Original Article



Reactivity of human antisera to codon optimized SARS-CoV2 viral proteins expressed in *Escherichia coli*

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INTRODUCTION

The current coronavirus disease 2019 (COVID-19) pandemic caused by the novel SARS-CoV2 coronavirus was first detected in China during late 2019 and has rapidly spread around the world since then. The Tzu Chi Foundation has around 100 service branches worldwide with more than 10 million volunteers, and these volunteers are exposed to high risk as they work to provide relief to those in need during the current pandemic. To provide safe working environments, accurate and rapid testing kits to identify uninfected, infected, and recovered populations are necessary, and here we describe the establishment of an *Escherichia coli*-based viral protein expression and purification platform that can produce codon-optimized SARS-CoV2 viral proteins for use in rapid antibody/antigen diagnostic kits.

The genome size of coronaviruses ranges between 26 and 32 kb, with a variable number of open reading

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ABSTRACT

Objective: The coronavirus disease 2019 (COVID-19) pandemic caused by the SARS-CoV2 virus continues to pose a serious threat to public health worldwide. The development of rapid diagnostic kits can assist the Tzu Chi Foundation in supporting global volunteers working to provide relief during the current pandemic. Materials and Methods: In this study, nucleotide sequences derived from publicly available viral genome data for several domains of the SARS-CoV2 spike and nucleocapsid (N) proteins were chemically synthesized, with codon optimization for Escherichia coli protein expression. No actual viral particles were involved in these experiments. The synthesized sequences were cloned into an E. coli expression system based on pQE80L, and expressed viral proteins were subsequently purified using Ni-affinity chromatography. Western blotting was conducted using human antiviral sera to assess the response of codon-modified viral proteins to COVID-19 patient sera. Results: N protein was expressed in amounts large enough to support large-scale production. The N-terminal domain, receptor-binding domain (RBD), Region 3, and the S2 domain were expressed in small but sufficient amounts for experiments. Immunoblotting results showed that anti-N IgG and anti-N IgM antibodies were detected in most patient sera, but only 60% of samples reacted with the recombinant RBD and S2 domain expressed by E. coli. Conclusion: The results indicated that codon-optimized SARS-CoV2 viral proteins can be expressed in E. coli and purified for rapid antibody detection kit preparation, with the codon-optimized N protein, RBD, and S2 protein demonstrating the most potential.

Keywords: Codon optimization, COVID-19, Escherichia coli expression system, N protein, SARS-CoV2 antisera

frames (ORFs) [1]. The SARS-CoV2 genome is about 30 kb in size, and has 14 ORFs that encode a total of 29 known proteins [2]. The first ORF, which covers 67% of the entire genome, encodes 16 non-structural proteins, while the other encode structural and accessory proteins [2]. The 3' end of the viral genome encodes four structural proteins, the spike (S), envelope (E), membrane (M), and nucleocapsid (N), as well as nine putative accessory proteins [2]. The S protein plays an important role in angiotensin-converting enzyme 2 (ACE2) receptor binding [3] and includes an N-terminal domain (NTD) of 271 amino acids (aa), receptor-binding domain (RBD) of 273 aa, and S2 domain of 600 aa. The RBD domain has a

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key functional role in ACE2 binding and is also important for neutralizing host antibody production as well. The N protein, which binds the RNA of the viral genome, is the most abundantly produced viral protein and is highly immunogenic during viral infection [2].

E. coli expression systems are distinguished for their ease of use and convenience, but have a key limitation in that posttranslational modification is absent, and this can impact the expression of proteins that require posttranslational modification. Certainly, protein expression systems that can perform posttranslational modification have been established in different eukaryotic cells, including *Saccharomyces cerevisiae* [4,5]. Chinese hamster ovary cells [6], and baculovirus expression systems [7]. These systems have an advantage in that protein phosphorylation and glycosylation are achievable, but *E. coli* expression systems are superior in terms of the ability to rapidly produce large amounts of viral proteins that can serve as antigens for diagnostic tests.

Comparative analysis of 13 different coronaviruses regarding preferred nucleotides, codon bias, nucleotide changes at the third position (NT3s), synonymous codon usage, and relative synonymous codon usage has revealed that 18 amino acids have preferred codons, of which eight of these were over-biased [8]. Since different organisms have their own bias regarding usage of the 61 available codons, when E. coli systems are forced to express proteins with "rare" codons under heterologous gene expression conditions, this can result in low expression levels or truncated protein products due to premature termination of protein translation. This issue of codon bias can be overcome through two possible approaches: the first involves cotransformation with a plasmid that encodes the transfer RNAs corresponding to the "rare" codons, while the second requires chemical synthesis of genetic sequences in which the majority of "rare" codons have been replaced with E. coli preferred codons [9].

SARS-CoV2 viral sequences have been published by research teams from several countries [10-12], and as of July 18, 2020, research teams from Taiwan alone have published 32 SARS-CoV2 genome sequences. In this study, we referenced the N protein and S protein coding sequences from this publicly available viral genomic data and proceeded to modify the codons based on E. coli preference. The original coding sequence for the SARS-CoV2 N protein and the codon-modified coding sequence of the S protein were chemically synthesized by Genomics (New Taipei City, Taiwan) and then cloned into an E. coli expression system. E. coli with modified N protein coding sequences were obtained from pET15b-NE cloned by the Academia Sinica. The viral protein production capacity of cloned E. coli expression systems was subsequently tested, and the reactivity of these purified viral proteins with patient sera was also assessed. The results indicated that E. coli expression systems were capable of expressing sufficient levels of viral proteins that could be used to develop rapid and accurate diagnostic tests.

MATERIALS AND METHODS

Bacterial strains and plasmids

The bacterial strains and plasmids used in this study are described in [Table 1].

Primers

The primers used in this study are listed in [Table 2].

Polymerase chain reaction and expression clone construction

DH5 α /pUC57-SE [Table 1] served as the template for the amplification of codon-modified S protein domain DNA fragments by colony polymerase chain reaction (PCR). DH5 α /pET15b-NE [Table 1] served as the template for amplification of codon-modified N protein coding sequences. The PCR products of different coding sequences were generated with equal amounts of *Pfu* and *Taq* DNA polymerase to reduce mutation rates following amplification. The PCR products of each DNA fragment were cloned into the respective restriction enzyme sites in pQE80L (Qiagen, Hilden, Germany) to generate the expression plasmids, pNE, pSE, pSE-NTD, pSE-RBD, pSE-R3, and pSE-S2 [Table 2]. After transformation, potential clones were evaluated with a previously described rapid screening method [13], followed by sequencing analysis (Genomics).

Recombinant protein expression and purification

To express viral proteins, transformants of each respective recombinant plasmid were expanded at 37°C by inoculating a 0.5-mL overnight culture into 50 mL of LB medium containing ampicillin. Incubation was continued at 37°C until the culture reached an OD₆₀₀ of 0.6. Protein expression was induced by adding isopropyl β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM or 1 mM. After incubating for 3 h at 37°C, cells were harvested by centrifugation at 4,000 × g for 15 min. The cells were then stored at – 80°C until use.

Protein purification was performed by a method described previously [14]. Frozen cells overexpressing target proteins were suspended in 10 mL of 1× binding buffer containing 5 mM imidazole, 0.5 mM NaCl, and 20 mM Tris-HCl (pH 8.0), and subjected to 3 cycles of freezing and thawing at -80°C and room temperature, respectively. The thawed cells were disrupted by sonication at 4°C using a Microson ultrasonic cell disruptor (Misonix, Farmingdale NY, USA). Cell extracts were separated from the cell debris by centrifugation at 17,000× g for 30 min at 4°C (Avanti J-25 Centrifuge, JA25.5 rotor; Beckman Coulter, Brea CA, USA). Recombinant proteins were then purified from the cell extract using Ni-affinity chromatography (Novagen, Madison WI, USA). Purified fractions were analyzed with different concentrations of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), based on the different molecular weights of each recombinant protein, and stained with Coomassie Brilliant Blue G-250.

Immunoblotting

Total proteins from bacteria cultured under different conditions were collected and separated by SDS-PAGE,

Table 1: Bacteria strains and plasmids used in this study

	Description	References or source
Bacteria		
DH5a	F ⁻ , supE44, hsdR17, recA1, gyrA96, endA1, thi-1, relA1, deoR, λ^{-}	ATCC53868
DH5a/pUC57-SE	Apr, DH5α containing pUC57-SE	This study
DH5a/pUC57-NO	Ap ^r , DH5α containing pUC57-NO	This study
DH5a/pET15b-NE	Apr, DH5α containing pET15b-NE	This study
DH5a/pQE80L	Apr , DH5α containing pQE80L	Qiagen
DH5a/pSE	Ap ^r , DH5α containing pSE	This study
DH5a/pSE-NTD	Apr , DH5α containing pSE-NTD	This study
DH5a/pSE-RBD	Apr , DH5α containing pSE-RBD	This study
DH5a/pSE-S2	Apr , DH5α containing pSE-S2	This study
DH5a/pSE-R3	Apr , DH5α containing pSE-R3	This study
DH5a/pNE	Ap ^r , DH5α containing pNE (E. coli modified)	This study
Plasmids		
pUC57-SE	Apr , pUC57 containing E. coli modified spike protein fragment	This study
pUC57-NO	Apr, pUC57 containing original nucleocapsid protein fragment	This study
pET15b-NE	Apr, pET15b containing E. coli modified nucleocapsid protein fragment	This study
pQE80L	Apr, expression vector, ColE1-ori, lacI ^q , His ₆ -N	Qiagen
pSE	Apr, pQE80L containing spike protein fragment	This study
pSE-NTD	Apr, pQE80L containing spike protein NTD domain fragment	This study
pSE-RBD	Apr, pQE80L containing spike protein RBD domain fragment	This study
pSE-S2	Apr, pQE80L containing spike protein S2 domain fragment	This study
pSE-R3	Apr, pQE80L containing spike protein, RBD domain region 3 fragment	This study
pNE	Apr, pQE80L containing E. coli modified nucleocapsid protein fragment	This study

E. coli: Escherichia coli, RBD: Receptor-binding domain, NTD: N-terminal domain

Table 2: Primers used in this study					
Primer	Sequence (5'-3')	Application			
pQE-F	GGC GTA TCA CGA GGC CCT TTC G	Sequencing			
pQE-R	CAT TAC TGG ATC TAT CAA CAG G	Sequencing			
pQE80L-Spike-F	AAG AGC TCA TGT TCG TTT TCC TGG TT	Spike amplification			
pQE80L-Spike-R	TTG GTA CCT TAG GTG TAA TGC AGC TT	Spike amplification			
pQE80L-NTD-F	TTG GAT CCG CGT ACA CCA ACA GCT TCA C	Spike protein NTD domain amplification			
pQE80L-NTD-R	TTA AGC TTC TTG GTT TCG CTC AGC GGG T	Spike protein NTD domain amplification			
pQE80L-RBD-F	ACG GAT CCC CGA ACA TCA CCA ACC TGT G	Spike protein RBD domain amplification			
pQE80L-RBD-R	TTA AGC TTG GTG CCC GGC GTG ATC AC	Spike protein RBD domain amplification			
pQE80L-S2-F	TTG CAT GCT GCG CGA GCT ACC AGA CCC A	Spike protein S2 domain amplification			
pQE80L-S2-R	AAG TCG ACC AGC TTC ACG CCT TTC AGA A	Spike protein S2 domain amplification			
pQE80L-R3-F	TGG GAT CCT GCT TTA CCA ACG TTT ACG C	RBD domain region 3 amplification			
pQE80L-R3-R	AAA AGC TTC GGG CCG CAC ACG GTC GCC G	RBD domain region 3 amplification			
pQE80L-NO-F	AAG GAT CCA TGT CTG ATA ATG GAC CC	Original nucleocapsid protein amplification			
pQE80L-NO-R	AAG TCG ACT TAG GCC TGA GTT GAG TC	Original nucleocapsid protein amplification			
pQE80L-NE-F	AAG GAT CCA TGA GCG ACA ACG GTC CG	E. coli modified nucleocapsid protein amplification			
pQE80L-NE-R	CCA AGC TTT TAC GCT TGG GTG CTA TCC G	E. coli modified nucleocapsid protein and E. coli modified nucleocapsid			
		protein with N-terminal deletion amplification			
pQE80L-NEd-F	AAG GAT CCC CGA ACA ACA CCG CGA GC	<i>E. coli</i> modified nucleocapsid protein fragment with N-terminal deletion amplification			

E. coli: Escherichia coli, RBD: Receptor-binding domain, NTD: N-terminal domain

and then transferred to a polyvinylidene difluoride (PVDF) membrane, after which specific antibodies were applied to detect protein expression. Human anti-SARS-CoV2 polyclonal antisera was obtained from Access Biologicals, USA (Mitek, COVID-19 Panel, 2X Panel 1.1, PW: 56435). Human subjects including any gender with age ranged from 18 to 90 years old. The confirmed infection with COVID-19 determined by a positive Roche swab results. Sample collected <10 days and more than 30 days after positive

results (https://accessbiologicals.com/news/covid-19/). Goat anti-human IgG Fc (HRP) ab97225 and goat anti-human IgM mu chain (HRP) ab97205 were obtained from Abcam (Cambridge, UK). Anti-His monoclonal antibody was obtained from Santa Cruz Biotechnology (Dallas TX, USA). After blocking the transferred membrane with 5% skim milk in 1×TTBS (0.25% Tween-20, 0.05 M Tris-HCl [pH 7.6], 0.9% NaCl), antiserum at 1:1000 dilution in 1×TTBS was added, followed by incubation at room temperature for 1

h. The membrane was washed six times for 10 min with ×TTBS. Goat anti-human IgG Fc (HRP) ab97225 and Goat anti-human IgM mu chain (HRP) ab97205 was added at 1:10,000 dilution in 1×TTBS, followed by incubation at room temperature for 1 h. The PVDF membrane was then washed five times with 1×TTBS, and then rinsed with 1×TBS (0.05 M Tris-HC1 [pH 7.6], 0.9% NaCl) before enhanced chemiluminescence (ECL) reagent (Pierce ECL Western Blotting Substrate, Thermo Fisher Scientific, Waltham MA, USA) was applied. Autography was obtained by UVP BioSpectrum 810 (Thermo Fisher Scientific).

RESULTS

In silico analysis of SARS-CoV2 genetic organization

The SARS-CoV2 genome contains about 29,000–30,000 bases, with four primary structural proteins [SEM, and N; Figure 1a]. These structural proteins are likely to be the main targets of immune responses, and we further broke down larger structural proteins into functional domains to better assess immunogenicity. Based on *in silico* functional domain analysis and a review of the literature, the S protein coding sequence (3,822 bases) was subdivided into the NTD, RBD, and S2 domain [Figure 1b]. Previous research has also shown that Region 3 (R3) of the RBD is functionally important for ACE2 receptor binding [15], and thus the R3 domain (comprising 135 amino acids) was also examined in this study.

Codon optimization

Previous studies have shown that viral sequences may not be properly expressed in *E. coli* due to codon usage differences with eukaryotic cells [9]. To resolve this issue, we designed sequences for chemical synthesis that replaced the original codons with *E. coli* preferred codons that still code for the same amino acid. *In silico* analysis of the codon-optimized NTD coding sequence showed that it shared 72% identity with the original viral sequence [Figure S1a], while the codon-optimized RBD, S2 domain, and N protein coding sequences respectively shared 71.1% [Figure S1b], 72.9% [Figure S1c], and 72.5% [Figure S1d] identity with the original viral sequences. The final translated amino acid sequences of all codon-optimized coding sequences shared 100% identity with the original viral amino acid sequences [Figure S1e and f].

Primer design and cloning

In this study, the Qiagen pQE80L expression system was used to express codon-optimized viral proteins, which were then purified with Ni-affinity chromatography. For the amplification of coding sequences for the S protein and related domains, pUC57-SE was used as a template for colony PCR; while for the amplification of N protein coding sequences, pET15b-NE was used as the template. Primers were designed according to the restriction enzyme sites for cloning the codon-optimized coding sequences of viral proteins into pQE80L [Table 2], and PCR was performed with Pfu and Taq DNA polymerases to ensure that the correct DNA sequences were amplified. PCR conditions were set at the respective melting temperatures for the pair of primers corresponding to each cloned coding sequence.

The sizes of the DNA coding sequences for the NTD, RBD, R3 domain, S2 domain, and N protein are respectively 813 base pairs (bp), 819 bp, 405 bp, 1,800 bp and 1,260 bp [Figure 2]. After restriction enzyme digestion and ligation with pQE80L, the resulting plasmids with insert were checked by electrophoresis following quick screening buffer treatment and colony PCR. Results showed that one transformant (lane 11) of the NTD coding sequence had the correct insertion size [Figure 3a], and this was subsequently confirmed by sequencing analysis. Three transformants carrying the correct insert size for the RBD coding sequence were identified [Figure 3b], and all were confirmed by sequencing analysis. Seven transformants carrying the correct insert size for the S2 domain coding sequence were identified [Figure 3c], of which three clones (lane 2, 5, and 7) were confirmed by sequencing analysis. Four transformants of the R3 domain coding sequence were identified via colony PCR [Figure 3e], of which three (lane 4, 6 and 11) were confirmed by sequencing analysis. For transformants of the original viral coding sequence of the N protein, only three transformants were identified out of more than 700 transformants



Figure 1: Schematic representation of the genome organization of SARS-CoV2 and functional domains of the S protein. (a) The single-strand RNA genome of SARS-CoV2 is mostly taken up by open reading frame 1 a and open reading frame 1b, and the structural protein open reading frames that encode the spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins are clustered near the 3' end. The structural proteins are common to all coronaviruses. (b) The S protein primarily consists of the S1 and S2 subunit (aa 671–1270). The S1 subunit can be further divided into an N-terminal domain (aa 27–297) and receptor binding domain (aa 310–583), and the latter includes the Region 3 domain (aa 391-525)

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Figure 2: Agarose gel electrophoresis of the PCR products of cloned sequences. (a) N-terminal domain; (b) receptor binding domain; (c) S2 domain; (d) N protein; (e) R3 domain. (a-d) were analyzed on a 0.8% agarose gel, while (e) was analyzed on a 2% agarose gel. The size of PCR products of N-terminal domain, RBD, S2, N gene and R3 is 813 bp, 819 bp, 1,800 bp, 1,266 bp and 405 bp, respectively



Figure 3: Agarose gel electrophoresis of plasmid quick screening results and colony PCR results of R3 clones. (a) N-terminal domain, lane 11(clone 10); (b) receptor binding domain, lane 3 (clone 17), lane 7 (clone 21), and lane 11(clone 25); (c) S2 domain, lane 2 (clone 27), lane 5 (clone 30), lane 7 (clone 32), lane 10 (clone 35), lane 12 (clone 37), lane 13 (clone 38), and lane 16 (clone 41); (d) N protein; lane 3 (clone 47), lane 5 (clone 49), lane 11 (clone 55), lane 13 (clone 57), lane 14 (clone 58), and lane 15 (clone 59). (a-d) The recombinant plasmid contained possible inserted PCR products with lower mobility than the rest of lanes with empty pQE80 L plasmid. (e) R3 domain, PCR product analyzed on 2% agarose gel. Lane 4(clone 129), 6 (clone 164), 8 (clone 178), 10 (clone 187) contained PCR products with the same size indicated they are correct recombinant clones for R3 region. Lane 14 is PCR positive control

screened, and the insert sequences still differed from the original sequences, perhaps due to codon bias in *E. coli*. One transformant (lane 13) was obtained and confirmed by sequencing analysis for the codon-optimized N protein coding sequence [Figure 3d].

Protein expression and purification

Confirmed clones were induced by 1 mM IPTG for 3 h, and at least 100 mL of bacterial culture was collected for protein purification by Ni-affinity chromatography. Subsequent 12%

150

SDS-PAGE results showed that the NTD, with size of about 30.4 kiloDaltons (kDa), was found in the induction fraction [Figure 4a, lane 2] and purified in lower amounts [Figure 4a, lane 3]. RBD (with size of about 30.6 kDa) was also found in the induction fraction [Figure 4a, lane 4, 5] and was successfully purified [Figure 4a, lane 6]. R3 (with size of about 15.4 kDa) expression could be induced after treatment with 1 mM IPTG for 3 h [Figure 4a, lane 8] and was also successfully purified [Figure 4a, lane 9]. Expression of the largest domain of the S protein, S2, was induced after IPTG treatment [Figure 4b, lane 2], and was successfully purified [Figure 4b, lane 3] even though the domain contains at least two transmembrane sections. Interestingly, despite the many difficulties encountered in cloning and deriving transformants with the N protein coding sequence, transformants with the codon-optimized N protein coding sequence demonstrated abundant expression after IPTG induction, with N protein representing about 30% of total protein expressed [Figure 4c, lane 2], and which was successfully purified [Figure 4c, lane 3]. Previously, a team at the Academia Sinica in Taiwan reported that they used the pET15b plasmid of the pET system (Novagen) to express N protein under induction conditions at 16°C for 10–12 h (personal communication); however, our results show that it is possible to obtain codon-optimized N protein using the pQE80L system (Qiagen) following induction at 37°C for 3 h, suggesting that expression efficiency can be enhanced through codon optimization.

Patient serum response to N protein and receptor-binding domain

A total of 0.5 μ g of purified N protein, 1.5 μ g of purified RBD, and 8.5 μ g of purified S2 domain was transferred to a PVDF membrane, which was divided into 18 strips for immunoblotting. With anti-human IgG used as secondary anti-serum, the results demonstrated that all tested serum samples responded to N protein, except those for patients 9011 [Figure 5a, lane 8] and 9013 [Figure 5a, lane 10]. Anti-N protein IgM was not found in the serum of patients 9011 [Figure 5b, lane 8], 9013 [Figure 5b, lane 10], and 9014 [Figure 5b, lane 11]. These results show that more than 80% of patient sera responded to codon-optimized N protein produced in *E. coli*, suggesting that this may serve

as a suitable antigen for rapid antibody detection tests. For RBD serum tests, anti-RBD IgG was detected in only seven samples out of 15, and anti-RBD IgM was detected in nine samples [Figure 6]. Interestingly, serum samples from patients 9011 and 9013 did not respond to N protein, but contained anti-RBD IgM [Table 3]. For S2 serum tests. 60% of samples contained anti-S2 IgG, but only patient 9099 had a high concentration of anti-S2 IgM [Figure 7]. Taken together, these results suggest that N protein and RBD would constitute a better combination for the construction of rapid antibody detection kit with greater accuracy and efficiency.

DISCUSSION

To date, many COVID-19 diagnostic kits have been developed for commercial production in other countries around the world, but as there is no commercial benefit for diagnostic kit production in Taiwan due to the low number of cases and excellent control of the pandemic, very few local companies have devoted resources to the development of such kits. However, since the start of the pandemic, the Tzu Chi Foundation has provided financial and healthcare support for many people in different countries, and the development of rapid antibody-based testing kits would not only help to protect Foundation volunteers working to provide relief, but could also be used in areas that lack quantitative reverse transcription PCR facilities to quickly identify the infected and enable the timely quarantine of patients. The use of an *E. coli* expression system can enable the rapid production of SARS-CoV2 viral proteins in sufficient quantities to support the development of testing kits that can be used by the Tzu Chi Foundation for nonprofit purposes.

Although RT-PCR detection of viral RNA remains the most accurate and sensitive testing method currently available, not all



Figure 4: Protein expression and purification analysis by SDS-PAGE. (a) N-terminal domain (30.4 kDa), receptor-binding domain (30.6 kDa) and R3 (15.4 kDa) analysis by 15%SDS-PAGE. (b) S2 (66.6 kDa) analysis by 12% SDS-PAGE. (c) N protein (46.8 kDa) analysis by 12% SDS-PAGE. NI: non induction fraction, I: induction fraction, P: purified fraction. Arrows indicated the purified proteins



Figure 5: N protein response to anti-sera. (a) Anti-N IgG, (b) anti-N IgM detection by western blotting. Each lane contained 0.5 µg of purified N protein. Anti-sera in each lane was diluted 1000-fold. Secondary anti-human IgG or IgM was diluted 10,000-fold. Lanes 1–18 of each strip used different anti-sera, as listed in [Table 3]



Figure 6: Receptor-binding domain response to anti-sera. (a) Anti-receptor-binding domain IgG, (b) anti-receptor-binding domain IgM detection by western blotting. Each lane contained 1.5 µg of purified receptor-binding domain protein. Anti-sera in each lane was diluted 1000-fold. Secondary anti-human IgG or IgM was diluted 10,000-fold. Lanes 1-18 of each strip used different anti-sera, as listed in [Table 3]

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Figure 7: S2 domain response to anti-sera. (a) Anti-S2 IgG, (b) anti-S2 IgM detection by western blotting. Each lane contained 8.5 µg of purified S2 domain. Anti-sera in each lane was diluted 1000-fold. Secondary anti-human IgG or IgM was diluted 10,000-fold. Lanes 1–18 of each strip used different anti-sera, as listed in [Table 3]

Table 5: Sum	able 3: Summary of western blotting							
Lane	Numbers	RBD (IgG)	RBD (IgM)	S2 (IgG)	S2 (IgM)	N (IgG)	N (IgM)	
1	9001			+		+	+	
2	9002	+	+			+	+	
3	9003	+	+	+		+	+	
4	9004	+	+	++		+	+	
5	9005	+				+	+	
6	9007		+	+		+	+	
7	9009	+	+	++		+	+	
8	9011		+	+				
9	9012					+	+	
10	9013		+					
11	9014		+			+		
12	9015	++	+	++		+	+	
13	9049					+	+	
14	9098			+		+	+	
15	9099	+		++	++	+	+	
16	Anti-His	+	+	+	+	+	+	
17	NCII-1				ND			
18	NCII-2				ND			
Percentage		46.6%	66.6%	60%	6%	86.6%	80%	

+: interaction with antibodies, ++: Intense interaction with antibodies. NC: negative control anti-serum. ND: Not determined

areas have access to the advanced facilities and equipment required to conduct such tests, and rapid antibody or viral protein detection kits would be a better choice in such cases. Previous research has shown that 100% of patients tested positive for antiviral IgG within 19 days, in a study that used magnetic chemiluminescence enzyme immunoassay (MCLIA) for viral-specific antibody detection in 285 patients with COVID-19 [16]. Serological detection will also be very helpful for the diagnosis of putative patients with negative RT-PCR results and for identifying asymptomatic infections and recovered infections. According to our immunoblotting results, codon-optimized N protein demonstrated an 80% positive response in serum samples against which it was tested, and the RBD of the S protein also exhibited a positive response in 50% of tested serum, indicating that these viral proteins produced in E. coli expression systems have strong potential to be used in serological testing kits.

To obtain large amounts of proteins in a short timescale, *E. coli* expression systems have a strong advantage over other expression systems. Cloning and validation can be completed within two weeks for an *E. coli* expression system [17]. However, the absence of posttranslational modification represents a major advantage and defect of *E. coli* expression systems [18-20], and codon bias may lead to truncated products because of premature termination of protein translation [21,22]. To overcome this issue, we prepared chemically synthesized viral protein coding sequences in which *E. coli* preferred codons were used. The results showed that these codon-optimized viral proteins were successfully produced and were capable of reacting with patient serum samples.

Previous studies have shown that the N protein of most coronaviruses is expressed abundantly during infection and is highly immunogenic [23,24]. High levels of IgG antiserum against N protein have been detected in patients with SARS infection [25], and SARS-specific T-cell proliferation and cytotoxic activity triggered by the N protein were typical T-cell responses in a vaccine developed for SARS [26]. IgM antiserum against the N protein of SARS-CoV2 was detected in 80% of serum samples in this study [Table 3], and most previous studies agreed with our observation that the N protein represents a good candidate for antibody detection [8]. Indeed, a previous study has used an N-terminal truncated N protein to produce specific antibodies against SARS N protein with no cross-reactivity with human coronaviruses OC43 and 229E [27]. In the future, we will also seek to generate N-terminal truncated N protein to reduce the possibility of cross-reactivity and improve the accuracy of antibody detection kits.

CONCLUSION

In conclusion, here we describe an *E. coli* expression system capable of rapidly and abundantly producing SARS-CoV2 viral proteins from chemically synthesized and codon-optimized sequences. Our results showed that the resulting codon-optimized N protein, RBD, and S2 domain were capable of reacting with patient sera, and have strong potential to be used in rapid and accurate serological tests for COVID-19.

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Conflicts of interest

There are no conflicts of interest.

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	1201 1201	ΕΛΑ ΓΩ ΑΛΑ ΓΩ ΑΛΑΤΤΟ ΑΛΑΛΤΤΟ ΕΙΕ ΕΤΟ ΕΓΕΛΤΤΟ ΕΙΕ ΑΤΓΟ ΑΤΟ ΓΟ ΑΛΑΛΟΓΟ ΑΛΑΛΟΓΟ ΑΓΟ ΕΓΕΓΟ ΤΟ ΕΓΕΓΟ ΕΓΕΓΟ ΤΙΤΟ ΕΙΤ ΕΑ ΑΓΩΑΛΑΑ ΑΛΑΓΙΤΟ ΑΛΑΓΟ ΕΓΕΓΟ ΈΝΕ ΕΘΕΟ ΈΝΑΤΘΕΙΟ ΕΙΑ ΤΟ ΕΙΑ ΤΟ ΕΠΑΛΑΛΟΓΟ ΈΛΟ ΕΓΙΟ ΕΙΟ ΕΙΟ ΕΛΑΛΟΓΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙ ΕΙ ΑΓΩΑΛΑΛΟ ΑΛΑΓΙΤΟ ΑΛΑΓΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙΟ ΕΙ	SO SE	361	370 380 380 400 410 410 410 KOTSTOL POROKKOTYLL PAROLDOD KSKOLOOSMSSADSTOA NO-38	
		TTACCCAGCGTAATTTTTATGAGCCGCAAATCATTACTACCGACAACACCTTTGTGTGTG	jority	301		
	1301 1301	TA A CACAA A GGA TTTTTTA GA A CCACAA A TCA TTA CTA CA GA CAA CA CATTTGTGTCTGTA A CTGTA TA GGA A TTGTGA CAA CAA CAA CAA CA CAGT Ta c g c a g g a a g a g a g a g a g a g	SO SE	:	ANNA ALIMAN ANA ALIMAN ANA ALIMANA ANA	
		<u>TTATGATCCTTTGCAACCTGAGTTGGACTGCTTCAAGGAGGAGTGGATAAATATTTTAAGAATCATACCTGCCCGGATGTTGATTTGGGTGACATTTGT</u> MA 1410 1429 1430 1440 1459 1410 1410 1410 1410 1410 1410 1410 141	jority	16		
	1401 1401	ITATGATGCTCTTTGCAACCTGAATTAGACTCATTCAAGGAGGAGTAGATAAATATTTTAAGAATCATACATCACCAGATGTTGATTTAGGTGACATCTT Giatgageggggggaacaggggggaaggtgaaggtcaaggaggaggagggag	SO SE	250 250		
		GGCATTAATGCTTGCGTTGTGAACATTCAAAAGGAGATTGACCGTCTGAATGAGGTTGCGAAGAATTTGAATGATGTCTGATCGATC	jority	2		
	1501 1501	GGCATTAAT GCTTCAGTTGTAAACATTCAAAAAGAAATTGACCGCCTCAATGAGGTTGCCAAGAATTTAATGAATCTCTCATGATCGCAGAAGATTG GGCATGAAGGCTTGTGTAACATTCAAAAGGAGATGGACCGGCTGAATGAGGCGAAGAATTTAATGAATCTCTCATCGATCG	SO SE	-	11111617/1164010119404014990041099011098011999991169946914601144099091(08-R-6.05 (4390/1091/294480440410-01940)1401141119111 1939 1949 1939 1939 1939 1939 1939 1949 194	
		GCAAGTATGAGGAGTATATTAAATGGCCGTGGTACATTTGGCTGGGTTGGTGGCTGGTTGATGCGATCGTTATGGTGACCATTATGCTTTGCTGTAT ISI0 IS20 IS30 IS60 IS50 IS50 IS50 IS50 IS50 IS50 IS50 IS5	jority	541		
	1601 1601	GAAA GTA TGAĞGA GTA TA TA AAA TGGC GA TGGTA CA TTT GGC TA GGT TTA TA GC TGGC TTGA TTGC CA TA GTA AA TGG TGACAA TTA TGATTGA TGATATGA GʻA A GTA TGA ŽCA TA GA TGAAA TGGC COTGGTA CA TTT GGC TGGGT TGA TGC GGC TGA TTGC GA TGC GA TGC GGA CA TTA TGATG GʻA A GTA TGA TGA TGA TGAAA TGGC COTGGTA CA TTT GGC TGGGT TGA TGC GGC TGA TTGC GA TGC GA TGC GGC GA TGA TGC GG	SO SE	18		
		GACCAGTTGTTGTAGTTGTTGTTGTTGTTGTTGTTGTTGTTGTGGCTGCTGCTAGTTGTAGGAGCGATTGTGAGCCGGTTCTGAAGGGGTGAAGTTG 1716 1228 1238 1246 1258 1258 1258 1258 1258 1258 1258	jority			
	1701 1701	GACCA GIT GÉT GIA GIT GIT GIT GIT GIT GIT GIT GIT GAT CCT GCI AGANT IT GAT GAGAGGACT CI GAGCGACI GCI AAAA GAGCGACT GI GAGCGACI GIT GAAAGGACT GAAAGGACT GAAAGGACT GI GAGCGACI GIT GAAAGGACT GI GAGCGACI GIT GAAAGGACT GI GAGCGACI GIT GAAAGGACI GAGCGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAGGACI GIT GAAAGGACI GIT GAAGGACI GIT GAAAGGACI GIT GAAGGACI GAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAGGACI GIT GAAAGGACI GIT GAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAAGGACI GIT GAAGGACI GIT GAAGGACI GIT GAAGACI GIT GAAGGACI GAAGGACI GIT GAAGGACI GAAGGACI GIT GAAGGACI GAAGGACI GAAGGACI GAAGGACI GIT GAAGGACI GIT GAAGGACI GAAGGACI GAAGGACI GAAGGACI GAAGGACI GIT GAAGGACI GAAGA	SO SE	***		
	corati	on "Decoration 21": Shade (with solid black) residues that match S2:S0 exactly.		1161	tila tila tila tila tila tila tila tila	f

Figure S1: (a) Nucleic acid alignment of NTD of Spike protein encoding genes. Identity between NTD-SO and NTD-SE is 72%. O is original sequence. E is *E. coli* modified sequences. (b) Nucleic acid alignment of RBD of Spike protein encoding genes. Identity between RBD-SO and RBD-SE is 71.1%. O is original sequence. E is *E. coli* modified sequences. (c) Nucleic acid alignment of S2 of Spike protein encoding genes. Identity between S2-SO and S2-SE is 72.9%. O is original sequence. E is *E. coli* modified sequences. (d) Nucleic acid alignment of Nucleocapsid protein encoding genes. Identity between NO and NE is 72.5%. O is original sequence. E is *E. coli* modified sequences. (e) Amino acid sequences alignment of nucleocapsid protein encoding genes. Identity between NO and NE is 100%. O is original sequence. E is *E. coli* modified sequences. (f) Amino acid sequences alignment of spike protein encoding genes. Identity between SO and SE is 100%. O is original sequence. E is *E. coli* modified sequences.