PCR Ct analysis for SARS-CoV-2 is key in deciding the therapeutic algorithm for positive patients [18,21,23,26]

5. Conclusion

UMELISA SARS-CoV-2 Antigen proved to be more useful in confirming the presence of the disease, while STANDARD F COVID-19 Ag FIA showed better performance in ruling out the presence of the disease.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Huang C, Wang Y, Li X, Ren I, Zhao J, Hu Y., et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395: 497-506.

[2] Shi S, Qin M, Yang B. Coronavirus disease 2019 (COVID-19) and cardiac injury-reply. JAMA Cardiol. 2020; 7: 802-810.

[3] Zang R, Gomez CMF, McCune BT, Zeng Q, Rothlauf PW, Sonnek NM., et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. Sci Immunol. 2020; 47: eabc3582.

[4] Bruzzone B, De Pace V, Caligiuri P, Ricucci V, Guarona G, Pennati BM., et al. Comparative diagnostic performance of rapid antigen detection tests for COVID-19 in a hospital setting. International Journal of Infectious Diseases. 2021; 107: 215-218.

[5] Kruger LJ, Tanuri A, Lindner AK, Gaeddert M, Koppel L, Tobian F. et al. Accuracy and ease-of-use of seven point-of-care SARS-CoV-2 antigen-detecting tests: A multi-centre clinical evaluation. eBioMedicine. 2022; 75(103774): 2352-3964.

[6] Liu J, Zhang Y, Han L, Guo S, Wu S, Doud EH., et al. Genome-wide analyses reveal the detrimental impacts of SARS-CoV-2 viral gene Orf 9c on human pluripotent stem cell-derived cardiomyocytes. Stem Cell Reports. 2022; 17: 1-16.

[7] Carcione D, Intra J, Riggio D, Sabella S, Rondelli L, Barbieri S, Leoni V, Biondi ML. Evaluation of a rapid diagnostic test for detection of SARS-CoV-2 antigen in nasopharyngeal swabs. Microbiología Médica. 2021; 36(1): 9623.

[8] Kiro VV, Gupta A, Singh P, Sharad N, Khurana S, Prak AS., et al. Evaluation of COVID-19 antigen fluorescence immunoassay test for rapid detection of SARS-CoV-2. J Global Infect Dis. 2021; 13(2): 91-93.

[9] Serrano CA, Ruiz GA, Segura FA, Olmo QV, Micó PRM, Barquilla GA., et al. Application of the threshold value of the number of cycles (Ct) of PCR in COVID-19. Family Medicine. SEMERGEN. 2021; 47: 337-341.

[10] Valdés GLE, Domínguez MA, Valdés VA, Sagaró CNM. Effect of COVID-19 control measures on other respiratory transmitted diseases. Rev Cubana Med Trop. 2022; 74 (1): e738.

[11] Andres AD, Feng Y, Campos AR, Yin J, Yang CC, James B., et al. SARS-CoV-2 ORF9c is a membrane-associated protein that suppresses antiviral responses in cells. bioRxiv. 2020; 8: 1-45.

[12] García FS, Marcos DP, Velasco de la Fuente S, Reina RMJ, Gonzalo M, Rodríguez LJ., et al. Evaluation of the rapid antigen detection test STANDARD F COVID-19 Ag FIA for diagnosing SARS-CoV-2: Experience from an Emergency. Department, Diagnostic Microbiology & Infectious Disease. 2022; 103 (2).

[13] World Health Organization (WHO). COVID-19: Cronología de la actuación de la OMS. 27 de abril de 2020. Available at: https://www.who.int/es/news/item/27-04-2020-who-timeline-covid-19

[14] World Health Organization (WHO). WHO Coronavirus (COVID-19).

[15] Carrazco MA, Herrera YA, Alarcón VD, Gutiérrez PD, Armendáriz CI, Andrade MD., et al. Omicron Sub-Lineages (BA.1.1.529+BA.*) Current Status in Ecuador. Viruses. 2022; 14: 1170-1177.

[16] Wu F, Zhao S, Yu B, Chen Y, Wang W, Song Z., et al. A new coronavirus associated with human respiratory disease in China. Nature. 2020; 579: 265-269.

[17] Hu B, Guo H, Zhou P, Shi Z. Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol. 2021; 19: 141-154.

[18] Ito K, Piantham C, Nishiura H. Relative instantaneous reproduction number of Omicron SARS-CoV-2 variant with respect to the Delta variant in Denmark. J Med Virol. 2022; 94: 2265-2268.

[19] Wu CR, Yin WC, Jiang Y, Xu HE. Structure genomics of SARS-CoV-2 and its Omicron variant: Drug design templates for COVID-19. Acta Pharmacol Sin. 2022; 0: 1-13.

[20] Raćnik J, Koćevar A, Slavec B. Transmission of SARS-CoV-2 from human to domestic ferret. Emerg Infect Dis. 2021; 27: 2450-2453.

[21] Yen HL, Sit TH, Brackman CJ, Chuk SSY, Gu H, Tam KWS., et al. Transmission of SARS-CoV-2 delta variant (AY.127) from pet hamsters to humans, leading to onward human-to-human transmission: a case study. Lancet. 2022; 399: 1070-1078.

[22] Cambra BI, Guardiola E, Baños JE. The COVID-19 Pandemic in Serial Medical Dramas. The Arts and Medicine. 2021; 20: 1-3.

[23] Flor LS, Friedman J, Spencer CN, Cagney J, Arrieta A, Herbert ME., et al. quantifying the effects of the COVID-19 pandemic on gender equality on health, social, and economic indicators: a comprehensive review of data from March, 2020, to September, 2021. The Lancet. 2022; 2: 1-17.

[24] Vadrevu KM, Reddy S, Jogdand H, Ganneru B, Mirza N, Tripathy VN., et al. Immunogenicity and reactogenicity of an inactivated SARS-CoV-2 vaccine (BBV152) in children aged 2-18 years: interim data from an open-label, non-randomised, age de-escalation phase 2/3 study. Lancet Infect Dis. 2022; 3: 1-10.

[25] Bi Q, Wu Y, Mei S. Epidemiology and transmission of COVID-19 in 391 cases and 1286 of their close contacts in Shenzhen, China: a retrospective cohort study. Lancet Infect Dis. 2020; 20: 911-919.

[26] Mallapaty S. Will COVID become a disease of the young? Nature. 2021; 595: 343-344.