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ORIGINAL ARTICLE

Evaluating the Performance of Four SARS-CoV-2 Commercial Chemiluminescence Immunoassays

[1]Sahar M. Khairat, [2]Nancy ELGuindy, [3]Amany E. Sheta, [4]Usama A. Bahaa Eldin, [5]Yasmin Adel El-Mahdy, [5]Noha S. Soliman*

[1]Professor of Clinical and Chemical Pathology. Faculty of Medicine, Cairo University. Director of the Microbiology Unit, Central Public Health Laboratories (CPHL), Ministry of Health, Egypt
[2]Professor of Clinical and Chemical Pathology. Faculty of Medicine, Cairo University. Head of Central Public Health Laboratories (CPHL), Ministry of Health, Egypt
[3]Director of Serology Unit, Central Public Health Laboratories (CPHL), Ministry of Health, Egypt
[4]Chemist of Serology Unit, Central Public Health Laboratories (CPHL), Ministry of Health, Egypt
[5]Lecturer of Clinical and Chemical Pathology. Faculty of Medicine, Cairo University

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Corresponding Author:
Noha S. Soliman
Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University.
Cairo, Egypt.
Tel.: 01016935707
nsal18@yahoo.com

ABSTRACT

Background: *COVID-19 is a pandemic of serious global threat that forced test developers to flood the market with various diagnostic assays that have been independently validated.* ***Objectives:*** *This study aims at assessing the performance of four SARS-CoV-2 chemiluminescence immunoassays.* ***Methodology:*** *The present study included sera from 96 Polymerase Chain Reaction (PCR)–confirmed COVID-19 cases collected at 2 time periods (5-9 days and 9-14 days post symptom onset) during patient follow-up, and 30 control sera from COVID-19 PCR–negative individuals. All sera were tested for SARS-CoV-2 antibodies using four high-throughput commercially available chemiluminescence immunoassays: YHLO Biotech Co, Ltd China (IgM and IgG); Abbott, Abbott USA (IgG); Roche, Roche US (total: IgM, IgG, and IgA), and Ortho, Ortho Clinical Diagnostics, USA (total and individual IgG).* ***Results:*** *For the detection of total antibodies (IgM, IgG, and IgA), the highest sensitivities were for Ortho followed by Roche assays (91.6% and 84.3% in 5-9 days period, respectively) raised to 96.8% and 92.7% in 9-14 days respectively with significant difference P-value <0.00001. Ortho, Roche, and YHLO iFlash (IgM) assays had specificities of 100%, 100%, and 90.3% respectively. Roche (Total) and Ortho (Total) assays showed perfect categorical agreement (94.4% in 5-9 days, and 96.4% in 9-14 days). As for the detection of individual IgG antibodies, the Abbott assay had the highest sensitivity (91.6% in 5-9 days, and 93.7% in 9-14 days), followed by Ortho and YHLO iFlash assays (84.3%, and 83.3% in 5-9 days respectively) that increased to 89.5% and 88.5% in 9-14 days respectively. Ortho assay was the best in specificity (100%), followed by Abbott (98.8%) and YHLO iFlash (96.3%). YHLO iflash (IgG) and Ortho (IgG) showed perfect agreement (96.8%) in the 2 time frames.* ***Conclusion:*** *Ortho assay showed the best performance in detecting total antibodies with perfect match to Roche assay, while Abott assay was the best performing in detecting individual IgG with perfect match to Ortho assay rendering them to be efficient diagnostic tools.*

INTRODUCTION

Coronavirus disease (COVID-19) is a worldwide threatening acute respiratory disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) that was first reported in Wuhan, China in 2019[1].

Reverse transcriptase-polymerase chain reaction (RT-PCR) is considered the gold standard method for the diagnosis of active COVID-19 infection[2]. However, the accuracy of the PCR test is impacted by several factors such as the type of sample, and its transportation and storage conditions[3]. Serological antibody tests can detect patients with past COVID-19 infection. These tests can provide a clearer picture of the prevalence of COVID-19 disease in any given population by identifying people who were previously exposed toSARS-COV-2[4,5].

Since the outbreak of the novel coronavirus, markets have been flooded with various types of commercially available serological tests[6]. Due to exceptional circumstances, the Food and Drug Administration (FDA) allowed test developers to independently validate Serological tests for SARS-COV-2 according to the Emergency Use Authorization (EUA) policy that was

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first issued in March, 2020[7,8]. The large number of commercially available serological tests as well as their fast production and release in the market with impaired process control may result in difficulty in reviewing their performance and assuring reliability[6]. In this perspective, the current study aims at evaluating the performance of four high-throughput commercial COVID-19 chemiluminescence immunoassays, in an effort to help selecting the best performing serological tests for COVID-19 diagnosis.

METHODOLOGY

Study population

The present study was conducted with four types of CLIA kits that were provided to CPHL to evaluate their performance in detecting SARS-CoV-2 antibodies. The 4 assays were tested on serum samples collected from 96 COVID-19 PCR positive patients admitted to isolation hospitals. The patients were under observation until they were discharged, and samples were collected at two time frames (a: 5-9 days, and b: 9-14 days) from the onset of disease symptoms. To assess possible false positivity of assays, a control group of 30 serum samples were collected in 2019 before the start of the COVID-19 outbreak and were stored at −20 °C, from patients who were suspected of microbial infections and were referred to CPHL for routine serological investigations. Control sera were positive for other viral or bacterial infections in the form of HAV IgM (n=8), IgG Rubella (n=2), HBV antibody (n=11), CMV IgM (n=4), HIV (n=2), and IgM *Mycoplasma pneumoniae* (n=3).

Ethical Statement

An institutional approval was received to conduct this research upon a request from the Central Public Health Laboratories (CPHL) of the Ministry of Health, Egypt to evaluate the provided four SARS-COV-2 antibodies chemiluminescence immunoassays prior to their implementation. The research was conducted retrospectively on serum samples that were submitted to the routine serology laboratory and stored at -20◦C for further testing. This study did not involve clinical trials or invasive procedures and did not involve laboratory animals.

Chemiluminescence Immunoassays:

Samples were tested by four chemiluminescence immunoassays in the form of YHLO Biotech Co, Ltd China for detection of individual IgM and IgG on iFlash 1800 CLIA analyzer, CHINA; Abbott, Abbott, USA for detection of individual IgG on Architect i2000SR analyzer; Roche, Roche US for detection of total antibodies (IgM, IgG, and IgA) on Cobas e601 analyzer; and Ortho, Ortho Clinical Diagnostics, USA for total antibodies (IgM, IgG, and IgA) and individual IgG on Vitros 3600. The Abbott anti-SARS-COV-2 IgG is a qualitative immunoassay based on chemiluminescence microparticle (CMIA) technology performed on Architect analyzer. Anti-SARS-COV-2 IgG antibodies in the serum bind to microparticles coated with SARS-COV-2 antigens; this was followed by a well washing process to remove any unbound material and provide optimized assay conditions. Then, a chemiluminescence reaction is created after adding anti-human IgG (acridinium labelled). Positive and negative reactions were decided according to the calculated index value of 1.4 signal-to-cutoff ratios as guided by the manufacturer[9]. The Ortho total anti-SARSCOV-2 antibody assay is a two-stage reaction chemiluminescence technology operated on Vitros 3600 analyzer. The first stage involves binding anti-SARS-COV-2 antibodies in serum to SARS-COV-2 antigen (Spike S1 protein) coated on wells, followed by well washing to remove any unbound particles. The second stage involves adding recombinant HRP labelled SARS-COV-2 antigen in conjugate reagent, which in turn binds to captured antibodies in the well in the first stage. A second wash step is done to remove unbound conjugate. Chemiluminescence reaction is produced after adding a luminogenic reagent. Light signals are measured as RLU, which is directly proportionate to the number of antibodies present in the sample. Results are interpreted as positive for anti-SARS-COV-2 at >1 signal to cut-off value, while <1 is considered non-reactive[9]. Roche total anti-SARS-COV-2 antibodies test is a qualitative fully automated chemiluminescence immunoassay operated on the Cobas e601 analyzer. The assay depends on the modified double antigen sandwich technique using recombinant SARS-COV-2 nucleocapsid protein (N). Results are interpreted according to signal to cut-off index value (COI), where COI >1 is considered reactive, while COI <1 is non-reactive for anti–SARS-COV-2 antibodies[10]. YHLO Biotech chemiluminescence immunoassay is a test used for individual detection of IgM and IgG anti-SARS-COV-2 antibodies using magnetic beads coated with SARS-COV-2 antigens (nucleocapsid and spike proteins). The tests were performed by a fully automated iFlash 1800 chemiluminescence analyzer that calculates the concentration of antibodies according to the amount of relative light units (RLU). According to the manufacturer, the proposed cut-off value is 10AU/ml, therefore samples with IgM or IgG antibodies concentration ≥10AU/ml are considered positive[6]. The analytical performance of these tests was evaluated according to the guidelines of the Clinical and Laboratory Standards Institute[11].

Statistical Analysis:

The performance of each test was evaluated in terms of sensitivity, specificity, and accuracy. Binomial 95% Confidence Intervals (CI) were calculated for proportions. The compared difference in performance between the four assays was measured using Chi-square

(X^2) test. A P-value of less than 0.05 was considered statistically significant. All statistical tests were performed with SPSS Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA version 15 for Microsoft Windows (Microsoft Corp., Redmond, WA).

RESULTS

The four provided chemiluminescence assays to CPHL were evaluated for detecting COVID-19 antibodies in serum samples collected from 96 SARS-COV-2 PCR positive patients over two time frames (5-9 days, and 9-14 days) from the onset of COVID-19 symptoms. As shown in **Table 1**, the sensitivities of Ortho and Roche CLIA assays in detecting total antibodies (IgM, IgG, and IgA) were found to be 91.6% (95%CI : 0.65-0.81) and 84.3% (95%CI : 0.63-0.80), respectively in the time frame of 5-9 days, increased to 96.8% (95%CI : 0.66- 0.82) and 92.7% (95%CI : 0.65-0.82), respectively in the time frame of 9-14 days (Fig. 1). YHLO iFlash (IgM) detected individual Ig M antibodies with sensitivities of 48.9% (95%CI : 0.53-0.75, and 53.12% (95%CI : 0.55-0.77) in the two time frames of 5-9 days and 9-14 days respectively. Regarding detection of IgG antibodies Abbott, Ortho and YHLO iFlash (IgG) assays recorded sensitivities of 91.6% (95%CI : 0.662-0.825), 84.3% (95%CI : 0.635-0.80) and 83.3% (95%CI : 0.65-0.82) in the time frame of 5-9 days, raised to 93.7% (95%CI : 0.667-0.828), 89.5% (95%CI : 0.65-0.816) and 88.5% (95%CI : 0.66-0.83) in the time frame of 9-14 days respectively **(Figure 1)**.

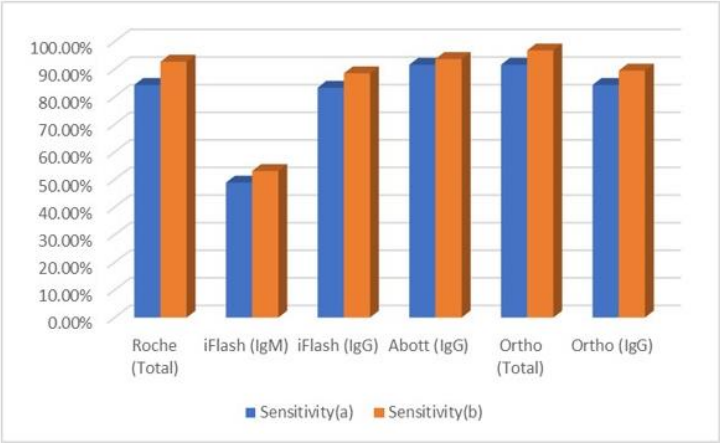

Fig. 1: Analytical sensitivities of the four chemiluminescence immunoassays for detection of COVID-19 antibodies in sera collected at time intervals of 5-9 days [sensitivity (a)] and 9-14 days [sensitivity (b)] from the onset of symptoms.

The recorded specificities of Ortho and Roche assays were found to be 100% in detecting total antibodies (IgM, IgG, and IgA) with 95% CI 0-0.69 for Ortho, while 95% CI: 0-0.43 for Roche assay. For detecting IgG antibodies, Ortho, Abbott and iFlash assays showed specificities of 100% (95% CI: 0-0.34), 96.6% (95%CI: 0.007-0.579) and 90% (95% CI: 0.057-0.51) respectively **(Figure 2)**.

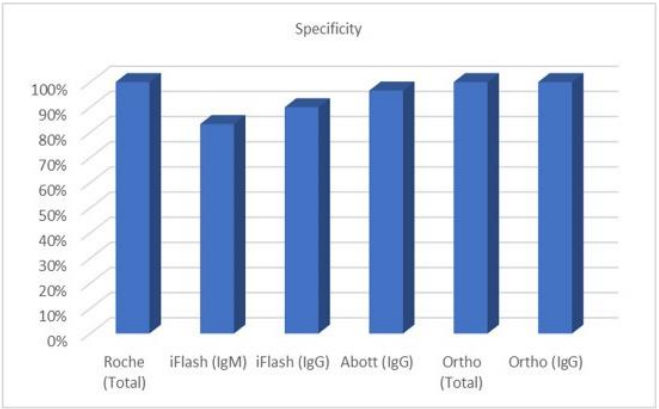

Fig. 2: Analytical specificity of the four chemiluminescence immunoassays for the detection of COVID-19 antibodies in 30 control serum samples.

A statistical significant difference was observed between sensitivities of Roche, iFlash, and ortho assays in detecting total or individual IgM antibodies with P-values of 0.000001 and 0.00021 in the frames of 5-9 days and 9-14 days respectively. However, no significant difference was recorded between the sensitivities of those assays in detecting individual IgG antibodies. Among the 4 CLIA assays, the iflash sensitivities in detecting IgM and IgG antibodies varied significantly between the two time frames with recorded P-values of <0.000001 and 0.049 respectively. No significant difference was observed for the recorded specificities between the evaluated CLIA assays.

The evaluated CLIA assays were ordered according to their accuracy in the rank of Ortho and Roche assays in detecting total antibodies followed by iFlash in the detection of IgM antibodies, while in the order of Abbott, Ortho, and iflash assays in detecting individual IgG as shown in **Table 1**.

Table 1: Analytical Performance of 4 CLIA in the detection of anti-SARS-COV-2 IgM and IgG antibodies at two time intervals.

Chemiluminescence immunoassays	Total Serum samples=126 (96 cases + 30 control sera)				
	Sensitivity[a/b] (95% CI)[a/b]	Specificity (95% CI)	NPV[a/b]	PPV	Accuracy[a/b]
Roche (Total)	84.3%/ 92.7% (0.63-0.80)/ (0.65-0.82)	100% (0- 0.43)	66.6%/ 81%	100%	88%/94.4%
YHLO iFlash (IgM)	48.9%/ 53.12% (0.53-0.75)/ (0.55-0.77)	83.3% (0.037- 0.225)	33.7%/ 35.7%	90.3%	57.1%/ 60.3%
YHLO iFlash (IgG)	83.3%/ 88.5% (0.65-0.82)/ (0.66-0.83)	90% (0.057- 0.51)	62.7%/ 71%	96.3%	84.9%/ 88.8%
Abott (IgG)	91.6%/ 93.7% (0.662- 0.825)/ (0.667- 0.828)	96.6% (0.007- 0.579)	78.3%/ 82.8%	98.8%	92.8%/ 94.4%
Ortho (total)	91.6%/ 96.8% (0.65- 0.81)/ (0.66- 0.82)	100% (0- 0.69)	78.9%/ 90.9%	100%	93.6%/ 97.6%
Ortho (IgG)	84.3%/ 89.5% (0.635- 0.80)/ (0.65-0.816)	100% (0- 0.34)	66.6%/ 75%	100%	88.1%/ 92.06%

CI: Confidence Interval, **NPV**: Negative PredictivV value, **PPV**: Positive Predictive Value, [a]: Collected sera within 5-9 days from onset of symptoms, [b]: Collected sera within 9-14 days from onset of symptoms

Regarding the detection of IgM antibodies, Roche (Total) and Ortho (Total) assays showed perfect categorical agreement in the detection of total antibodies including IgM of 94.4% (Kappa: 0.87) and 96.8% (kappa: 0.92) in the time frames of 5-9 days and 9-14 days respectively. Roche (Total) and iFlash (IgM) showed poor agreement of 57.9% (Kappa: 0.19) in 5-9 days, while fair agreement of 65.8% (Kappa: 0.34) in 9-14 days. Ortho (Total) and iFlash (IgM) showed poor agreement of 52.3% (Kappa: 0.109) in 5-9 days, while fair agreement of 62.6% (kappa: 0.29) in 9-14 days. As for the detection of IgG antibodies, a perfect agreement was recorded between iflash (IgG) and Ortho (IgG)(96.8%; Kappa: 0.93) in the two time frames. Abbott (IgG) and Ortho (IgG) showed perfect agreement of 93.6% (Kappa: 0.93) in 5-9 days and 96.03% (Kappa: 0.90) in 9-14 days. Abbott (IgG) and iFlash (IgG) showed good agreement of 90.47% (Kappa:0.78) in 5-9 days, while perfect agreement of 94.4% (Kappa:0.86) in 9-14 days.

DISCUSSION

COVID-19 is a worldwide highly spreading acute respiratory infection with adverse socio-economic and health consequences. Currently, the two major categories of assays for COVID-19 diagnosis are PCR and immunoassays[2].

Unlike PCR, serological antibody tests are privileged by recognizing people who had a past infection and became immune to SARS-COV-2. SARS-COV-2 antibodies are considered more stable than RNA due to their potential existence in blood for a long time and are less affected by sample transportation or storage factors[4]. However, the peak of anti-SARS-COV-2 antibodies may not be reached before 9–11 days from the start of infection, so less likely indicate active infection[12]. Moreover, cross-reactivity with non-SARS-COV-2 coronaviruses (HKU1, NL63, or OC 43, 229E) may lead to false-positive results[12,13].

The present study introduces performance evaluation of four commercially available high-throughput, chemiluminescence immunoassays (ROCHE total antibodies, YHLO iFlash individual IgM and IgG, Abbott IgG, Ortho total and individual IgG antibodies) for detection of anti-SARS-COV2 antibodies in serum samples collected from 96 COVID-19 PCR positive patients admitted to isolation hospitals at two time intervals (a: 5-9 days, and b: 9-14 days) from the onset of disease symptoms. In the present study, the highest recorded sensitivity in detecting total antibodies (IgM, IgG, and IgA) antibodies was observed for Ortho followed by Roche assay (91.6% and 84.3% in 5-9 days period respectively) raised to 96.8% and 92.7% in 9-14 days period respectively. YHLO iFlash assay (IgM) had the least sensitivity (48.9% in 5-9 days, 53.12% in 9-14 days). A statistically significant difference between assays was shown with P-values of 0.000001 in 5-9 days and 0.0000021 in 9-14 days. Ortho and Roche assays had 100% specificity, while YHLO iFlash (IgM) showed a lower specificity (90.3%). Regarding the detection of individual IgG antibodies, the highest recorded sensitivity was Abbott assay (91.6% in 5-9 days, 93.7% in 9-14 days), followed by Ortho and YHLO iFlash assays that had comparable sensitivities of 84.3% and 83.3% in 5-9 days respectively, increased to 89.5% and 88.5% in 9-14 days respectively with no statistical significant difference. As for specificity, Ortho assay was the best (100%), followed by Abbott (98.8%) and YHLO iFlash (IgG) (96.3%). It was observed that there was an improvement in the sensitivity of immunoassays in the samples that were collected in the time frame (9-14 days) than those in 5-9 days, which could be explained by the production of antibodies with higher concentrations as time goes allowing for better detection. The sensitivities of YHLO iflash assay sensitivities in detecting IgM and IgG antibodies varied significantly between the two time frames with recorded P- values of <0.000001 and 0.049 respectively.

Perfect categorical agreement in detecting total antibodies was found between Roche (Total) and Ortho (Total) assays (94.4% in 5-9 days, and 96.4% in 9-14 days). In the detection of individual IgG, YHLO iflash (IgG) and Ortho (IgG) showed perfect agreement (96.8%) in the two time frames.

Published data are still limited on commercially available SARS-COV-2 serological tests. However, according to an evaluation done by Public Health England, Ortho and Roche assays for detection of total antibodies showed sensitivities of 85% and 88.1%, while specificities of 99.5% and 100% respectively, which was close to the results of the current study[14,15]. Higher sensitivity for Roche assay was reported by Haselmann et al., 2020 (92.3%)[16]. YHLO iFlash assay was evaluated by a study in Italy that recorded higher sensitivity (73.3%) and specificity (92.2%) in detecting IgM antibodies than the current study (48.9% in 5-9 days; 53.12 in 9-14 days)[6]. In the present study, the sensitivity of the Abbott assay in the detection of IgG antibodies (91.6% in 5-9 days; 93.7% in 9-14 days) was higher than other several studies that recorded sensitivities of 43.6%[17], 49.5%[13], and 82.4%[18], while specificities ranged from 99.4%-100%[18,19] which was higher than the present study (98.8%). In contrast to the current study, Theel et al.[13] recorded lower sensitivity of Ortho assay in the detection of IgG antibodies (38.5%) than that of the present study (84.3% in 5-9 days; 89.5% in 9-14 days) and also, a lower specificity (99.3%) than the current study (100%). Unlike IgM, the sensitivity and specificity of YHLO iFlash in the detection of IgG was higher in the present study (84.3% and 96.3%, respectively) than that reported by Infantino et al. (76.7% and 100% respectively)[6]. An improvement in the sensitivity of the evaluated CLIA assays was observed in samples collected at longer time frames from the onset of symptoms which was similarly reported by several studies[13,17,18].

Any discrepancy in the technical performance of evaluated assays in the current study from other published data could be attributed to a difference in the study population and their immune response, sample size, or inter-laboratory performance.

The performance of serological is challenged by several issues. According to previous studies, coronavirus antibodies can be produced as early as four days post symptoms onset[20], though they may be produced late with minimal concentrations leading to false-negative infected patients[6,8]. Another challenge is false-positive results that may occur due to cross-reaction with other respiratory infections[6]. Variation in technical performance between different immunoassays can be substantially related to the following: 1) the test's capacity of early detecting SARS-COV-2 antibodies which depends on the number of test antigens, 2) avoiding cross-reaction with other respiratory viruses by adding a specific type of SARS-COV-2–derived antigens, N: Nucleocapsid protein and S: Spike glycoprotein (S1 and S2 subunits, receptor-binding domain)[3,5].

Selecting the appropriate threshold for the sensitivity and specificity of a given antibody test depends on the purpose of that test, whether deciding the need for isolation of an actively infected patient or the release of a patient from quarantine after developing immunity to SARS-COV-2[21].

The role of immunoassays in diagnosing (SARS-CoV-2) infection is considered crucial for public health. Antibody immunoassays can be useful surveillance tools for distinguishing non-immune from immune individuals, which in turn can guide health authorities to proper decisions[22]. The clinical and public health settings will continue to evolve providing increasing insight into the immune response to the virus[13].

Antibody tests can be used mainly as surveillance tools to distinguish immune individuals from non-immune ones, and identify hotspots in low-immunity individuals. This can guide governmental health authorities to take proper socioeconomic decisions allowing better allocation of resources to affected areas[22]. Notably, the presence of anti–SARS-COV-2 antibodies does not necessarily confirm immunity against COVID-19 infection[23]. For the diagnosis of active SARS-COV- 2 infection, it is recommended to use PCR and serological antibody tests together and not the serological tests only[7,24]. There is still a dire need for further researches to clarify the dynamics and components of the humoral immune response to SARS-COV-2[13].

The present study is considered the first Egyptian performance evaluation of four commercially available COVID-19 chemiluminescence immunoassays to help users select the best available in the market.

CONCLUSION

There are various independently validated commercial COVID-19 serological tests in the market. The present study evaluated four SARS-CoV-2 chemiluminescence assays and concluded that the best performing was the Ortho assay in detecting total antibodies with a perfect match to Roche assay. For detecting individual IgG, the Abbott assay was the best performing with a perfect match to Ortho assay rendering them to be efficient diagnostic tools.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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