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## ORIGINAL ARTICLE

# Establishment of SARS-CoV-2 Immunoglobulins (IgM, IgG) Reference Intervals for Elder Population in China based on 3,733 Samples

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### SUMMARY

***Background:*** **Establishment of reference intervals (RIs) for different biomarkers is essential for clinical monitoring. The purpose of this study was to establish laboratory RIs of SARS-CoV-2 IgM and IgG for elder population.**
***Materials:*** **Performance verification was conducted with reference to the Clinical and Laboratory Standards Institute (CLSI) guidelines, including linearity, imprecision, and allowable dilution ratio. Based on CLSI C28-A3 document, a total of 3,734 serum samples were collected, and 3,733 serum samples were used for the establishment of RIs for SARS-CoV-2 IgM and IgG. The subjects were grouped by gender and age. The age groups were as follows: 60 - 69 years, 70 - 79 years, 80 - 89 years, and 90 - 101 years. The RI was defined by nonparametric 95th percentile intervals.**
***Results:*** **Percentage deviation of all the seven dilutions were all less than 12.5% during linearity evaluation. The inter-assay and intra-assay imprecision were all less than 5%. There is no significant difference between different gender and age groups for IgM (p = 0.0818, p = 0.7094), and there is significant difference between different gender and age groups for IgG (p = 0.0011, p = 0.0013). Harris-Boyd's test did not indicate partitioning for IgM and IgG. Cutoff values of RI for SARS-CoV-2 IgM and IgG were defined as 0.1523 S/CO and 0.2663 S/CO, respectively.**
***Conclusions:*** **RIs of SRAR-CoV-2 IgM and IgG were established for elder population, which can play an important role in the prevention and control of the epidemic.**
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### KEYWORDS

SARS-CoV-2 IgM and IgG, COVID-19, elder population, reference interval, chemiluminescence immunoassay (CLIA)

### INTRODUCTION

In early December 2019, a new coronavirus virus, namely severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in Wuhan city of China and quickly attracted our attention for its highly

infective and rapid spread rate all over the world [1-3]. Coronavirus disease 2019 (COVID-19), which is caused by SARS-CoV-2, was declared a pandemic on March 12, 2020 [4]. Up to March 29, 2021, more than 127 million confirmed cases and 2.79 million death cases have been reported worldwide. SARS-CoV-2 can infect people of all ages, from infants to the elderly. The elderly over the age of 60 are a special population whose immune system is different from that of other age groups [5]. Elderly patients with COVID-19 have more severe clinical symptoms and a worse prognosis [6].

Because there are no specific drugs for COVID-19 at present, timely and accurate diagnosis, together with diagnosis and isolation of positive cases, is the most effective approach to limit further spread of the virus [7-9]. The detection of viral RNA is the main approach for diagnosis of SARS-CoV-2 infection because of its high accuracy, and real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) is one of the preferred tools for viral RNA detection [10,11]. However, a complete nucleic acid detection process includes the steps of nucleic acid extraction, reverse transcription, nucleic acid amplification, and result analysis, which requires 4 - 6 hours. At the same time, real-time RT-PCR requires expensive equipment, special room to avoid contamination, and trained operators [12]. Therefore, many medical and health institutions cannot carry out SARS-CoV-2 RNA detection.

Besides nucleic acid detection, serological testing for specific antibodies is a simple and feasible approach in clinical laboratory for the diagnosis of virus infection [13,14]. As for COVID-19, studies have shown that two kinds of virus-specific antibodies, immunoglobulin-M (IgM) and immunoglobulin-G (IgG), can be detected in serum of SARS-CoV-2 infected cases [1]. IgM is the earliest antibody that is produced by the immune system after SARS-CoV-2 infection. IgG appears later than IgM in serum, but its concentration can reach 4 - 8 times of IgM and can maintain a high level in serum for a long time post-infection [15,16]. Therefore, simultaneous detection of virus-specific IgM and IgG is helpful to identify those infected by SARS-CoV-2 infectors.

Different kinds of immunoassays have been used to detect SARS-CoV-2 IgM and IgG, such as colloidal gold immunochromatographic assay (GICA), immunochromatographic assay (ICA), enzyme linked immunosorbent assay (ELISA), indirect immunofluorescence tests (IIFT), and luciferase immunoprecipitation system (LIPS) [17-19]. However, most of the mentioned methods can only be used for qualitative detection instead of accurate quantification. The chemiluminescence immunoassay (CLIA) is currently the most widely used method for the quantitative detection of SARS-CoV-2 IgM and IgG [20].

The appropriate interpretation of SARS-CoV-2 serological test results for elder people requires that it be compared with a reference interval (RI) [14]. All commercial kits used in clinical laboratories have their own RI. However, the RI for SARS-CoV-2 IgM and IgG used in China is supplied by the manufacturer, which is determined based on 206 clinical samples. The upper limit of RI for IgM and IgG were all set as 1 S/CO (signal-to-cut-off ratio). In fact, the basic levels of IgM and IgG in serum is different, thus the RI of IgM and IgG should be different theoretically. Besides, 206 specimens used for the establishment of RI is probably too small to represent all populations. The basic immune status of elder persons acts differently from children and middle-aged populations. As a result, reliable SARS-CoV-2 IgM and IgG RI for elder people determined from large population is an essential task for clinical laboratories. Thus, the aim of the present study was to establish the appropriate RI for SARS-CoV-2 IgM and IgG for elder people based on the large population in China.

## MATERIALS AND METHODS

### Subjects

We included a total of 3,734 subjects, who were referred to the Beijing Tiantan Hospital, Capital Medical University (Beijing, China) from January 2021 to March 2021 for a COVID-19 screening. The inclusion criteria were as follows: balanced diet, did not inject the COVID-19 vaccine, and negative for SARS-CoV-2 RNA tests. Among the 3,734 subjects, there were 2,005 males (ages 60 - 97 years) and 1,729 females (ages 60 - 101 years).

### Blood sampling and measurement

According to standard operating procedures in clinical laboratories, venous blood of each subject was drawn and collected into a separator tube. The tubes were centrifuged for 10 minutes at 3,200 rpm to separate the serum, which was tested immediately or stored at -20°C for a period of time before detection. SARS-CoV-2 IgM and IgG of different samples was measured by the CLIA system (Antu Biotechnology Co., Ltd, Beijing, China). According to the manufacturer's instructions of the analyzer and immune kit, standard methodologies and dedicated reagents were used, and the analyzer was routinely maintained every week. The standard curve for SARS-CoV-2 IgM and IgG was established, and two levels of quality controls were run every day. The Westgard rules were used in the internal quality control procedure to evaluate the stability of the measurement process during the entire period of the study. All measurements were performed under the guidance of the standard and routine operation protocols of the clinical laboratory.

### Measurement analysis

Before the establishment of RI for SARS-CoV-2 IgM and IgG, performance verification of the CLIA system was conducted, including detection linearity, allowable dilution ratio, and imprecision. All the evaluation studies were repeated three times.

According to the CLSI document EP06-A, the linearity of SARS-CoV-2 IgM and IgG were evaluated for eight pools of serum, besides the serum samples with the low level and high level concentrations (0.01 S/CO and 5.029 S/CO for IgM, 0.01 S/CO and 34.762 S/CO for IgG), the other six pools were from a mixture of the mentioned serum with a ratio of 1:6, 2:5, 3:4, 4:3, 5:2, and 6:1, respectively [21]. Linear-fit IgM and IgG values were calculated using the equation of the best-fitted line, and the percentage deviation from the linearity of each pool for IgM and IgG were calculated by the equation: 100% - (predicted value/linear fit value) × 100%.

Regarding the allowable dilution ratio, with reference to the CLSI document EP34-A, serum samples with concentrations of 25.4017 S/CO, 21.178 S/CO for SARS-CoV-2 IgG and 3.169 S/CO for SARS-CoV-2 IgM were diluted at different ratios (2, 5, 10, 15, 20, 30, and 40) to compare the predicted value with the actual value and assess the bias [22].

According to CLSI document EP 15-A3, the imprecision of IgM and IgG were assessed with two samples (low and high concentration). Over a time period of 5 working days, five runs of one plate each were performed daily [23].

**Statistical analysis**

All analyses were performed using SPSS 26.0 (IBM SPSS Statistics for Windows; IBM Corp., Armonk, NY, USA) and GraphPad Prism 7.0 (GraphPad, San Diego, CA, USA) statistical software. Intra- and inter-assay components of error were calculated with a fully nested analysis of variance. As for the RIs of SARS-CoV-2 IgM and IgG, according to CLSI document C28-A2, the D/R ratio was used in the estimation of the reference value, and D was the absolute difference between an extreme observation and the next largest (or next smallest) observation, and R was the range of all observations (extreme values included). The extreme value was deleted if the D/R ratio was more than 1/3. The D/R ratio would be calculated again until the value of D was less than one/third of the value of R, and then all the values would be kept in the establishment of the RI [24]. The Shapiro-Wilk normality test was used to evaluate whether the detected IgM and IgG value were normally distributed. The correlation between age and values of IgM and IgG were assessed by Spearman's rank correlation coefficient, respectively.

Results of SARS-CoV-2 IgM and IgG samples were first divided into different gender and age groups (60 - 69 years, 70 - 79 years, 80 - 89 years, and 90 - 101 years). Then according to Harris-Boyd's method, the z-values for two groups were used to determine whether it was essential to keep separate group. If the results of the Harris-Boyd's test did not demonstration partition, the subgroup should be combined. At the same time, analyses of difference between gender and age groups were conducted by Mann-Whitney U test, one-way analysis of variance, or Kruskal-Wallis test, appropriately. The RI was calculated using the 95th percentile for the high reference limit. A value of $p \leq 0.05$ was defined as statistically significant.

## RESULTS

**Measurement analysis**

Regarding the SARS-CoV-2 IgM, as is shown in the Table 1, the percentage deviation of the first pool between the predicted 2$^{nd}$ value and predicted 3$^{rd}$ value exceeded 150% when the linearity was evaluated by all the eight pools. It indicated that the concentration of the first pool should not be set as the lower starting point of the linear range. Thus, the value of the first pool was deleted and linearity of IgM was analyzed again by the other seven pools. Results in Figure 1 showed that the percentage deviations of all dilutions were less than 12.5%. As for the SARS-CoV-2 IgG, the percentage deviation of the first pool between the predicted 1$^{st}$ value and predicted 2$^{nd}$ value exceeded 150% when the linearity was evaluated by all the eight pools (Table 2). As a result, the first pool was deleted and the linearity was evaluated again by the other pools. Results in Figure 1 F showed that the percentage deviations of all dilutions were less than 12.5%.

Regarding the dilution ratio for SARS-CoV-2 IgM, as shown in Table 3, the bias would exceed 30% when the dilution is 2, so clinical samples for IgM detection cannot be diluted. Regarding the dilution ratio for SARS-CoV-2 IgG, as is shown in the Table 4, when the dilution ratio is less than 5, the bias is less than 10%. However, the bias caused by dilution exceeded 12.5% when the ratio is 10.

As is shown in Figure 2 and Table 5, with regard to the within-run and between-run, the CVs, namely relative standard deviation, for SARS-CoV-2 IgM were 2.45% and 2.54% with the concentration of 3.6502 S/CO, 2.01% and 1.36% with the concentration of 6.8566 S/CO, respectively. CVs for SARS-CoV-2 IgG were 2.52% and 3.56% with the concentration of 6.3045 S/CO, 3.67% and 1.72% with the concentration of 11.0050 S/CO, respectively. All CV results were in the allowable range.

**Distribution of reference values**

The values were obtained from all reference samples (n = 3,734), and one extreme value for SARS-CoV-2 IgG was discarded as outlier for the D/R ratio is more than 1/3. The Shapiro-Wilk test results indicated that all results of IgM and IgG detection were non-parametric ($p < 0.001$). Details of measuring values are shown in the Table 6, and we can see that all the grouped values were non-parametric.

**Comparison of gender and age**

The detection results for SARS-CoV-2 IgM and IgG were firstly divided into different gender groups (males, females). As for IgM detection, we can see from the

**Table 1. Preliminary linearity evaluation of SARS-CoV-2 IgM.**

| Serum sample concentrations | Predicted 1st order | Predicted 2nd order | Predicted 3rd order | Difference S/CO | Difference % |
|---|---|---|---|---|---|
| 0.0133 | -0.3676 | 0.0161 | 0.0057 | 0.0104 | 184.20 |
| 0.3867 | 0.3468 | 0.4009 | 0.4091 | -0.0082 | -2.00 |
| 0.9283 | 1.0612 | 0.8955 | 0.9070 | -0.0115 | -1.27 |
| 1.5010 | 1.7756 | 1.4999 | 1.5056 | -0.0057 | -0.38 |
| 2.1950 | 2.4900 | 2.2140 | 2.2109 | 0.0032 | 0.14 |
| 3.0647 | 3.2044 | 3.0379 | 3.0289 | 0.0090 | 0.30 |
| 3.9333 | 3.9188 | 3.9715 | 3.9659 | 0.0056 | 0.14 |
| 5.0380 | 4.6332 | 5.0149 | 5.0280 | -0.0131 | -0.26 |

**Note: The predicted 1st order, predicted 2nd order, and predicted 3rd order represent the predicted result of linear equation, the quadratic equation, and the third-order equation, respectively. Difference S/CO is the difference value between the Predicted 2nd order and the Predicted 3rd order. Difference % means the percentage difference between the Predicted 2nd order and the Predicted 3rd order.**

**Table 2. Preliminary linearity evaluation of SARS-CoV-2 IgG.**

| Serum sample concentrations | Predicted 1st order | Predicted 2nd order | Difference S/CO | Difference % |
|---|---|---|---|---|
| 0.1203 | 0.1040 | -0.1771 | 0.2811 | -158.71 |
| 4.5043 | 5.0920 | 5.0514 | 0.0406 | 0.80 |
| 10.2067 | 10.0800 | 10.1997 | -0.1197 | -1.17 |
| 15.5910 | 15.0680 | 15.2678 | -0.1998 | -1.31 |
| 20.2063 | 20.0560 | 20.2555 | -0.1995 | -0.98 |
| 25.2267 | 25.0440 | 25.1630 | -0.1190 | -0.47 |
| 29.8630 | 30.0320 | 29.9901 | 0.0419 | 0.14 |
| 34.7620 | 35.0200 | 34.7370 | 0.2830 | 0.81 |

**Note: The predicted 1st order, predicted 2nd order represent the predicted result of linear equation and the quadratic equation, respectively. Difference S/CO is the difference value between the Predicted 1st order and the Predicted 2nd order. Difference % means the percentage difference between the Predicted 1st order and the Predicted 2nd order.**

**Table 3. Definition of the allowable dilution ratios for SARS-CoV-2 IgM.**

| Dilution ratio | Serum sample concentrations | Predicted S/CO | SD S/CO | Actual S/CO | Bias % |
|---|---|---|---|---|---|
| 1 | 3.1678 | 3.1687 | 0.0766 | 3.1687 | 0.00 |
| 2 | 1.5839 | 2.0683 | 0.0746 | 1.5844 | 30.55 * |
| 5 | 0.6336 | 0.9977 | 0.0144 | 0.6337 | 57.43 |
| 10 | 0.3168 | 0.4580 | 0.0212 | 0.3169 | 44.54 |
| 15 | 0.2112 | 0.2587 | 0.0067 | 0.2112 | 22.45 |
| 20 | 0.1584 | 0.1970 | 0.0069 | 0.1584 | 24.34 |
| 30 | 0.1056 | 0.1260 | 0.0044 | 0.1056 | 19.29 |
| 40 | 0.0792 | 0.0913 | 0.0021 | 0.0792 | 15.29 |

**Note: * Exceeds the 12.5% requirement of the clinical laboratory.**

**Table 4. Definition of the allowable dilution ratios for SARS-CoV-2 IgG.**

| Dilution ratio | Serum sample concentrations | Predicted S/CO | SD S/CO | Actual S/CO | Bias % |
|---|---|---|---|---|---|
| 1 | 25.4017 | 25.4017 | 0.9829 | 25.4017 | 0.00 |
| 2 | 12.7009 | 12.4063 | 0.1460 | 12.7009 | -2.32 |
| 5 | 5.0803 | 4.7833 | 0.1005 | 5.0803 | -5.85 |
| 10 | 2.5402 | 1.9123 | 0.0930 | 2.5402 | -24.72 * |
| 15 | 1.6934 | 1.3190 | 0.0321 | 1.6934 | -22.11 |
| 20 | 1.2701 | 0.8663 | 0.1361 | 1.2701 | -31.79 |
| 30 | 0.8467 | 0.5633 | 0.0427 | 0.8467 | -33.47 |
| 40 | 0.6350 | 0.4673 | 0.0272 | 0.6350 | -26.41 |

**Note: * Exceeds the 12.5% requirement of the clinical laboratory.**

**Table 5. Imprecision of SARS-CoV-2 IgM and IgG.**

| | SARS-CoV-2 IgM S/CO | | SARS-CoV-2 IgG S/CO | |
|---|---|---|---|---|
| | 3.6502 | 6.8566 | 6.3045 | 11.0050 |
| Within-run | 2.45% | 2.01% | 2.52% | 3.67% |
| Between-run | 2.54% | 1.36% | 3.56% | 1.72% |

**Note: Four clinical samples with different concentrations of IgM and IgG were used here.**

Figure 3A that there was no significant difference between male and female groups ($p = 0.0818$). Besides, the result of the Harris-Boyd's test in Table 7 did not indicate partitioning between males and females. Since $z < z^*$ ($-0.09 < 11.38$), the groups were not separated but combined ($n = 3,733$) and reevaluated. Regarding the male and female groups for IgG, we can see from the Figure 3B that there was significant difference between males and females ($p = 0.0011$). However, the Harris-Boyd's test result in Table 8 did not indicate partitioning because $z < z^*$ ($3.33 < 11.38$), thus IgG value of the male group and female group were combined ($n = 3,733$) and revaluated.

The analysis values of SARS-CoV-2 IgM and IgG were then divided into different groups based on age (60 - 69-years-old, 70 - 79-years-old, 80 - 89-years-old, and 90 - 101-years-old). With regard to IgM, Spearman's rank correlation analysis in Figure 4A indicated that the SARS-CoV-2 IgM values were not correlated with age ($R^2 = 0.0007$; $p = 0.1053$), and the result of the Kruskal-Wallis test in Figure 4B indicated that there was no significance among different age groups ($p = 0.7094$). At the same time, the result of the Harris-Boyd's test in Table 7 did not indicate partitioning since all the z-values were less than the z* values. Thus, all the age groups for SARS-CoV-2 IgM were combined, and the RI for IgM is 0 ~ 0.1523 (S/CO).

Regarding to IgG, the result of the Spearman's rank correlation analysis in Figure 4C indicated that the SARS-CoV-2 IgG values were not correlated with age ($R^2 = 0.0000$; $p = 0.9920$). In Figure 4D, the Kruskal-Wallis test results showed significant differences among the four age groups ($p = 0.0011$). However, the result of the Harris-Boyd's test shown in Table 8 did not indicate partitioning because all the z-values were less than the z* values. Thus, all the age groups for SARS-CoV-2 IgG were combined, and the RI for IgG is 0 - 0.2663 (S/CO).

## DISCUSSION

COVID-19, caused by SARS-CoV-2 has spread all over the world and is a global public threat nowadays [1]. There is no specific therapeutic method for COVID-19. Moreover, SARS-CoV-2 may persist in some asymptomatic individuals or cured patients, which may cause a continued pandemic [25-28]. As was mentioned before, elder people are a special group with low immunity. The basal metabolism and the basic content of various biomarkers in the body of the elderly may be different from those of other populations [5]. Xi Chen, et al. pointed that elder population is more susceptible to SARS-CoV-2 [29]. At the same time, elder patients

**Table 6. SARS-CoV-2 IgM and IgG values of reference subjects.**

| Groups | Number of samples | SARS-CoV-2 IgM | | | SARS-CoV-2 IgG | | |
|---|---|---|---|---|---|---|---|
| | | Median ($X_{25\%}$, $X_{75\%}$) S/CO | S-W test | Range S/CO | Median ($X_{25\%}$, $X_{75\%}$) S/CO | S-W test | Range S/CO |
| All | 3,733 | 0.014 (0.011, 0.022) | p < 0.0001 | 0.010 - 4.341 | 0.021 (0.012, 0.041) | p < 0.0001 | 0.010 - 8.481 |
| Male age, year | | | | | | | |
| 60 - 69 | 1,317 | 0.014 (0.011, 0.021) | p < 0.0001 | 0.010 - 2.545 | 0.020 (0.011, 0.038) | p < 0.0001 | 0.010 - 6.296 |
| 70 - 79 | 480 | 0.014 (0.012, 0.024) | p < 0.0001 | 0.010 - 0.518 | 0.020 (0.012, 0.0408) | p < 0.0001 | 0.010 - 2.184 |
| 80 - 89 | 174 | 0.0155 (0.001, 0.023) | p < 0.0001 | 0.010 - 0.243 | 0.025 (0.013, 0.040) | p < 0.0001 | 0.010 - 4.026 |
| 90 - 99 | 34 | 0.0125 (0.0108, 0.020) | p < 0.0001 | 0.010 - 0.047 | 0.023 (0.0125, 0.0388) | p < 0.0001 | 0.010 - 0.262 |
| Total | 2,005 | 0.014 (0.011, 0.022) | p < 0.0001 | 0.010 - 2.545 | 0.020 (0.012, 0.038) | p < 0.0001 | 0.010 - 6.296 |
| Female age, year | | | | | | | |
| 60 - 69 | 1,127 | 0.014 (0.011, 0.022) | p < 0.0001 | 0.010 - 4.341 | 0.022 (0.012, 0.040) | p < 0.0001 | 0.010 - 8.481 |
| 70 - 79 | 399 | 0.013 (0.010, 0.020) | p < 0.0001 | 0.010 - 1.634 | 0.024 (0.013, 0.048) | p < 0.0001 | 0.010 - 3.103 |
| 80 - 89 | 182 | 0.014 (0.011, 0.024) | p < 0.0001 | 0.010 - 0.578 | 0.029 (0.014, 0.0613) | p < 0.0001 | 0.010 - 3.88 |
| 90 - 101 | 20 | 0.0125 (0.011, 0.0205) | p < 0.0001 | 0.010 - 0.172 | 0.0155 (0.013, 0.0503) | p < 0.0001 | 0.010 - 0.158 |
| Total | 1,728 | 0.014 (0.010, 0.021) | p < 0.0001 | 0.010 - 4.341 | 0.023 (0.012, 0.044) | p < 0.0001 | 0.010 - 8.481 |

**Table 7. Harris-Boyd's test of IgM for different subgroups.**

| Subgroup | z | z * | Partition |
|---|---|---|---|
| Female vs. male | -0.09 | 11.83 | no |
| 60 - 69 vs. 70 - 79 | 0.16 | 11.16 | no |
| 60 - 69 vs. 80 - 89 | 0.40 | 10.25 | no |
| 60 - 69 vs. 90 - 99 | -1.62 | 9.68 | no |
| 70 - 79 vs. 80 - 89 | 0.28 | 7.08 | no |
| 70 - 79 vs. 90 - 99 | -1.63 | 5.92 | no |
| 80 - 89 vs. 90 - 99 | -1.67 | 3.92 | no |

**Note: Harris Boyd's test was calculated with the logarithms of the S/CO. The z and z * value was calculated by the formula according to CLSI C28 - A3 document. All the z-values were less than that of z * for SARS-CoV-2 IgM, which indicated that there was no need for partitioning.**

were more susceptible to severe diseases. Under these circumstances, timely and accurate detection of the SARS-CoV-2 is a high priority for treatment of elder patients. SARS-CoV-2 IgM and IgG are immunological biomarkers for current infection or post infection for the diagnosis of COVID-2019 [30,31]. However, there are no special reference intervals of SARS-CoV-2 IgM and IgG for elder population, which may cause missed diagnosis of the infected person or unnecessary panic for the non-infected person, and increase the difficulty of epidemic prevention and control simultaneously [32, 33]. Therefore, establishment of SARS-CoV-2 RIs for the elderly based on large batch of samples is of vital importance.

**Table 8. Harris-Boyd's test of IgG for different subgroups.**

| Subgroup | z | z * | Partition |
|---|---|---|---|
| Female vs. male | 3.33 | 11.83 | no |
| 60 - 69 vs. 70 - 79 | 1.55 | 11.16 | no |
| 60 - 69 vs. 80 - 89 | 3.28 | 10.25 | no |
| 60 - 69 vs. 90 - 99 | 0.14 | 9.68 | no |
| 70 - 79 vs. 80 - 89 | 2.27 | 7.08 | no |
| 70 - 79 vs. 90 - 99 | -0.33 | 5.92 | no |
| 80 - 89 vs. 90 - 99 | -1.29 | 3.92 | no |

**Note: Harris Boyd's test was calculated with the logarithms of the S/CO. The z and z * value was calculated by the formula according to CLSI C28 - A3 document. All the z-values were less than that of z * for SARS-CoV-2 IgG, which indicated that there was no need for partitioning.**

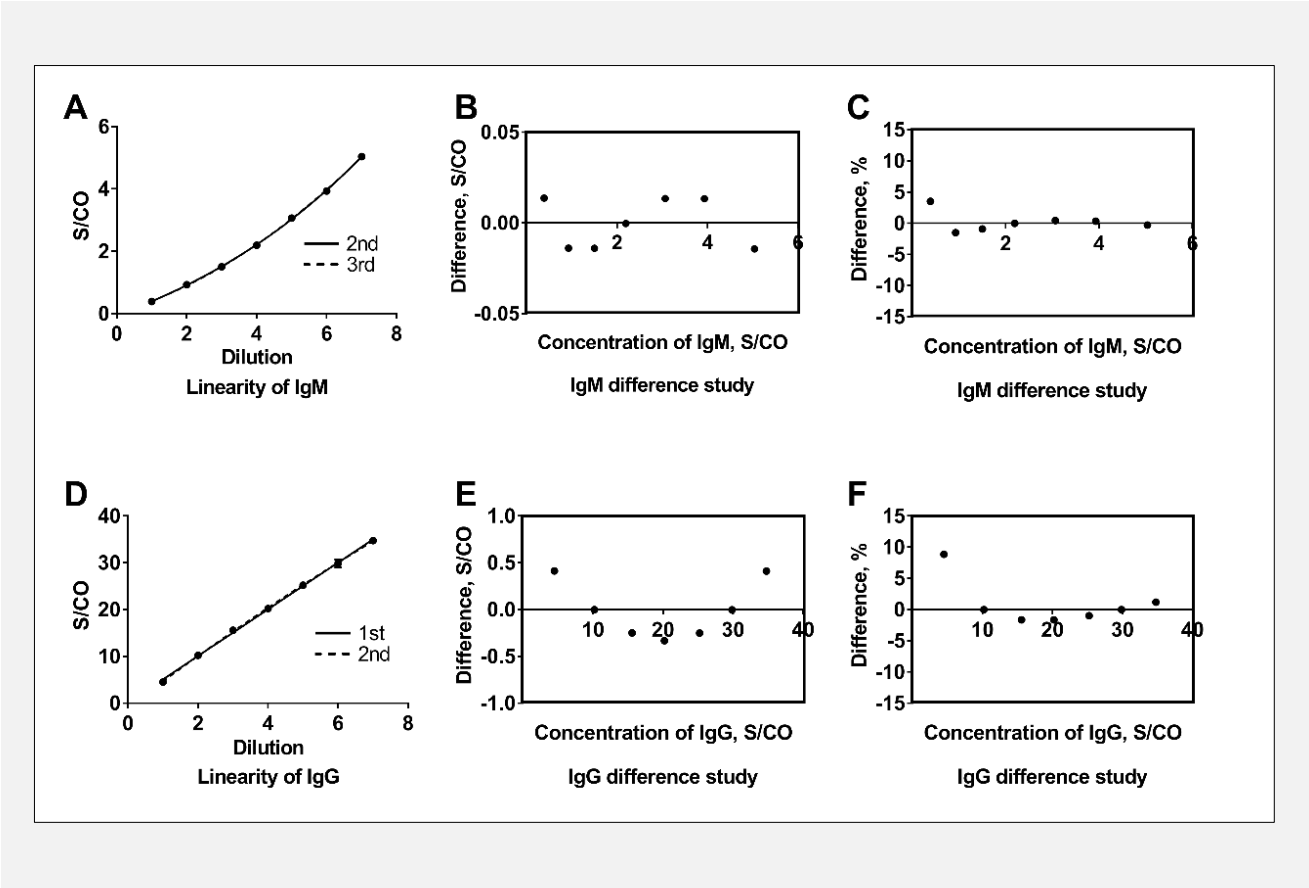


**Figure 1. Evaluation of the linearity.**

**(A) The second- and the third-order models of the statistical analysis for SARS-CoV-2 IgM - the equation of the two models was Y = 0.05531*X*X + 0.3263*X + 0.02367 and Y = 0.002296*X*X*X + 0.02776X*X + 0.4205*X - 0.059, respectively. (B) The concentration differences between the second- and the third-order models of SARS-CoV-2 IgM; (C) The differences between the second- and the third-order models of SARS-CoV-2 IgM, and all did not exceed 5%. (D) The first- and second-order models of the statistical analysis for SARS-CoV-2 IgG - the equation of the two models was Y = 4.99*X + 0.09124 and Y = - 0.0827*X*X + 5.652*X - 0.9011, respectively. (E) The concentration differences between the first- and second-order models of SARS-CoV-2 IgG; (C) The differences between the first- and second-order models of SARS-CoV-2 IgG, and all did not exceed 10%.**

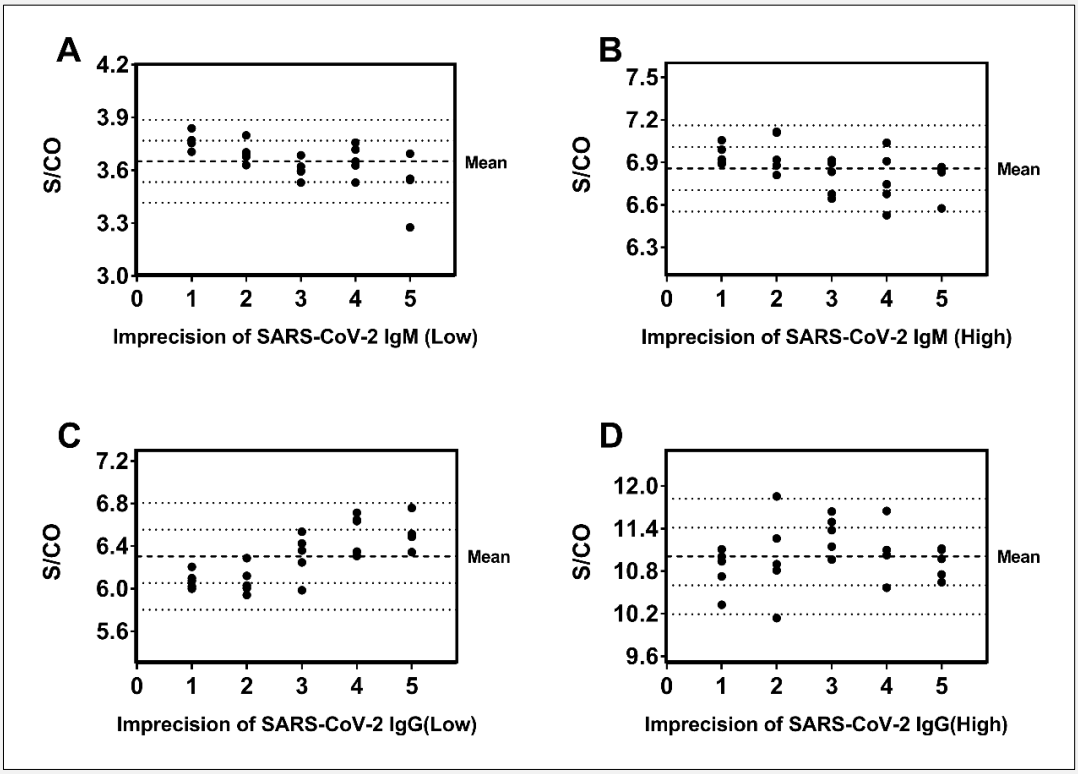


**Figure 2. Imprecision evaluation for SARS-CoV-2 IgM and IgG.**

**(A) Imprecision of SARS-CoV-2 IgM for low concentration; (B) Imprecision of SARS-CoV-2 IgM for high concentration; (C) Imprecision of SARS-CoV-2 IgG for low concentration; (D) Imprecision of SARS-CoV-2 IgG for high concentration.**

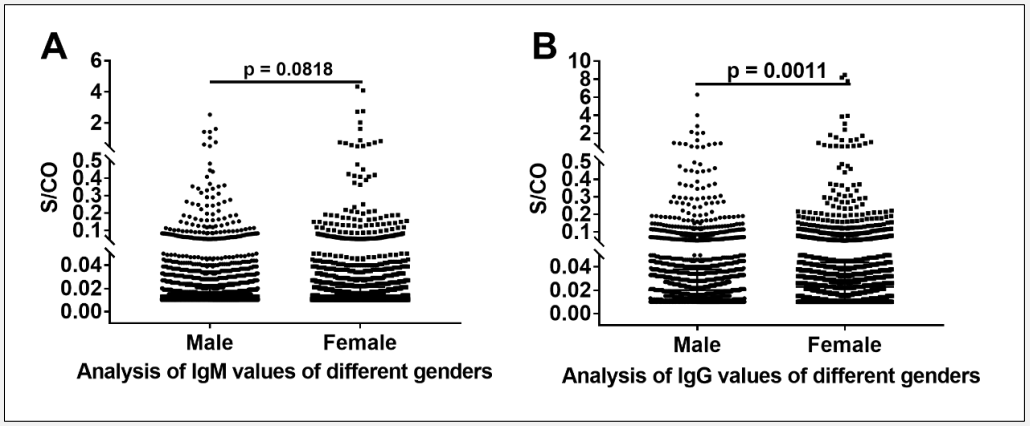


**Figure 3. Comparison of gender.**

**(A) Analysis of IgM values of different genders, there was no significant difference between males and females, p = 0.0818; (B) Analysis of IgG values of different genders, shows there is a significant difference between males and females, p = 0.0011.**

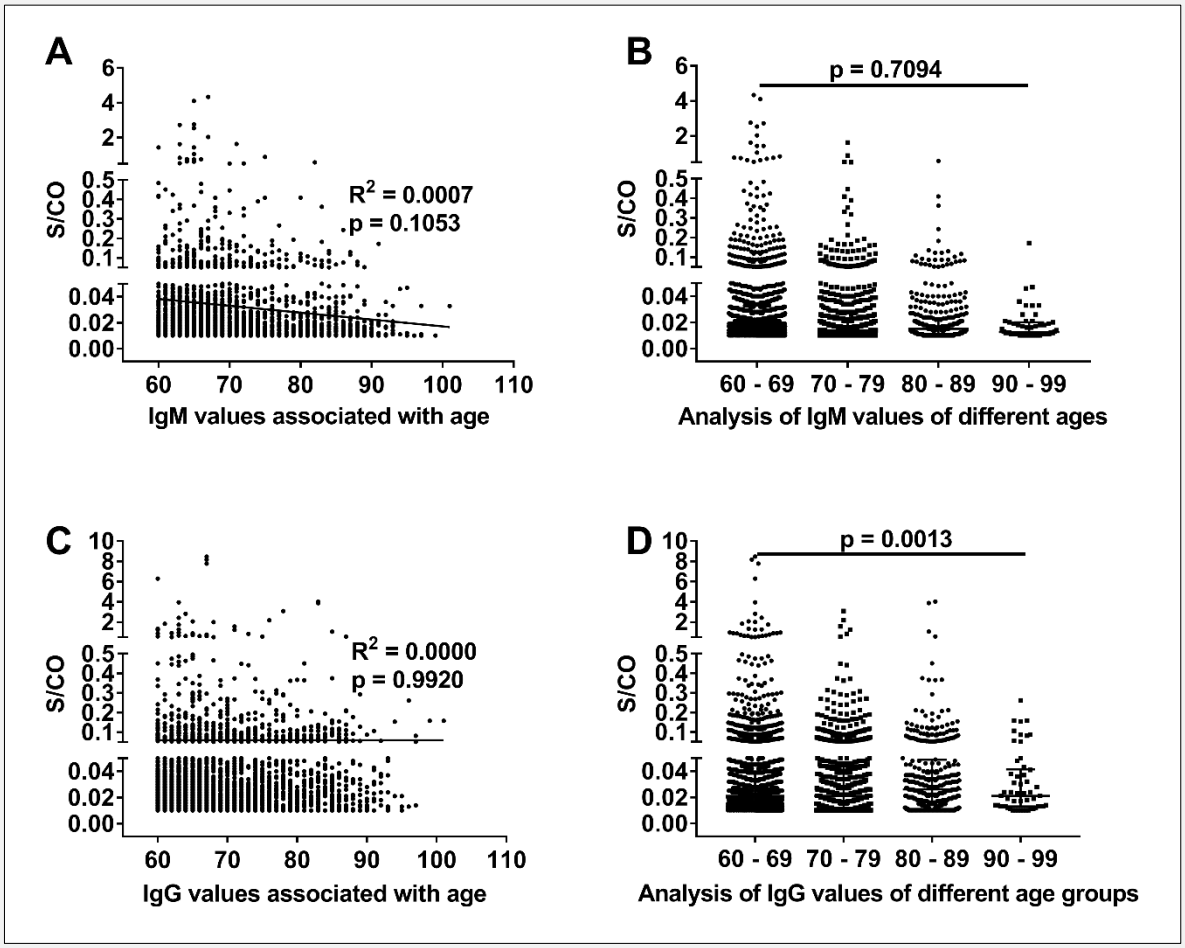


**Figure 4. Comparison of age.**

**(A) SARS-CoV-2 IgM values associated with age; (B) Analysis of SARS-IgM values of different ages, there's no significant difference between different age groups, p = 0.7094; (C) SARS-CoV-2 IgG values associated with age; (D) Analysis of SARS-IgG values of different age groups, there is significant difference between different age groups, p = 0.0013.**

In order to ensure the accuracy of the establishment of RI, we first evaluated the performance of the CLIA detection system before conducting the experiment. The linearity, allowable dilution ratio, and precision of the CLIA kit for SARS-CoV-2 IgM and IgG used in this study were in compliance with laboratory requirements. According to the CLSI document C28-A3, 3,734 subjects with the age of more than 60 in our hospital or outpatients were selected. The serum SARS-CoV-2 IgM and IgG were assessed to establish the RI for elderly population. Strict calibrations of the detection system and quality control procedures were performed during the testing process. Finally, 3,733 qualified serum samples were obtained and analyzed for the study after one result was deleted as outlier.

The SARS-CoV-2 IgM level has no significant difference among different gender groups. Harris-Boyd's test result in Table 7 did not indicate partitioning for different gender groups. Based on that, the results of IgM were divided into different age groups and analyzed again. Results in Figure 4A and 4B indicated that there is no significant difference between different age groups. At the same time, the Harris-Boyd's test result in Table 7 did not indicate partitioning for different age groups. Thus, the upper limit of RI for SARS-CoV-2 IgM for elder population was defined as 0.1523 (S/CO) based on a total of 3,734 subjects according to the Shapiro-Wilk test. In contrast, the SARS-CoV-2 IgG has significant differences among different gender groups and age groups (p = 0.0011 and 0.0013, respectively).

However, the result of Harris-Boyd's test in Table 8 did not indicate partitioning for different gender or age groups. Therefore, the RI of SARS-CoV-2 IgG for elder population can be defined as 0 - 0.2663 (S/CO).

As we can see from the established RIs for SARS-CoV-2 IgM and IgG, referring to IgM detection, if the result is more than 0.1523 (S/CO), it can be defined as a positive result; similarly, referring to IgG detection, if the result is more than 0.2663 (S/CO), it can be defined as a positive result. The upper limits of RI for IgM and IgG in this study are different, which is consistent with the basic content of IgG in the human body being higher than that of IgM. The RIs of SARS-CoV-2 IgM and IgG claimed by the manufacturer are all 0 - 1 S/CO, which differs from the RIs established by the present study. The major explanation is that the claimed RI of the CLIA kit was based on measurements of 206 subjects, which is not a sufficient number compared with the present study. In addition, RIs supplied by the manufacturer are not specifically established for the elderly. As a result, the establishment of a new RI of SARS-CoV-2 IgM and IgG for elder population is vital for clinical diagnoses.

Reference intervals are conventionally used to differentiate normal vs. abnormal, or positive vs. negative. S/CO value > 1 is normally used to differentiate between positive and negative decision. However, the S/CO values supplied by the manufacture may not be applicable in some cases. So, we should adjust the S/CO calculation process or establish new RIs for antibody detection.

There were two limitations in this study. First, defined cutoff values for IgM and IgG were only suitable for clinical kits using the CLIA system. Second, clinical samples used in our study were all collected in our hospital, which may reduce the representation of the conclusion.

For the CLIA detection method we use here, there is a big difference between the RIs we established and the RIs provided by the manufacturer, which undoubtedly suggests that for other detection systems or assays, we had better not used the RIs provided by the manufacturer directly. Instead, try to re-establish each detection system as much as possible, or verify the clinical RIs with a large sample, so as to provide a better reference for clinical diagnosis.

In a word, based on the performance verification and quality control of the CLIA system, the RIs of SARS-CoV-2 IgM and IgG were successfully established for the elder population in our laboratory, which can provide a reliable and accurate reference for clinical judgment, medical intervention, and epidemic prevention. However, further studies are also needed for multicenter and multiethnic research to make the results more representative of different populations.

**Declaration of Interest:**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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