

1 **A Peptide-based Magnetic Chemiluminescence Enzyme Immunoassay for** 2 **Serological Diagnosis of Corona Virus Disease 2019 (COVID-19)**

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47 **Abstract**

48 A respiratory illness has been spreading rapidly in China, since its outbreak in
 49 Wuhan city, Hubei province in December 2019. The illness was caused by a novel
 50 coronavirus, named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).
 51 Clinical manifestations related to SARS-CoV-2 infection ranged from no symptom to
 52 fatal pneumonia. World Health Organization (WHO) named the diseases associated
 53 with SARS-CoV-2 infection as COVID-19. Real time RT-PCR is the only laboratory
 54 test available till now to confirm the infection. However, the accuracy of real time
 55 RT-PCR depends on many factors, including sampling location and of methods,
 56 quality of RNA extraction and training of operators etc.. Variations in these factors
 57 might significantly lower the sensitivity of the detection. We developed a
 58 peptide-based luminescent immunoassay to detect IgG and IgM. Cut-off value of this
 59 assay was determined by the detection of 200 healthy sera and 167 sera from patients
 60 infected with other pathogens than SARS-CoV-2. To evaluate the performance of this
 61 assay, we detected IgG and IgM in the 276 sera from confirmed patients. The positive
 62 rate of IgG and IgM were 71.4% (197/276) and 57.2% (158/276) respectively. By
 63 combining with real time RT-PCR detection, this assay might help to enhance the
 64 accuracy of diagnosis of SARS-CoV-2 infection.

65 **Key words:** SARS-CoV-2, COVID-19, Immunoglobulin G, Immunoglobulin M,
 66 Detection, Chemiluminescence Immunoassay

67 Introduction

68

69 In December 2019, a novel coronavirus, labeled as severe acute respiratory
70 syndrome coronavirus 2 (SARS-CoV-2) the Coronavirus Study Group [1], has been
71 identified as the causative agent of the Corona Virus Disease 2019 (COVID-19) [2]
72 outbreak in Wuhan, Hubei province of China [3, 4, 5, 6, 7]. This disease spread
73 rapidly by human-to-human transmission [4] from Wuhan to other regions [8],
74 resulting in more than 44,000 COVID-19 cases in mainland China based on the
75 statistical data until Feb 12, 2020 [9]. Additionally, a total of 78 cases were identified
76 in Hong Kong, Macao and Taiwan, and more than 400 cases were identified in
77 Thailand, Japan, South Korea, United States, Vietnam, Singapore, Nepal, France,
78 Australian and Canada [9]. So far, the number of infected people is still growing
79 significantly.

80 Given that effective anti-viral therapeutics are unavailable currently, the first line
81 of defense is to identify infected-patients as early as possible. Currently, laboratory
82 diagnosis of SARS-CoV-2 has been carried out by detecting viral RNA in throat swab
83 samples based on real-time reverse transcription polymerase chain reaction assay
84 (real-time RT-PCR assay). This real-time RT-PCR method is sensitive and does not
85 require live virus present in the specimen. Despite these advantages, such method can
86 give results that are falsely-negative or falsely-positive due to several limitations, such
87 as quality of specimen collection, multi-steps of RNA preparation and virus mutation.
88 Another most widely used method is a serological test for the presence of antibodies

89 against viral proteins. Like severe acute respiratory syndrome CoV (SARS-CoV) and
 90 Middle East respiratory syndrome CoV (MERS-CoV), SARS-CoV-2 are enveloped
 91 positive-sense single-stranded RNA viruses [5, 10]. Genomic analysis of
 92 SARS-CoV-2 reveals four major structural proteins including Spike (S) protein,
 93 Nucleocapsid (N) protein, Envelope (E) protein, and Membrane (M) protein, as well
 94 as a number of accessory open reading frame (ORF) proteins [5, 10]. In this study, we
 95 developed a magnetic chemiluminescence enzyme immunoassay (MCLIA) which has
 96 good specificity for immunological reaction and high sensitivity for detecting serum
 97 immunoglobulin G (IgG) and IgM against SARS-CoV-2.

98 **Materials and methods**

99 **Human sera**

100 A total of 276 sera were collected from 276 inpatients from three designated
 101 hospitals, Chongqing Three Gorges Central Hospital, Yongchuan Hospital Affiliated
 102 to Chongqing Medical University (CQMU), and The Public Health Center, in
 103 Chongqing, China. These patients were confirmed to be infected with 2019-nCoV by
 104 real time RT-PCR detection of virus RNA. Among these sera samples, 168 were
 105 taken from patients with fever symptom. The time points of sampling range from day
 106 2 to day 27 from the onset of fever. Ninety-nine of these patients reported exposure to
 107 persons with confirmed infection latter. The 200 normal human sera were collected
 108 from healthy people more than 1 year before 2019-nCoV outbreak. 167 sera from
 109 patients with infection with other pathogens were collected from the Second Hospital
 110 Affiliated to CQMU and Children's Hospital Affiliated to CQMU. These pathogens

111 include influenza A virus (25), respiratory syncytial virus (7), parainfluenza virus (8),
 112 influenza B virus (5), adenovirus (6), *Klebsiella pneumonia*
 113 (8), *Streptococcus pneumonia* (3), *Mycoplasma* (5), *Acinetobacter baumannii*
 114 (10), *Candida albicans* (2), *Staphylococcus aureus* (3), *Mycobacterium*
 115 *tuberculosis*(4), Hepatitis B virus (33), Hepatitis C virus (22), Syphilis (23) and
 116 *Saccharomycopsis* (3).

117 All sera samples were inactivated at 56 °C for 30 min. This study was approved
 118 by the Ethics Commission of Chongqing Medical University (CQMU-2020-01).
 119 Written informed consent was waived by the Ethics Commission of the designated
 120 hospital for emerging infectious diseases.

121 **Synthetic peptide-based luminescent immunoassay.**

122 We developed a luminescent immunoassay for the detection of 2019-nCov
 123 antibody using synthetic peptide antigens as the immunosorbent. Twenty peptides,
 124 deduced from the genomic sequence from GenBank (NC_045512.1), were
 125 synthesized as candidate antigens from the orf1a/b, spike (S), and nucleocapsid (N)
 126 proteins. Each kind of peptide was labelled with biotin and the biotinylated peptide
 127 was purified and used to bind to streptavidin-coated magnetic beads. For antibody
 128 assay, serum samples (100µl/each sample) were mixed with the beads carrying
 129 corresponding peptides for 10 min at 37°C. Beads were washed 5 times, reacted to the
 130 antibody conjugate, again washed 5 times, and reacted to substrate. Reactivity was
 131 determined by a luminescence reader (Peteck 96-I, Bioscience, China).

132 **Evaluation of the luminescent immunoassay.**

133 Cut-off value of the test was determined as the mean luminescence value of the
134 200 normal sera plus 5 folds of SD. Sera from 276 COVID-19 patients 167 patients
135 with irrelevant pathogens were used to evaluate the performance of the assay. The
136 luminescent immunoassay was performed as described above. Results were
137 determined as positive if the signal/cutoff (S/C) ratio ≥ 1 .

138

139 **Result**

140 **Evaluation of Synthetic peptide-based MCLIA for SARS-CoV-2**

141 Twenty synthetic peptides, derived from the amino acid sequence of ORF1a/b,
142 Spike (S) protein and Nucleocapsid (N) protein, were used to develop MCLIA for
143 detecting IgG and IgM antibodies against SARS-CoV-2. To screen these peptides, 5
144 sera from confirmed patients and 10 normal sera were used to react with these
145 peptides respectively. Among these peptides tested, one from S protein showed the
146 best performance. We used the assay based on this peptide for the following study. To
147 determine the cut-off value of this assay, serum samples from 200 healthy blood
148 donors who donated blood 1-2 years ago were first tested. The mean
149 chemiluminescence (CL) values for IgG and IgM were 0.152 ± 0.109 and 0.151 ± 0.107 ,
150 respectively (Fig.1). Cut-off value for IgG and IgM detection were determined as 0.7
151 and 0.7 respectively.

152 To test the specificity of the assays, the serum samples from 167 people infected
153 with other respiratory pathogens such as influenza A virus, influenza B virus,
154 parainfluenza virus, adenovirus, respiratory syncytial virus, mycoplasma,

155 *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Acinetobacter baumannii*, *Candida*
156 *albicans*, *Staphylococcus aureus* were tested. The mean chemiluminescence (CL)
157 values for IgG and IgM in non-SARS-CoV-2 infected people were 0.121 ± 0.062 and
158 0.120 ± 0.065 , respectively (Fig.1A-B). These results showed that no cross-reactivity
159 was observed for these 20 pathogens, indicating a very good specificity.

160 To test stability of this MCLIA based serological diagnosis method, serum
161 samples with different concentrations were measured 10 times (Fig 2. A, B, C, D),
162 coefficient of variation (CV) of IgG and IgM detection in different concentration
163 samples were all below 6% (Fig 2. E, F), which meant a perfect stability of this assay
164 in IgG/IgM detection. Furthermore, series dilutions for 6 serum samples(3 for IgG, 3
165 for IgM) were performed and S/co values were collected, regression analysis revealed
166 S/co value range from 1 to 200 linear reflected serum antibody concentration (IgG,
167 $R^2 = -0.902$, $P < 0.001$, Fig 3A; IgM, $R^2 = -0.946$, $P < 0.001$, Fig 3B), assured the
168 rationality in further quantitative comparison based on S/co values.

169 Serological Diagnosis for SARS-CoV-2 in patients with confirmed infection

170 IgG and IgM were further examined by MCLIA in serum from 276 patients with
171 confirmed SARS-CoV-2 infection. The median age of these patients was 48 years
172 (IQR, 37-56; range, 0.66-84years), and 151/276 (54.71%) were men. The mean (CL)
173 values for IgG were 18.62 ± 32.87 , ranging from 0.05-194.56; IgM were 5.50 ± 22.60 ,
174 ranging from 0.04-318.16. Majority (197/276; 71.4%) of patients were positive for
175 IgG antibody against the SARS-CoV-2, while 57.2% (158/276) patient were positive

for IgM antibody against the SARS-CoV-2. Overall, 225 patients showed positive for IgM or IgG test and the total positive rate reached 81.52% (225/276) (Table1).

Discussion

We developed a luminescent immunoassay for IgG and IgM against SARS-CoV-2, which, to our knowledge, was the first such assay allowed us to study the antibody response to the newly identified coronavirus. This assay was based on a peptide from S protein, screened out from 20 candidate peptides deduced from the genomic sequence. Using synthetic peptide as antigen helps to enhance the stability and repeatability of an assay, and theoretically would be more specific than using virus as antigen. Indeed, this peptide showed a very good specificity in our assay: none of the 167 sera from patients infected with other pathogens than SARS-CoV-2 reacted with this peptide. This high specificity may be attributed to the relatively low homology of this region to other coronaviruses (data not shown).

Real time RT-PCR was the only test to confirm the infection of SARS-CoV-2 till now. We detected both IgM and IgG in the same sera from the 276 infection-confirmed patients. IgG was detected in 71.4% (197/276) of all the sera, higher than the detection rate of IgM (57.2%, 158/276). A combination the two antibodies enhanced the detection rate to 81.5% (225/276). Different sensitivity of the detection of IgG and IgM had been reported in SARS [11]. IgG can be detected as early as 2 days after the onset of fever. IgM was not detected earlier than IgG, similar to the situation in MERS [12], which limits its diagnostic utility. It was reported that

198 20%-50% SARS patients cannot be confirmed by RT-PCR [13], and this elicited a
199 speculation that there might be a comparable part of infection cannot be detected by
200 real time RT-PCR. Failure of detection by real time RT-PCR can be caused by the
201 problems from sampling, RNA extraction and PCR amplification, while detecting
202 antibodies in serum sample avoid a large part of these problems.

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212

213 **Disclosure statement**

214 No potential conflict of interest was reported by the authors.

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223 Reference

224 1. Alexander E. Gorbalenya SCB, Ralph S. Baric, et al. Severe acute respiratory
225 syndrome-related coronavirus: The species and its viruses – a statement of the
226 Coronavirus Study Group. medRxiv. 2020. doi:
227 <https://doi.org/10.1101/2020.02.07.937862>.

228 2. WHO. 2020. Available from:
229 [https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-](https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020)
230 [briefing-on-2019-ncov-on-11-february-2020](https://www.who.int/dg/speeches/detail/who-director-general-s-remarks-at-the-media-briefing-on-2019-ncov-on-11-february-2020)

231 3. Chan JF, Yuan S, Kok KH, et al. A familial cluster of pneumonia associated with the
232 2019 novel coronavirus indicating person-to-person transmission: a study of a family
233 cluster. Lancet. 2020 Jan 24. doi: 10.1016/S0140-6736(20)30154-9. PubMed PMID:
234 31986261.

235 4. Li Q, Guan X, Wu P, et al. Early Transmission Dynamics in Wuhan, China, of Novel
236 Coronavirus-Infected Pneumonia. N Engl J Med. 2020 Jan 29. doi:
237 10.1056/NEJMoa2001316. PubMed PMID: 31995857.

238 5. Zhou P, Yang XL, Wang XG, et al. A pneumonia outbreak associated with a new
239 coronavirus of probable bat origin. Nature. 2020 Feb 3. doi:
240 10.1038/s41586-020-2012-7. PubMed PMID: 32015507.

241 6. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel

- 242 coronavirus in Wuhan, China. Lancet. 2020 Jan 24. doi:
243 10.1016/S0140-6736(20)30183-5. PubMed PMID: 31986264.
- 244 7. Wei-jie Guan Z-yN, Yu Hu, et. al. Clinical characteristics of 2019 novel coronavirus
245 infection in China. medRxiv. 2020. doi:
246 <http://dx.doi.org/10.1101/2020.02.06.20020974>.
- 247 8. Yang Yang Q-BL, Ming-Jin Liu, et al. Epidemiological and clinical features of the 2019
248 novel coronavirus outbreak in China. medRxiv. 2020. doi:
249 <https://doi.org/10.1101/2020.02.10.20021675>.
- 250 9. WHO. Coronavirus disease 2019 (COVID-19) Situation Report – 23. Available from:
251 https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200212-sitrep-23-ncov.pdf?sfvrsn=41e9fb78_2
252
- 253 10. Chan JF, Kok KH, Zhu Z, et al. Genomic characterization of the 2019 novel
254 human-pathogenic coronavirus isolated from a patient with atypical pneumonia after
255 visiting Wuhan. Emerg Microbes Infect. 2020 Dec;9(1):221-236. doi:
256 10.1080/22221751.2020.1719902. PubMed PMID: 31987001.
- 257 11. Woo PC, Lau SK, Wong BH, et al. Differential sensitivities of severe acute respiratory
258 syndrome (SARS) coronavirus spike polypeptide enzyme-linked immunosorbent
259 assay (ELISA) and SARS coronavirus nucleocapsid protein ELISA for serodiagnosis
260 of SARS coronavirus pneumonia. J Clin Microbiol. 2005 Jul;43(7):3054-8. doi:
261 10.1128/JCM.43.7.3054-3058.2005. PubMed PMID: 16000415; PubMed Central
262 PMCID: PMC169156.
- 263 12. Corman VM, Albarak AM, Omrani AS, et al. Viral Shedding and Antibody Response

264 in 37 Patients With Middle East Respiratory Syndrome Coronavirus Infection. Clin
 265 Infect Dis. 2016 Feb 15;62(4):477-483. doi: 10.1093/cid/civ951. PubMed PMID:
 266 26565003.

267 13. Yam WC, Chan KH, Poon LL, et al. Evaluation of reverse transcription-PCR assays
 268 for rapid diagnosis of severe acute respiratory syndrome associated with a novel
 269 coronavirus. J Clin Microbiol. 2003 Oct;41(10):4521-4. doi:
 270 10.1128/jcm.41.10.4521-4524.2003. PubMed PMID: 14532176; PubMed Central
 271 PMCID: PMC254368.

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274 **Figure Legends**

275 Fig1. Evaluation of the synthetic peptide-based MCLIA for the detection of IgG and
276 IgM against SARS-CoV-2. Serum samples were obtained from 200 healthy blood
277 donors, 167 people infected with other respiratory pathogens and 276 patients with
278 confirmed SARS-CoV-2 infection. The serum IgG (A) and IgM (B) were analyzed by
279 MCLIA.

280 Fig 2. Assessment methodological quality of MCLIA. A. repeated detection analysis
281 of high antibody concentration serum sample (time of replication=10). B. Detection
282 analysis of middle antibody concentration serum sample (time of replication=10). C.
283 Detection analysis of low antibody concentration serum sample (time of
284 replication=10). D. Detection analysis of negative control(healthy) serum sample
285 (time of replication=10). E. Percent coefficient of variation for different concentration
286 serum samples. F. Mean percent coefficient of variation for the different concentration
287 serum samples.

288

289 Fig 3. The correlation between the serial dilution ratio and calculated S/co values. A.
290 correlation between serial dilution ratio and S/co values in IgG detection in 3 serum
291 samples ($n=3$ replicates in each dilution for each sample). B. correlation between
292 serial dilution ratio and S/co values in IgM detection in 3 serum samples ($n=3$
293 replicates in each dilution for each sample).

294

Table 1. Positive rates of serum IgG or IgM in 276 patients with confirmed SARS-CoV-2 infection

	Positive No.	Negative No.	Positive rate (%)
IgG	197	79	71.37
IgM	158	118	57.24
IgG or IgM	225	51	81.52

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2

Figure 1

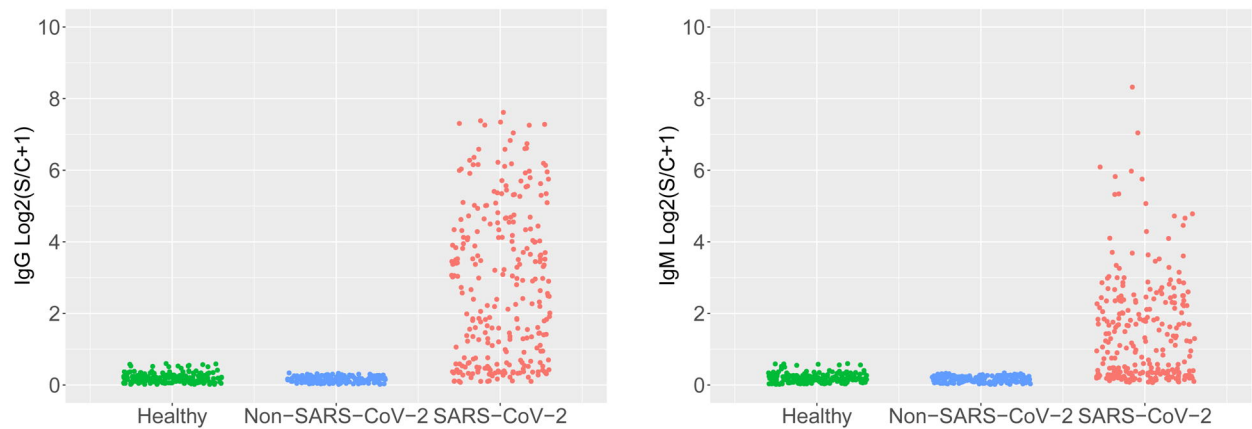


Figure 2

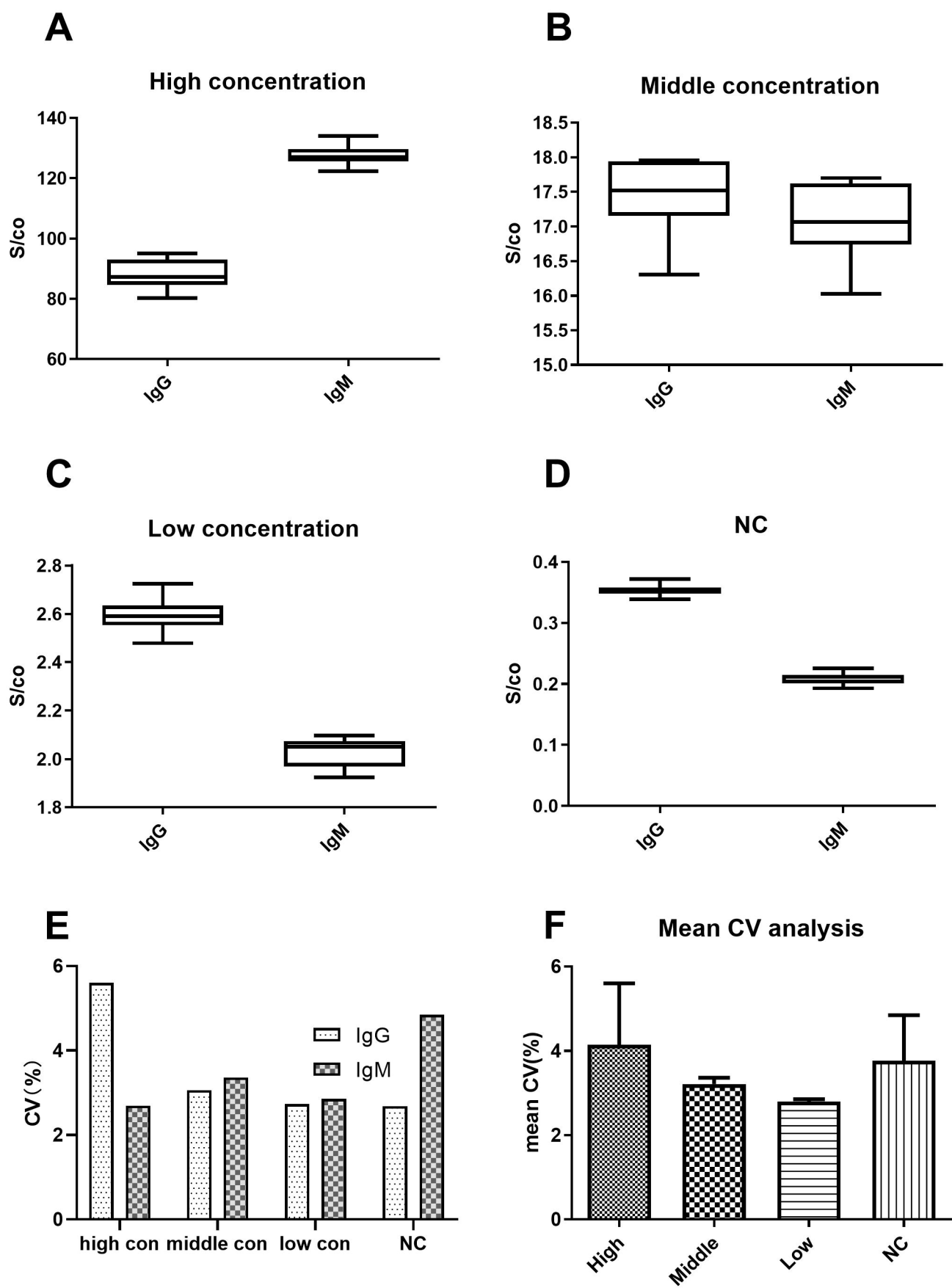


Figure 3

