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Development of Enzyme-linked Immunosorbent Assay for Serodiagnosis of COVID-19

Lapasrada Mungmongkol[1] Porntip Chaiya[1] Parnuphan Panyajai[1] Sakulrat Soonthornchartrawat[1] Sumalee Chanama[2] Archawin Rojanawiwat[1] and Panadda Dhepakson[2]

[1]Medical Life Science Institute, [2]National Institute of Health, Department of Medical Sciences, Tiwanond Road, Nonthaburi 11000, Thailand

ABSTRACT Emerging of pandemic coronavirus disease-19 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) leading to diverse respiratory pathological disorders particularly severe pneumonia worldwide. Serological tests are essential approach to support diagnosis. We developed immunological assays based on capture ELISA to detect immunoglobulin M (IgM) and immunoglobulin G (IgG) against SARS-CoV-2 nucleocapsid protein presented in sera of COVID-19 patients. Four main conditions were optimised including concentration of capture molecule, sample dilution ratio, blocking reagent and concentration of conjugated protein. Anti-SARS-CoV-2 IgM ELISA showed 41.46% sensitivity and 99% specificity while sensitivity and specificity of anti-SARS-CoV-2 IgG ELISA were 60.98% and 100%, respectively. In addition, the cut-off thresholds were established at 3.2 for IgM ELISA and 2.2 for IgG ELISA, sensitivity of IgM and IgG assays was increased to 51.28% and 89.74% based on duration of onset, respectively. It is suggested that our developed ELISA could be used to determine antibody to SARS-CoV-2.

Keywords: COVID-19, ELISA, SARS-CoV-2, IgM, IgG

Corresponding author E-mail: Lapasrada.m@dmsc.mail.go.th

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Introduction

The global pandemic coronavirus disease2019 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) which belongs to Betacoronavirus in Coronaviridae family[1]. COVID-19 considerably widespread via airborne transmission in human to human with zoonotic origin and have been found R0 between 2.2-2.6. Fever, fatigue, pneumonia and respiratory symptoms are a typical manifestation in COVID-19[2]. On the contrary, certain number of confirmed cases reveal asymptomatic or other associated pathological findings such as multiple patchy, Guillain-Barré and hyposmia. Previous research indicated that the average of incubation period is about 5-6 days as related to 14 days for quarantine recommendation of public measures[3].

Thai Ministry of Public Health has worked co-ordinately with World Health Organisation to prevent the outbreak that contributes the outcomes as follow; approximately 3000 confirmed cases with 1.8% fatal cases but 2945 recovered cases have been reported or around 95%[4]. Aftermath, the emergence of viral infection affects not only health and well-being in population but in socio-economic scheme as well.

Mucosal droplets containing viral germs have been reported as mode of transmission which affected respiratory airway and may be caused multi-organ failure. Meanwhile, viral particle is composed of diverse fundamental proteins in replication, formation and host attachment. Depending on cellular proteases, there are two major receptors including dipeptidyl peptidase 4 (DPP4) and angiotensin-converting enzyme 2 (ACE2) for entry mechanisms by binding to viral receptor-binding domain (RBD)[5]. After infection, host innate immunity recognises pathogen associated molecular patterns (PAMs) through endosomal receptors, leading to downstream signalling and antiviral cytokine expression in first line of defence[6]. Thereafter, both cell-mediated and humoral immunity are triggered in adaptive immune response. T-lymphocyte obtains the antigens through major histocompatibility complex and subsequently transfers antigens to B-cell, leading to cell proliferation and differentiation. Indeed, T-lymphocyte is divided into subpopulation including cytotoxic T-cells (CD3+CD8+), regulatory T-cells (FOXP3) and helper T-cells (CD3+CD4+) with different functions[7]. Likewise, excessive pro-inflammatory cytokines and chemokines such as Interferon family, IL-6 and CCL2 in cell-cell interaction cause cytokine storm in some severe cases[8]. Being stimulated by helper T-cells, B-lymphocytes differentiate into plasma cells to generate diverse serotypes of immunoglobulin. Similarly, SARS-CoV-2 antigens are also presented to B-cells via MHC class II molecules and thereafter plasma cells produce antibodies against various epitopes of SARS-CoV-2 into circulation for neutralisation as well as enhancement of phagocytosis[9,10].

Early detection of COVID-19 leads to effective treatment and emerging control. There are different diagnostic tools with different principles to be used in diagnosis, for instance, patient history, vital signs, thoracic imaging and laboratory results. Likewise, reverse-transcription polymerase chain reaction (RT-PCR), particularly real-time RT-PCR, is an assay to ensure viral infection by detection of SARS-CoV-2 viral RNA, spike protein and nucleocapsid encoded genes with high sensitivity and high specificity. Even though molecular techniques provide reliable

results, high throughput and less time consumption in early detection, but limitation was found. Moreover, molecular assays required labour intensive for good specimen collecting and performing excellent analytical process[11].

Immunological assays are available for monitoring the progressive of disease and evaluation of host immune status. Increasing of immunoglobulin M (IgM) and immunoglobulin G (IgG) could be distinguished early stage or chronic phase of infection. Antibodies and viral proteins as target biomarkers have been detected in blood samples and body fluids by various serological methods for example lateral flow immunoassay, enzyme-linked immunosorbent assay (ELISA), luminescent immunoassay, neutralisation assay as well as biosensor test. The ELISA method has been designed to quantify antibodies, antigen or hormone in sera binding specifically to target analyte and then antigen-antibody complexes are detected throughout their colorimetric or fluorescent signals.

COVID-19 IgM/IgG ELISA could provide some beneficial aspects. It detects specific antibodies which show higher stability in sera than viral RNA. Unlike molecular techniques, patient sera could be collected through standard phlebotomy which could reduce contamination of interferences in samples. Additionally, presence of specific antibodies could be tracked previous infection in some cases. ELISA tests for COVID-19 IgM/IgG are recommended to interpret in parallel with molecular test results by healthcare professional for reliable result[12]. Therefore, this study aimed to develop and optimise the ELISA to detect levels of anti-SARS-CoV-2 IgM and IgG. Additionally, cross-reactivity and method precision were also evaluated possibilities of being the diagnostic tool for COVID-19 infection.

Materials and Methods

Source of specimens

A total of 123 blood samples of COVID-19 patients that confirmed positive with real-time RT-PCR and 200 negative serums that were collected from healthy volunteers under restrict criteria, 100 negative samples were collected before pandemic period and another 100 specimens collected during the outbreak. Similarly, 40 specimens of diverse infectious diseases including Mycoplasma infection, mumps, measles, HFMD, autoimmune disorders, hepatitis as well as HIV were used to analyse cross-reactivity of the COVID-19 ELISA test kit. All specimens were transferred under cold chain system and sera were separated by centrifugation at 1500xg for 10 minutes, inactivated at 56°C for 30 minutes and then stored at -20°C until use.

This study was approved by Ethical Committee of Department of Medical Sciences (0644/EC 190) on June 22, 2020.

Recombinant antigens

Recombinant nucleocapsid protein (NP), expressed in HEK293F mammalian cell line, was provided by Research and Development team of Medical Biotechnology Centre, Department of Medical Sciences.

Statistical analysis

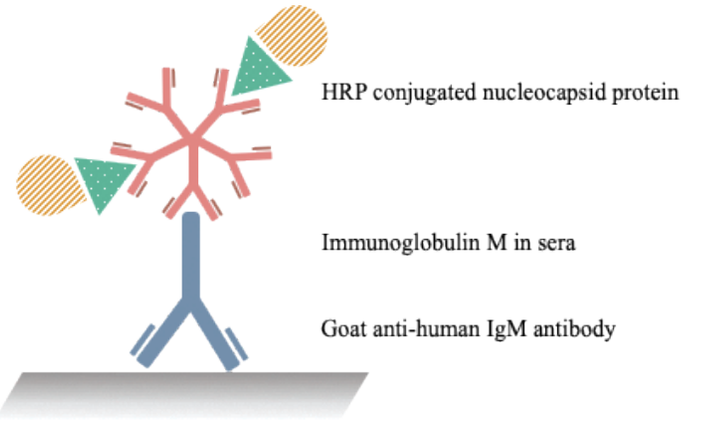
GraphPad prism version 6.0 was used for statistical analysis. The variables are concluded as the median along with Mann-Whitney test. Statistical significance was considered at P value <0.01.

Capture ELISA

Optimisation of COVID-19 IgM ELISA

To immobilise the primary antibody, goat anti-human IgM antibody (MP Biomedicals, Illkirch, France) was diluted in binding solution (0.6mM carbonate bicarbonate buffer pH9.4) into final concentrations of 5 and 10 µg/ml and 100 µl of each was added to each ELISA well (Nunc, Roskilde, Denmark). The coated plate was incubated for 16-18 hours at 4°C and further washed for three times with 400 µl of 1x phosphate buffered saline pH7.4 (PBS)+0.1% Tween. Blocking was performed using 0.5% casein and 3% skim milk (sample diluent). After blocking procedure, 50 µl of each sera (1:100 and 1:200 dilution in sample diluent) was added and then incubated at room temperature for an hour before washing with wash buffer. HRP conjugated NP was prepared to final concentrations of 0.5, 0.75 as well as 1 µg/ml and 100 µl of the conjugated NP was added. The plate was incubated in a dark chamber at room temperature for an hour. After washing, 85 µl of TMB substrate (KPL, Gaithersburg USA) was added, further incubated in dark at room temperature for 30 minutes. The colour reaction was stopped by adding 50 µl of 1 N sulfuric acid. Colour development was read at 450/630 nm by an ELISA plate reader (Sunrise TM Absorbance Reader, TECAN, Switzerland). (Figure 1)

(A)



Factor	Assigned condition	Variable condition
Concentration of capture antibody	- Sample dilution 1:100 - Blocking solution: 3% skim milk - Sample diluent: 3% skim milk - Conjugated NP dilution: 0.75 μg/ml	- 5 μg/ml - 10 μg/ml
Sample dilution ratio	- Coating antibody: 10 μg/ml - Blocking solution: 3% skim milk - Sample diluent: 3% skim milk - Conjugated NP dilution: 0.75 μg/ml - Coating antibody: 10 μg/ml	- 1:100 - 1:200
Blocking solution	- Sample dilution 1:100 - Sample diluent: 3% skim milk - Conjugated NP dilution: 0.75 μg/ml	- 0.5% casein - 3% skim milk
Sample diluent	- Coating antibody: 10 μg/ml - Sample dilution 1:100 - Blocking solution: 3% skim milk - Conjugated NP dilution: 0.75 μg/ml	- 0.5% casein - 3% skim milk
Dilution of conjugated NP	- Coating antibody: 10 μg/ml - Sample dilution 1:100 - Blocking solution: 3% skim milk - Sample diluent: 3% skim milk	- 0.5 μg/ml - 0.75 μg/ml - 1 μg/ml

Figure 1 (A) Scheme of a COVID-19 IgM ELISA that the solid phase is coated by anti-human IgM antibody to capture target analyte in blood sample. Then, conjugated NP acts as detection molecule in specific binding with IgM. (B) COVID-19 IgM ELISA was optimised in various conditions.

Evaluation of COVID-19 IgM ELISA

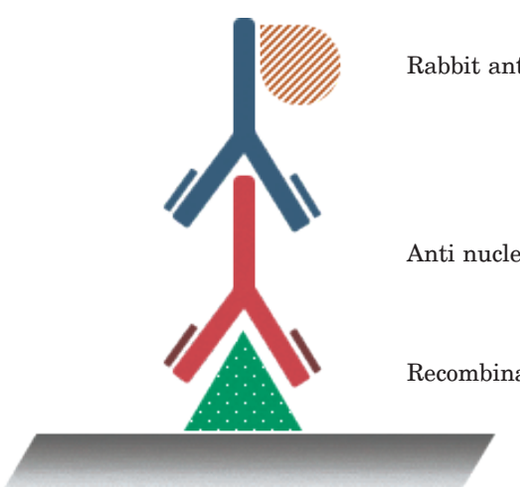
To evaluate COVID-19 IgM ELISA, 100 μl of 10 μg/ml Goat-anti human IgM antibody in binding solution was added into each ELISA well and incubated at 4°C for 16-18 hours before flushing off and washing with 400 μl washing buffer (1X PBS+0.1% Tween-20) for three times. The plate was then blocked with 3% skim milk for at least 1 hour at room temperature. Patients' sera and control samples were diluted at 1:100 in 3% skim milk. After washing, 50 μl of diluted samples was applied into each ELISA well and incubated under room temperature for an hour before washing with wash buffer. Afterwards, HRP conjugated NP (0.75 μg/ml) was prepared and 100 μl of conjugated NP was added into ELISA well and further incubated away from light for an hour at room temperature. After 1 hour, the ELISA plate was washed and 85 μl of TMB substrate

was added, then incubated for 30 minutes in the dark chamber to develop colour of reaction. Subsequently, 50 µl of 1 N sulfuric acid was added into each well to stop the reaction and the absorbance was read at 450/630 nm.

Optimisation of COVID-19 IgG ELISA

Regarding to the conventional procedure, NP antigen was diluted in binding solution to final concentrations of 1 µg/ml, 5 µg/ml and 10 µg/ml and then 100 µl of capture molecule in coating buffer (0.6 mM carbonate bicarbonate buffer pH 9.4) was coated in 96 flat-bottom ELISA plate. Then, coated ELISA plate was incubated at 4 °C for overnight before flushing off and washing with 400 µl washing buffer (1X PBS+0.1% Tween-20) for three times. Blocking was performed with 0.5% casein, 2% BSA, 3% skim milk and 5% skim milk (sample diluent) for 1 hour at room temperature. After blocking procedure, 50 µl of each sera (1:100 and 1:200 dilution in sample diluent) was added and then incubated at room temperature for an hour before washing with wash buffer. HRP conjugated-anti human IgG antibody (Dako, Denmark) was prepared in blocking solution at various dilution including 1:5000, 1:8000, 1:12000 and 1:16000 and 100 µl of the conjugated protein was added. The plate was incubated in the dark chamber at room temperature for an hour. After washing, 85 µl of TMB substrate (KPL, Gaithersburg USA) was added, further incubated in dark at room temperature for 30 minutes. The colour reaction was stopped by adding 50 µl of 1 N sulfuric acid. Colour development was read at 450/630 nm by an ELISA plate reader (Sunrise TM Absorbance Reader, TECAN, Switzerland). (Figure 2)

(A)



(B)

Factor	Assigned condition	Variable condition
Concentration of capture NP	- Sample dilution 1:200 - Blocking solution: 5% skim milk - Sample diluent: 5% skim milk - Conjugated antibody dilution: 1:16000	- 1 μg/ml - 5 μg/ml - 10 μg/ml
Sample dilution ratio	- Capture NP: 1 μg/ml - Blocking solution: 5% skim milk - Sample diluent: 5% skim milk - Conjugated antibody dilution: 1:16000	- 1:100 - 1:200
Blocking solution	- Capture NP :1 μg/ml - Sample dilution 1:200 - Sample diluent: 5% skim milk - Conjugated antibody dilution: 1:16000	- 0.5% casein - 2% BSA - 3% skim milk - 5% skim milk
Sample diluent	- Capture NP: 1 μg/ml - Sample dilution 1:200 - Blocking solution: 5% skim milk - Conjugated antibody dilution: 1:16000	- 0.5% casein - 2% BSA - 3% skim milk - 5% skim milk
Dilution of conjugated antibody	- Capture NP :1 μg/ml - Sample dilution 1:200 - Blocking solution: 5% skim milk - Sample diluent: 5% skim milk	- 1:5000 - 1:8000 - 1:12000 - 1:16000

Figure 2 (A) Scheme of a COVID-19 IgG ELISA that the solid phase is coated by recombinant NP to capture target analyte in blood sample. Then, HRP conjugated anti-human IgG antibody act as detection molecule in specific binding with IgG. (B) COVID-19 IgG ELISA was optimised in various conditions.

Evaluation of COVID-19 IgG ELISA

To evaluate COVID-19 IgG ELISA, 100 μl of 1 μg/ml recombinant NP in binding solution was added in each ELISA well and incubated at 4°C for 16-18 hours before flushing off and washing with 400 μl washing buffer (1X PBS+0.1% Tween-20) for three times. The plate was blocked by 5% skim milk for at least 1 hour at room temperature when patients' sera and control sample were diluted at 1:200 in 5% skim milk. After washing, 50 μl of diluted samples was applied into each ELISA well and incubated under room temperature for an hour before washing with wash buffer. Afterwards, polyclonal rabbit anti-human IgG antibody-HRP was diluted at 1:200 in sample diluent and 100 μl of conjugated antibody was added and further incubated away from

light for an hour at room temperature. After 1 hour, the ELISA plate was washed and 85 µl of TMB substrate was added, then incubated for 30 minutes in the dark chamber to develop colour of reaction. Subsequently, 50 µl of 1 N sulfuric acid was added into each well to stop the reaction and the absorbance was read at 450/630 nm.

Results

COVID-19 IgM ELISA

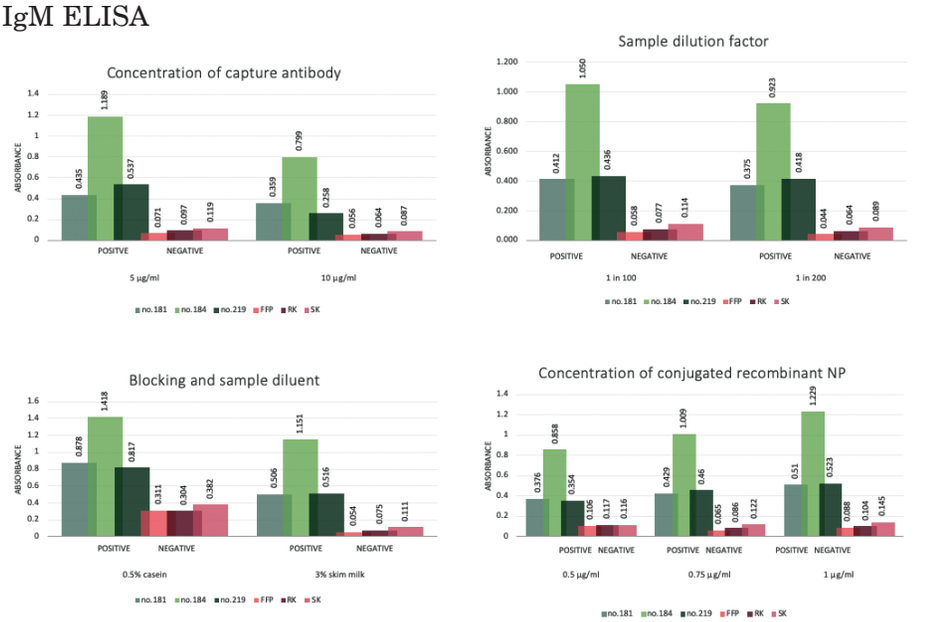
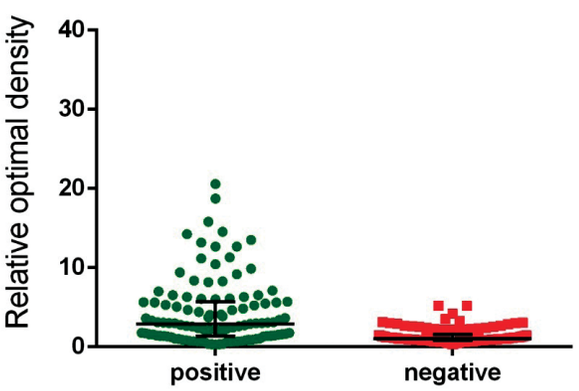


Figure 3 Optimisation of COVID-19 IgM ELISA conditions

Considering optimal conditions of COVID-19 IgM ELISA was based on (1) minimisation the absorbance value of negative sample (2) maximisation of absorbance in positive group to clearly distinguish positive sample from negative group and (3) lower concentration of protein was selected in case of indifferent outcome[13]. Therefore, the optimisation of COVID-19 IgM ELISA was found to be 5 µg/ml of goat anti-human IgM polyclonal antibody, 3% skim milk, 1:100 sample dilution and 0.75 µg/ml of HRP conjugated recombinant NP. (Figure 3)



Study group	Mean ± SD	Median (IQR)	*P* value
RT-PCR positive cases (n=123)	4.283 ± 4.209	2.910 (1.379-5.725)	<0.01
Healthy volunteers (n=200)	1.251 ± 0.650	1.025 (0.800-1.568)	<0.01

Figure 4 Relative optimal density plot between positive and healthy population as well as statistical analysis from data set of COVID-19 IgM ELISA.

The figure 4 demonstrated the comparison of relative optical density between two groups which were positive and healthy that one plot represents one ELISA unit. Each individual OD_{sample} is normalised by baseline unit which is derived from OD value of pooled negative plasma. Then, the outcomes show as the relative OD or ELISA unit to consider the method cut-off. Extended scatter in positive group have been shown when compared with healthy population. Likewise, statistic mean in both groups provides clearty distinguish including 4.297 in positive and 1.251 in negative groups whereas the median (IQR) examines more reliable parameter when *P* value less than 0.0001

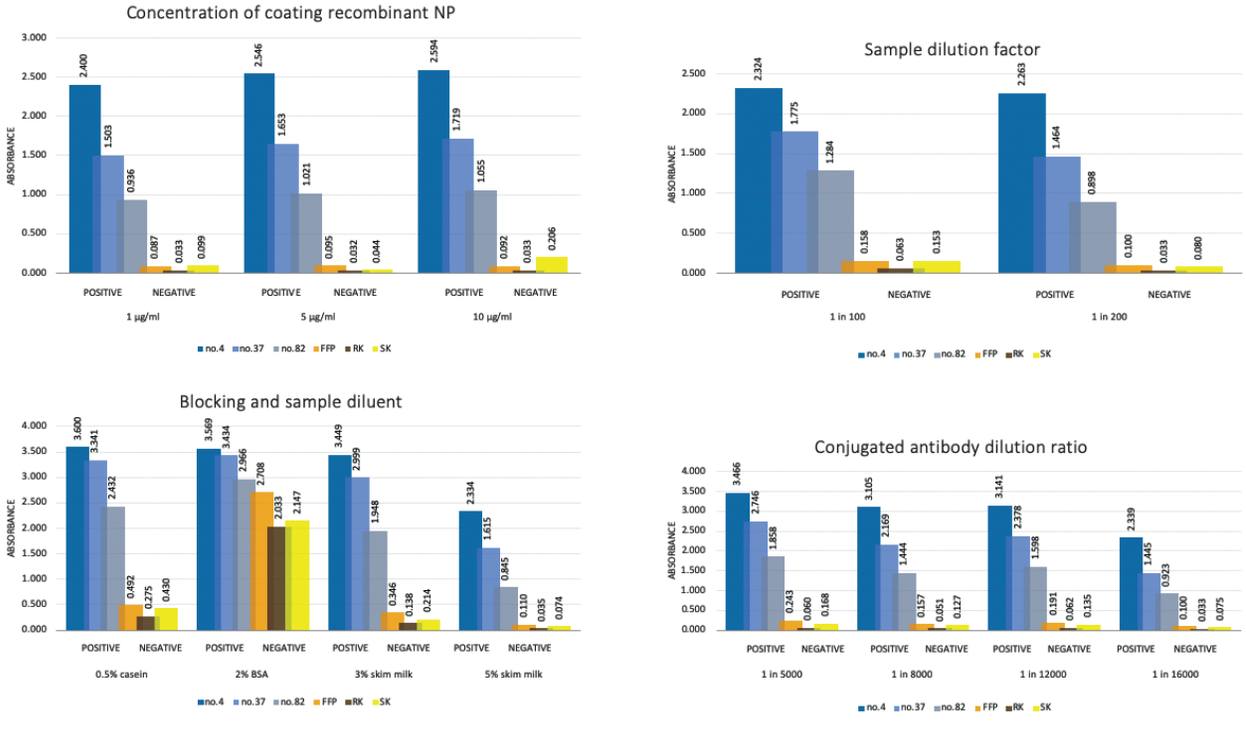
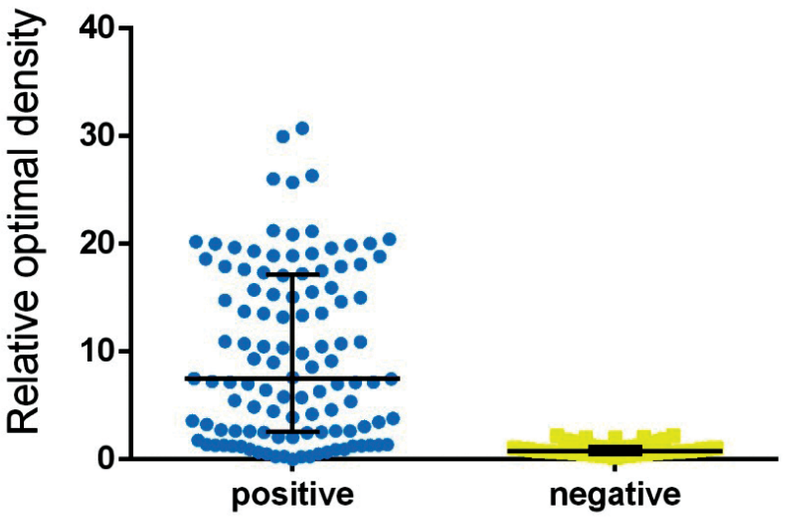


Figure 5 Optimisation of COVID-19 IgG ELISA conditions

Considering the condition of COVID-19 IgG ELISA was comparable to COVID-19 IgM ELISA based on minimisation of $OD_{negative}$ along with maximisation of $OD_{positive}$ to categorise two distinct group. Additionally, the concentration of protein was another influential factor to be concerned. With regards to our results (Figure5), optimal conditions of COVID-19 IgG ELISA were 1 µg/ml recombinant NP, 5% skim milk, 1:200 sample dilution and 1:16000 of HRP conjugated rabbit anti-human IgG.



Study group	Mean ± SD	Median (IQR)	*P* value
RT-PCR positive cases (n=123)	9.757 ± 7.889	7.507 (2.583-17.20)	<0.01
Healthy volunteers (n=200)	0.858 ± 0.431	0.740 (0.527-1.161)	<0.01

Figure 6 Relative optimal density plot between positive and healthy population as well as statistical analysis from data set of COVID-19 IgG ELISA.

The sets of raw data were normalised into relative OD or ELISA unit before statistical analysis and graph plotting. Both mean values were examined and found clearly distinguished between positive group (9.757) and healthy individuals (0.858). Moreover, median value in positive population was approximately higher than that in the negative group by 10 times. P value between two groups was less than 0.01. (Figure 6)

Determining the cut-off value in ELISA

According to Table 1, specificity of IgM ELISA at the threshold "mean+3 standard deviation of healthy" demonstrated the high value at 99.0 whereas their sensitivity was 41.46%. Besides, the sensitivity of the IgM ELISA was increased to 51.28% or approximately 10% when considered about the onset period at >14 days. Whilst the COVID-19 IgG ELISA showed 100% assay specificity at meanneg +3SD threshold despite the fact that assay sensitivity was 60.98%. However, the sensitivity rose roughly 30% to 89.74% in IgG ELISA by considering patients' onset. Thereby, meanneg +3SD was a capable point to set the cut-off value when standards were not available. Thus, the cut-off for COVID-19 IgM set at 3.2 while COVID-19 IgG established the cut-off at 2.2.

Table 1 The cut-off value of ELISA

COVID-19 ELISA	Cut-off	Sensitivity (95% CI) N=123	Sensitivity (>14 day onset) N=39	Specificity (95% CI) N=200
COVID-19 IgM ELISA	3.2	41.46 (32.48-59.84)	51.28 (29.37-73.19)	99.00 (97.62-100.00)
COVID-19 IgM ELISA	2.2	60.98 (49.94-72.01)	89.74 (79.69-99.79)	100.00 (100.00-100.00)

Cross reactivity

Comparable three-dimensional structure in variant antigens possibly lead to cross reactivity particularly in binding of polyclonal immune response. From previous studies, T-cell receptor (TCR) cross-reactivity carries around 10^5 diverse peptides[14]. Therefore, serological diagnostic tool required cross-reactivity assessment. This study performed cross-reactivity with 40 positive cases of 7 infectious and inflammatory diseases including mycoplasma pneumoniae, mumps, measles, HFMD, autoimmune disorders, HCV as well as HIV. From the results, there are positive for mumps, autoimmune disorders in COVID-19 IgM ELISA. On the contrary, none of cross-reactivity was shown in COVID-19 IgG ELISA.

Table 2 Cross-reactivity results of both ELISA test systems.

Diseases	COVID-19 IgM ELISA	COVID-19 IgG ELISA
Mycoplasma pneumoniae (n=2)	-	-
Mumps (n=9)	1/9	-
Measles (n=9)	-	-
HFMD (n=5)	-	-
Autoimmune diseases (n=5)	2/5	-
Hepatitis C (n=5)	-	-
HIV (n=5)	-	-

Precision

To measure random error of the assay, ELISA tests for both IgM and IgG detection were completed within-run and between-run performance when within-run precision contributes the estimation of daily performance. Likewise, between-run provides the estimation between-day performance which carries more factors of operating error. Typically, less than 10 of %CV indicates excellent performance of the method and over 30% of coefficient of variation point out non-precise procedure[15,16]. Hence this in-house ELISA tests are acceptable to use for analysis since their %CV of selected samples are in the range from 0.3 to 23 (Table 3 & 4)

Table 3 Standard deviation (SD) and coefficient of variation (CV) in COVID-19 IgM ELISA in intra-lab study

precision	Strong positive				Adequate positive				Weak positive				Negative			
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Repeatability	0.21	0.6	0.10	0.3	1.07	6.7	0.33	2.5	0.26	2.7	0.20	2.3	0.03	3.9	0.01	1.0
Within day	6.11	16.9	5.86	15.7	2.21	13.9	1.91	14.1	2.19	18.0	0.70	7.2	0.05	9.7	0.05	8.3
Between day	3.35	8.5	3.17	7.7	1.06	6.2	1.29	8.9	1.86	16.1	1.48	16.8	0.09	12.9	0.05	8.0

Table 4 Standard deviation (SD) and coefficient of variation (CV) in COVID-19 IgG ELISA in intra-lab study

precision	Strong positive				Adequate positive				Weak positive				Negative			
	Sample 1		Sample 2		Sample 3		Sample 4		Sample 5		Sample 6		Sample 7		Sample 8	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Repeatability	0.64	3.1	0.61	3.1	1.03	5.2	1.00	5.7	0.47	4.8	0.13	1.7	0.05	3.1	0.04	4.0
Within day	0.50	2.4	0.48	2.3	0.89	4.4	0.52	3.0	0.45	3.7	0.16	1.5	0.05	2.3	0.04	3.7
Between day	2.77	12.3	2.57	11.6	3.10	14.2	2.73	14.1	1.76	14.7	1.39	15.3	0.09	4.8	0.05	4.2

Discussion

To develop COVID-19 ELISA, four main factors were examined which were capture-molecule concentration, blocking solution, sample dilution and concentration of detection molecule. In COVID-19 IgM ELISA, 5 μg/ml anti-human IgM was coated in ELISA well and blocked with 3% skim milk when patients' sera were diluted by 1:100 ratio and then 0.75 μg/ml of HRP-conjugated recombinant NP was added to bind with antigen-antibody complex before measuring the colorimetric reaction. Whilst, immunoglobulin IgG against SARS-CoV2 in 1:200 diluted sample would be measured in 1 μg/ml recombinant NP coated plate that was blocked in 5% skim milk before binding to 1:16000 prepared anti-human IgG HRP. Both ELISA tests contributed colorimetric reaction and were measured at 450/630 to obtain absorbance values.

Without primary standard method to compare, raw data have to normalise by absorbance value of pooled negative sample (OD pooled negative) resulting in one ELISA unit or relative OD for further analysis. In this point, meanpositive −3SD and meannegative +3SD are considered to be use as formula for cut-off calculation[17]. Using meanneg +3SD in our study as the cut-off value could provide higher specificity, i.e., 99% specificity in COVID-19 IgM ELISA and 100% specificity in COVID-19 IgG test and decreased possibilities of false positive results. The assays developed were suitable to use in low prevalence countries such as Thailand[18] which high specificity test is required to ensure infection.

As for cross-reactivity, there were positive for mumps, autoimmune disorders in COVID-19 IgM ELISA, while, none of cross-reactivity was shown in COVID-19 IgG ELISA. Thus, false positive results from other infectious diseases might occur for the COVID-19 IgM ELISA. Moreover, within-run and between-run performances revealed the value of %CV which were in the range from 0.3 to 23 indicating adequate precision of the method. From our results, both systems for antibody detection should be further developed to ensure high precision of analytical results with less than 10% of CV.

Conclusion

COVID-19 IgM ELISA was able to be used for detection of immunoglobulin M against SARS-CoV-2 with 41.46% sensitivity and 99% specificity. Additionally, COVID-19 IgG ELISA could use to detect immunoglobulin G against SARS-CoV-2 in blood sample with 60.98% sensitivity and 100% specificity. The possibility of cross-reactivity with mumps and auto-immune diseases were demonstrated with COVID-19 IgM ELISA.

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การพัฒนาชุดตรวจหลักการอีไลซาสำหรับตรวจทางห้องปฏิบัติการทางน้ำเหลืองวิทยาโรคโควิด-19

ลภัสรดา มุ่งมงคล[1] พรทิพย์ ไชยยะ[1] ภาณุพันธ์ ปัญญาใจ[1] สกุลรัตน์ สุนทรฉัตราวัฒน์[1]
สุมาลี ชะนะมา[2] อาชวินทร์ โรจนวิวัฒน์[1] และปนัดดา เทพอัคศร[1]
[1]สถาบันชีววิทยาศาสตร์ทางการแพทย์ [2]สถาบันวิจัยวิทยาศาสตร์สาธารณสุข กรมวิทยาศาสตร์การแพทย์ ถนนติวานนท์ นนทบุรี 11000

บทคัดย่อ การแพร่ระบาดใหญ่ของโรคโควิด-19 จากเชื้อ SARS-CoV-2 ก่อให้เกิดพยาธิสภาพในระบบทางเดินหายใจที่หลากหลายโดยเฉพาะก่อให้เกิดโรคปอดบวมรุนแรงทั่วโลก การศึกษานี้ได้พัฒนาวิธีวิเคราะห์ด้วยหลักการอีไลซาเพื่อตรวจวัดแอนติบอดีต่อเชื้อไวรัสโคโรนาสายพันธุ์ใหม่ 2019 ในตัวอย่างเลือดของผู้ติดเชื้อและศึกษาปัจจัยที่มีผลต่อการวิเคราะห์ ได้แก่ ความเข้มข้นของโมเลกุลที่ถูกตรึงไว้จับแอนติบอดีเป้าหมาย, อัตราส่วนการเจือจางตัวอย่าง, สารละลายบล็อกกิ้ง และความเข้มข้นของโมเลกุลที่ติดฉลากด้วยเอนไซม์ ผลการวิเคราะห์พบว่าอีไลซาสำหรับไอจีเอ็มที่ค่าจุดตัดที่ 3.2 หน่วยอีไลซา ซึ่งให้ค่าความไวที่ร้อยละ 41.46 และความจำเพาะร้อยละ 99 โดยในส่วนของอีไลซาเพื่อตรวจไอจีจีที่ค่าจุดตัด 2.2 หน่วยอีไลซาให้ค่าความไวที่ร้อยละ 60.98 และความจำเพาะร้อยละ 100 นอกจากนี้พบว่าค่าความไวของอีไลซาสำหรับไอจีเอ็มและไอจีจีเพิ่มขึ้นเป็นร้อยละ 51.28 และ 89.74 ตามลำดับ ขึ้นกับระยะเวลาของการติดเชื้อ การศึกษานี้แสดงว่าชุดตรวจที่พัฒนาขึ้นสามารถนำมาใช้ตรวจหาแอนติบอดีต่อเชื้อ SARS-CoV-2 ได้

คำสำคัญ: โควิด-19, อีไลซา, SARS-CoV-2, อิมมูโนโกลบูลิน เอ็ม, อิมมูโนโกลบูลิน จี