Cloning, Expression and Functionality evaluation of Recombinant Monoclonal antibody against VP1 capsid protein of FMD virus

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Abstract

Foot-and-Mouth Disease (FMD) is one of the most contagious viral disease with potentially devastating economic, social and environmental impacts that caused by a virus of the genus Aphthovirus, family Picornaviridae. FMD virus (FMDV) is a highly variable RNA virus, and there is little or no cross-protection between serotypes and even between different strains of the same serotype.

Monoclonal antibody (mAb) has a pivotal role in detection and serotyping of FMDV in pathological specimens, and also protection evaluation against FMD after vaccination. This study explore the expression and function of an engineered recombinant single-chain variable fragment (scFv-mAb) in Escherichia coli (*E.coli*) BL21(DE3) Rosetta strain. Production of recombinant mAb against FMDV in prokaryotic system is simple, with high yield and cost effective.

Designed scFv-mAb gene ordered into pET28a (+) expression plasmid. Expressed protein purified using Ni^{2+}-NTA resin column and quality assessed by 12% SDS-PAGE. Finally, efficiency and functionality of scFv-mAb confirmed by indirect sandwich (capture) ELISA.

The transformed *E. coli* BL21(DE3) Rosetta strain induced with 0.5 mM IPTG and incubated for 12 hr at 37 $^{\circ}C$. Significant protein with purity of > 90% expressed. The concentration of purified scFv-mAb in optimum condition calculated approximately 2.00 mg/ml by Bradford assay. By analyzing chequerboard results and mean of negative serum in 1:10 dilution, 400 ngr of scFv-mAb coated in each well. Eventually optical density (OD) of 0.3 selected as cut-off in the indirect Sandwich ELISA assay. The ELISA results showed that the scFv-mAb fragment successfully detected serotype O of FMDV with signal ranges of $0.3 \leq X \leq 1.5$ at 450nm wavelength in different positive control treatments.

Key words: FMDV, G- H loop, VP1, Recombinant scFv-mAb, Indirect sandwich ELISA

کلونینگ، بیان و ارزیابی عملکرد آنتی بادی مونوکلونال نوترکیب علیه پروتئین VP1کپسید ویروس FMD

چکیده

بیماری تب برفکی (FMD) یکی از مسری ترین بیماری های ویروسی با اثرات بالقوه مخرب اقتصادی، اجتماعی و زیستی است که توسط ویروسی از جنس آفتوویروس، خانواده Picornaviridae ایجاد می شود. ویروس تب برفکی(FMDV) یک RNA ویروس بسیار متغییر است و بین سروتیپ‌ها و حتی ساب تایپ های هر سروتایپ، حفاظت متقاطع کمی وجود داشته یا اصلاً وجود ندارد.

آنتی بادی مونوکلونال mAb نقش مهمی در شناسایی و تعیین سروتیپ FMDV در نمونه های پاتولوژیک و همچنین ارزیابی محافظت در برابر FMD پس از واکسیناسیون را دارد. این مطالعه بیان و عملکرد قطعه نوترکیب، تک زنجیره ای و مهندسی شده scFv-mAb در سویه روزتا اشریشیا کلی *E.coli* BL21(DE3) را بررسی می کند. تولید mAb نوترکیب بر علیه FMDV در سیستم پروکاریوتی ساده، با عملکرد بالا و مقرون به صرفه می باشد.

ژن scFv-mAb طراحی شده در پلاسمید بیانی pET28a (+) سفارش داده شد. پروتئین بیان شده با استفاده ازستون رزین Ni^{2+}-NTA خالص‌سازی گردید و کیفیت آن با ۱۲٪ SDS-PAGE ارزیابی شد. در نهایت، کارایی و عملکرد scFv-mAb با روش ساندویچ الایزای غیرمستقیم تأیید شد.

سویه روزتا *E.coli* BL21(DE3) ترانسفورم شده، با ۰/۵ میلی مولار IPTG القا شد و به مدت ۱۲ ساعت در دمای ۳۷ درجه سانتی گراد انکوبه شد. پروتئین قابل توجهی در هر دو بخش محلول و نامحلول با خلوص بیش از ۹۰٪ بیان شد. غلظت scFv-mAb خالص شده در

شرایط بهینه تقریباً ۲ میلی گرم بر میلی لیتر با روش برادفورد محاسبه شد. با آنالیز نتایج چکربورد و میانگین سرم منفی در رقت ۱:۱۰، ۴۰۰ نانوگرم scFv-mAb درهرچاهک پوشش داده شد. درنهایت جذب نوری 0.3 به عنوان cut off در روش Sandwich ELISA غیر مستقیم انتخاب شد. نتایج ELISA نشان داد که قطعه scFv-mAb با موفقیت سروتیپO-FMDV را با سیگنال‌هایی بین ۰/۳ تا ۱/۵ در طول موج ۴۵۰ نانومتر در تیمارهای مختلف کنترل مثبت شناسایی کرد.

کلمات کلیدی: FMDV، لوپ G-H، VP1 ، scFv-mAb نوترکیب، الایزای ساندویچ غیرمستقیم

1. Introduction

FMD is a highly contagious viral disease of mammals and causes significant economic losses in susceptible cloven-hoofed animals. The virus belongs to the genus Aphtho virus, in the family Picornaviridae. Its genome is a positive single stranded RNA, 8.5 kbp length with a single open reading frame (1).

There are seven different serotypes, A, O, C, Asia1, Southern African Territories (SAT) 1, 2, 3. Infection with one serotype does not confer immunity against another. FMD cannot be differentiated clinically from other vesicular diseases and its laboratory diagnosis is important (1,2).

The intact virion included an icosahedral capsid structure, containing 60 copies of structural proteins VP1-VP4 and 7 non-structural proteins. VP1capsid protein has the highest copy number among all FMDV proteins and consist of 213 amino acids. G±H loop (residue 141-160) of VP1 capsid protein is highly variable and main antigenic region contain highly conserved triplet amino acids of Arg±Gly±Asp (RGD) among FMDV types which cause production of protective antibodies against FMDV types. Studies show, 40% of secreted antibodies against FMDV stimulated by this loop (2-4).

In this study, designed cross-reactive recombinant mAb against conserved RGD region of the G±H loop as a scFv with a flexible linkers for detection of FMDV serotype O expressed. Our goal was possibility

assessment of recombinant scFv-mAb production using *E.coli* expression system with low cost and in a short time (2,3,5).

2. Materials and Methods

2.1 Engineering and prediction of physicochemical properties of recombinant scFv-mAb

For engineering of mAb against G±H loop region of FMD virus VP1 capsid protein (Arg±Gly±Asp , RGD motif), PDB ID "1ejo" retrieved and subjected to required truncations to achieve the desired scFv. Advantages of this antibody were having low folding complexities, well annotated of its sequence and 3D structure and also low post-translational modifications especially, glycosylations (3).

The truncation was performed at ARG 2112 (arginine amino acid) in light chain and Lys 2621 amino acid in heavy chain. The antibody domain were fused together by poly Gly-Ser linker (GSGGGGS).

The physicochemical properties of finally engineered FMD virus mAb were analyzed using the ProtParam tool on the ExPasy web server (www.expasy.org). In this web server, various parameters can be calculated, including: molecular weight (kDa), theoretical isoelectric point (pI), and estimated half-life under in vitro and in vivo conditions, stability index, aliphatic index, and grand average of hydrophobicity (GRAVY)(6).

2.2. Bacterial strains and culture media

E.coli DH5α (Novagen Co.) used as a cloning host for production and maintenance of expression vector and *E.coli* BL21(DE3) Rosetta strain (Novagen Co.) used as expression host of eukaryotic proteins that contain codons rarely used in *E.coli*. Luria Bertani (LB) used as bacterial culture media (7).

2.3. Plasmid preparation

Recombinant scFv-mAb antibody (50.306kD) gene cassette designed using bioinformatic tools and was considered between NcoI and BamHI restriction sites, then chemically synthesized by Shine Gene of Molecular Biotech co. (Shanghai, China) into pET28a(+) expression vector. Chloramphenicol and kanamycin antibiotics anticipated as selectable markers of Rosetta strain and pET28a(+) expression vector respectively.

2.4. Plasmid Cloning and extraction

E.coli DH5a strain competent cells (200µl) prepared using $CaCl_2$ method and transformed with pET28a(+) vector (5µl) using heat shock Novagen transformation method (6).Then selection for transformants accomplished by plating on LB agar plates containing kanamycin (35µg/ml) and incubation at 37℃ for 18-24 hr.

Then extraction and purification of plasmid from transformants employed using Favor Prep™ Plasmid DNA Extraction Mini Kit based on kit instructions, included; 3 ml of well-grown transformant culture centrifuged at 11000×g for 1 minute, discarded supernatant completely. Added 200 µl of FAPD1 Buffer (RNase A added) to cell pellet and suspended completely by pipetting. Centrifuged at 18000×g for 5 min to clarify the lysate. During centrifugation, a FAPD Column placed in a Collection Tube. Then, transferred the supernatant carefully to the FAPD Column and centrifuge at 11000×g for 30 seconds. In following, the flow-through discarded and placed the column back to the collection tube and added 400 µl of WP Buffer to the FAPD Column and centrifuged at 11000×g for 30 seconds. Then, discarded the flow-through and placed the column back to the Collection Tube. Next, 700 µl of wash Buffer added to the FAPD Column and centrifuged at 11000×g for 30 seconds. This process continued by discarding flow-through and replacing column back to collection tube. Centrifuged at full speed (18000×g) for an additional 3 minutes to dry FAPD Column. Then, FAPD column placed to a new 1.5 ml microcentrifuge tube. In continue, added 50 µl ~ 100 µl of Elution Buffer or ddH_2O to the membrane center of the FAPD Column. Standed the column for 1 minute and then centrifuged at full speed (18000×g) for 1 minute to elute plasmid DNA. Finally stored the DNA at -20℃. Also, 5 microliters of the extracted plasmid was run on a 0.8% agarose gel, at 85 V for 90 min and its quality checked (8).

2.5. Cloning and protein expression

E.coli BL21 (DE3) Rosetta strain (Novagen) competent cells (200µl) prepared using CaCl2 method and transformed with pET28a(+) vector (5µl) using heat shock Novagen transformation method. Then selection for selection for transformants accomplished by plating on LB agar plates containing chloramphenicol (70 µg/ml), kanamycin (35µg/ml) and incubated at 37℃ for 18-24 hr (7).

Then, transformants cultured in LB broth containing chloramphenicol (70 µg/ml), kanamycin (35µg/ml) and incubated at 37°C by stirring 210 rpm for overnight. Sub-culture was done at 1:50 (v/v) ratio in 100 ml of fresh LB broth containing chloramphenicol (70 µg/ml), kanamycin (35µg/ml) and incubated under the above-mentioned conditions. As soon as optical density (OD) at 600 nm reached ~0.8, 1 ml of culture was withdrawn as expression negative control. So, the expression of the target protein was induced by the

addition of isopropyl-β-d-Galactopyranoside (IPTG; Sigma-Aldrich, St. Louis, USA) at a final concentration of 0.5, 0.75 and 1.0 mM. And culture incubated at 30°C, 210 rpm. The expression time-course studies performed in 0, 4, 8 and 12 hr. after induction. Finally, pellets harvested by centrifugation of each sample at 7000×g, 15min at 20 $^{\circ}$C, and pellets were stored at -80°C for further processes (9).

2.6. Protein expression analysis and extraction

Collected pellets were re-suspended in protein sample buffer (5X) plus 2-mercapto ethanol (2ME) according to the Laemmli method (7). Based on hypothetical molecular weight of scFv (50.306 kDa), the resolving and stacking SDS-PAGE gel concentration was selected as 12% and 4%, respectively. Electrophoresis was done in running buffer 25 mM Tris-base, 192 mM glycine, 1% SDS, pH 8.3; (CinnaGen Co., Tehran, Iran) at 90 V for 2–3 hr. The gel was stained by staining solution, 1% Coomassie blue R-250 (Merck, Darmstadt city, Germany) and de-stained by 7% acetic acid (Merck, Darmstadt city, Germany), 5% methanol (Merck, Darmstadt city, Germany) and 88% water solution. The standard molecular marker (CinnaGen Co., Tehran, Iran) was run in parallel with other samples in order to estimate the molecular weights of the proteins. Moreover, for the periplasmic proteins extraction sonication (Sonicator; Hielscher Ultra-sound Technology, Brandenburg, Germany) method include 5 times × 1min sonication and 1min interval on ice was followed. Also, 1.0 mM phenyl methyl sulfonyl fluoride (PMSF), (Merck, Darmstadt, Germany) added to each sample for inhibition of the possible proteases in extracted samples (7).

2.7. Western blotting (WB) for confirmations of specificity

Specificity of recombinant scFv-mAb confirmed by western blotting method. In brief, SDS-PAGE was followed as described above condition without gel staining. So, the sandwich was arranged as follows in cathode to anode direction: support pad, Whatman NO.1 filter paper, SDS-PAGE gel, nitrocellulose membrane, Whatman NO.1 filter paper and support pad. The blotting was implemented at 20 V for 2–3 hr. by transfer buffer (25 mM Tris, 192 mM glycine, 20% (v/v) methanol for 1L double-distilled water, pH 8.3).Then, the nitrocellulose membrane was blocked by 5% (w/v) skim milk powder in PBS-T buffer (PBS with 0.05% tween 20 (v/v)) overnight at 4°C. In continue, washing was done for three times with PBST and then the membrane was incubated by anti-poly histidine monoclonal antibody at 1:1000 dilutions for 2 hr, at room temperature. Washing for three times and soaking of paper in horseradish

peroxidase (HRP) conjugated anti-His tag antibody (Sigma-Aldrich) solution. Then DAB solution (Merck, Darmstadt city, Germany) was added as the enzyme chromogen substrate. After incubation in dark place at room temperature and appearing a color scFv-mAb band, the reaction stopped by distilled water (7).

2.8. Protein purification and concentration measurement

Production of recombinant scFv-mAb, either in solubilized or insolubilized form, analyzed by 12% SDS-PAGE gel electrophoresis (Hercules, USA).Then, Ni^{2+}-NTA affinity chromatography (nickel-nitrilotriacetic acid) (QIAGEN, USA) resin was used for protein purification based on affinity to 6×His-tagg in C-terminus of scFv-mAb. The purification performed under native condition with equilibration of washing (plus 20 mM imidazole) and elution (plus 250 mM imidazole) buffers according to the manufacturer's protocol. Collected samples from various purification runs were analyzed using SDS-PAGE as described before.

The concentration of purified scFv-mAb measured at 595 nm ($25^{\circ C}$) using the colorimetric Bradford assay method in comparison with bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, USA) as standard (0-20 mg/ml). Standard curve was constructed with a serial dilution from 0.00 to 20.00 mg/ml by Graph Pad online server regression tools (www.graphpad.com) by linear regression calculator and plotted versus BSA (7,10,11).

2.9. Chequerboards and Evaluation of scFv-mAb function by using sandwich (capture) ELISA

Diagnostic value analysis of purified recombinant scFv-mAb was the most important part of present study which it shows its proper folding and functionality. It is performed by indirect sandwich ELISA (S-ELISA, Capture antibody ELISA) that reflects affinity, folding, function, and specificity of scFv-mAb. The 96-well flat bottom polystyrene high bind microplate (Corning Co. , USA) coated with 400ng/well of scFv-mAb (as a capture antibody) in coating buffer (1.50 g/L Na2CO3, 2.93 g/L Na2HCO3 in 1000 ml distilled water, pH 9), and incubated at $4^{\circ C}$, overnight. Also, BRS ((Bovine Reference Serum against FMDV serotype O (BRS-O)) used as standard antibody positive control for FMD virus (Virus neutralization test (VNT) Log10 ≥1/8, 1:10 dilution/well).

Then, supernatant removed and washed three times with PBS-T buffer. Then 150 µl blocking buffer (PBST plus 2% sodium caseinate) was added for 2 hr. and incubated at room temperature. Supernatant removed and wells washed three times by PBS-T. In chequerboard assay test different concentration (µg/well) of r-mAb antibody 1.5, 1, 0.5, 0.25, 0.125, 0.061 was used. Also, 2, 1.5, 1, 0.5 µg/well of ultra-

centrifuged concentrated viral antigen FMDV serotype O_{2016} (previously prepared by Razi Vaccine and Serum Research Institute, Department of FMD) mixed with 0.03 µg/well of PEG+NACL were added (Data not shown). After 45 minute incubation at 37$^{\circ C}$ and three times washing with PBS-T, 100 µl/well of secondary antibody BRS with VNT 50% ≥Log10 1.8) was added in 1:10 dilution. Also, 4 negative (non-vaccinated calf serum, age below 6 month) and 2 positive controls (BRS) used. Then, the plate incubated at 37$^{\circ C}$ for 45 minute and then washed three times with PBS-T. Next 100 µl/well (Goat anti bovine HRP conjugated antibody) conjugated Horseradish peroxidase (HRP) in 1:10000 dilution with PBS-T. After 30 minute incubation at 37$^{\circ C}$, reactions were developed by adding 100 µl/well of TMB substrate (3, 3′, 5, 5′-tetra methyl benzidine) (IDvet Co., Grabels, France). Finally, 50 µl/well of 1M H_2So_4 (Merck, Darmstadt city, Germany) was added to stop the reaction. The absorbance at 450 nm was determined by an ELISA microtitre plate reader ((Denly, well Scan Co.). The OD of highest dilution of each sera that was 2.5 time bigger than OD of negative control serum (Mean of negative ±2SD) considered as the end point titer (7,12,13).

3. Results

3.1. scFv-mAb Structure analysis

The final scFv-mAb with 470 amino acid lengths was designed and linked together using a peptide linker (GSGGGGS). These nucleotide sequence of engineered recombinant anti-FMD virus antibody was as follow;

*CCATGG*GCaaatacctattgcctacggcagccgctggattgttattactcgcggcccagccggccatgGAAGTTATGCTGGTTGA
ATCTGGTGGTGGTCTGGTTAAACCGGGTGGTTCTCTGAAACTGTCTTGCACCGCTTCTGGTT
TCATCTTCAACCGTTGCGCTATGTCTTGGGTTCGTCAGACCCCGGAAAAACGTCTGGAATGG
GTTGCTACCATCTCTTCTGGTGGTACCTACACCTACTACCCGGACTCTGTTAAAGGTCGTTT
CACCATCTCTCGTGACAACGCTAAAAACACCCTGTACCTGCAGATGTCTTCTCTGCGTTCTG
CTGACACCGCTATGTACTACTGCGTTCGTCGTGAAGACGGTGGTGACGAAGGTTTCGCTTA
CTGGGGTCAGGGTACTGTTGTTACCGTAAGCGCTGCTAAAACCACCCCGCCGTCTGTTTACC
CGCTGGCTCCGGGTTCTGCTGCTGCTGCTGCTTCTATGGTTACCCTGGGTTGCCTGGTTAAA
GGTTACTTCCCGGAACCGGTTACCGTTACCTGGAACTCTGGTTCTCTGTCTTCTGGTGTTCA
CACCTTCCCGGCTGTTCTGCAGTCTGACCTGTACACCCTGTCTTCTTCTGTTACCGTTCCGTC

TTCTACCTGGCCGTCTGAAACCGTTACCTGCAACGTTGCTCACCCGGCTTCTTCTACCAAAG
TTGACAAAAAAATCGTTCCGCGTGGTGGTGGTTCTGGTGGTGGTTCTGGTGGTGGTTCTGGT
GGTGGTTCTGACATCGTTCTGACCCAGTCTCCGGCTTCGCTCGCTGTAAGCCTGGGTCAGCG
TGCTACCATCTCTTGCCGTGCTTCTGAATCTGTTGACTCTTCTGGTCACTCTTTCATGCACTG
GTACCAGCAGAAACCGGGTCAGCCGCCGAAACTGCTGATCTACCGTGCTTCTAACCTGGAA
TCGGGTATCCCAGACAGGTTCTCTGGTTCTGGCTCTCGTACCGACTTCACCCTGACCATCGA
CCCGGTTGAAGCTGACGACGTTGCTACCTACTACTGCCAGCAGTCTAACGAAGTTCCGCTG
ACCTTCGGTGCTGGTACCAAACTGGACCTGAAACGTGCTGACGCTGCTCCGACCGTTTCTAT
CTTCCCGCCGTCTTCTGAACAGCTGACCTCTGGTGGTGCTTCTGTTGTTTGCTTCCTGAACAA
CTTCTACCCGAAAGACATCAACGTTAAATGGAAAATCGACGGTTCTGAACGTCAGAACGGT
GTTCTGAACTCTTGGACCGACCAGGACTCTAAAGACTCTACCTACTCTATGTCTTCTACCCT
GACCCTGACCAAAGACGAATACGAACGTCACAACTCTTACACCTGCGAAGCTACCCACAAA
ACCTCTACCTCTCCGATCGTTAAATCTTTCAACCGTAACGAATGCGGTCCGGGTGGTCAGCA
CCACCACCACCACCACCACCACTGCTAA**GGATCC**

As shown in the sequence, the signal peptide (PelB signal peptide) at the beginning of the sequence is showed as lowercase in 5' terminal before NcoI restriction enzyme (RE) site (C↓CATGG). A dash is drawn below the start and end codon. At 3' terminal of ordered sequence His-tag, stop codon and BamHI RE site (G↓GATCC) has been located.

After initial analysis, final confirmation suitable physicochemical characteristics of construct structure based on were reported in table 1(3).

Table 1. Calculated parameters for recombinant scFv-mAb by using the ExPASy ProtParam tool.

Synthetic fusion protein parameters	**Recombinant mAb against FMD virus** (G±H loop region of the VP1 capsid protein)
Number of amino acids	470
Molecular weight (MW) of synthetic fusion protein	50.306 kDa
isoelectric point (pI)	6.91

Overall +R & -R	40 & 39
Instability index and half-life estimation (in *E.coli*)	45.24 and >10 hours
Aliphatic index	63.60
Grand average of hydropathicity (GRAVY)	-0.358

3.2. Plasmid dilution, amplifying and extraction

Plasmid dilution implemented based one manufacturer instruction with some modifications. So, lyophilized vector dissolved in 100 µl of distilled water to obtain a final concentration of 2µg/100µl. 75 µl stored at -20$^{\circ C}$ as stock solution and 25 µl used as working solution. Also, pET28a(+) successfully cloned in *E.coli* DH5α, screened with antibiotic rich media and extracted by FavorPrep™ Plasmid DNA Extraction Mini Kit. At the end of this process, 90 µl stored at -20$^{\circ C}$ as extracted plasmid stock and 10 µl used as working solution. Concentration of extracted plasmid solution using nanodrop device was 100 ng/ml. 8 µl of extracted plasmid solution used for 0.8% (w/v) agarose gel electrophoresis, results revealed the typical distinct high purity band of circular and linear plasmids (~6958bp) (Figure 1) (14.15).

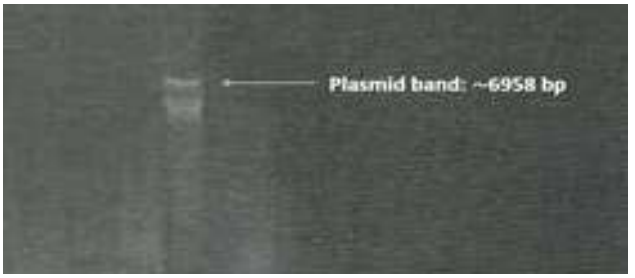

Figure 1. Recombinant plasmid extraction from *E.coli* DH5α was showed in 0.8% (w/v) agarose gel electrophoresis.

3.3. Transformation

The selection of transformants showed the growth of *E.coli* DH5a on LB agar medium containing (30 µg/ml kanamycin) and *E.coli* BL21(DE3) Rosetta on LB agar medium containing kanamycin (35µg/ml) and chloramphenicol (70 µg/ml) after 18-24 hr. incubation at 37℃ in comparison to negative control. This confirm successful transformation of pET28a(+) vector to its hosts (Data not shown)(16).

3.4. Expression of recombinant scFv-mAb

The transformed *E.coli* BL21(DE3) Rosetta strain induced with 0.5, 0.75 and 1mM IPTG and incubated at 37°C for 12 hr, expressed a significant protein fraction in both soluble and sonicated (inclusion body) (Figs. 2A and 2B). All expression samples were run on 12% under reducing condition to confirm the expression of scFv. The results of SDS-PAGE clearly showed distinct band at predicted position in induced bacterial cell extract.

As depicted in Fig.2 and evaluating from the presence of ~50 kDa band the expression was successful for both (0.5 and 1 Mm) IPTG concentrations and duration time of 4 to 12 hr. Also presence of scFv bands was confirmed by western blotting (16,17).

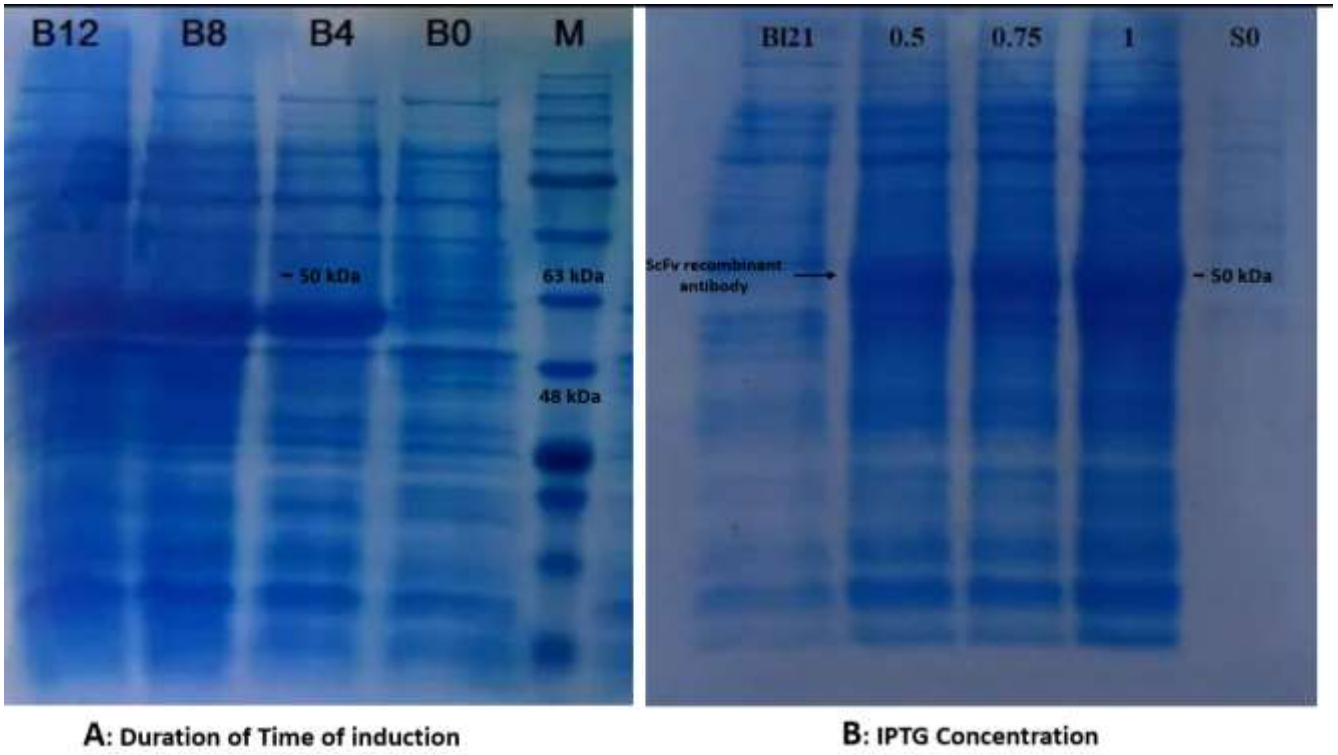

Figure 2. SDS-PAGE of scFv expression results. Fig (A) Duration of time at 0, 4, 8 and 12 hours after induction. Lane M: Protein marker (10–250 kDa). Fig (B) Expression of total protein in 0.5, 0.75 and 1 mM of IPTG concentration. The arrow shows the corresponding ~50 kDa band of recombinant scFv antibody.

3.5. Concentration calculation of purified scFv-mAb

The optimum conditions for purification of recombinant scFv-mAb by Ni^{2+}-NTA agarose affinity chromatography included three-times binding process, five times washing in presence of 25 mM imidazole to remove non-specific contaminants, and then elution buffer with a pH of 7.5 and 0.3 M imidazole for maximum efficiency implemented.

By a single purification step, SDS-PAGE analysis showed more than 90% purity. The concentration of purified scFv-mAb protein in optimum condition was calculated approximately 2.00 mg/ml by Bradford assay (14).

3.6. Efficiency evaluation of purified scFv by Sandwich (capture) ELISA

By analyzing chequerboard results, and based on curve break pattern, optical absorption jump, and optical absorption preferences between 0.8 and 1, optimum concentration of virus (0.5 µg/well of O_{2016} serotype) and scFv-mAb (0.4 µg/well) with the highest signal to noise ratio for Capture ELISA determined (Fig. 3)

By assessing mean of negative serum in 1:10 dilution against 0.4 µg/well coated scFv, eventually OD of 0.3 selected as cut-off. ELISA results showed that scFv-mAb fragment detected FMDV serotype O_{2016} with signals above 0.3 (optimum concentration of antigen was 0.5 µg/well). Also, mean OD of 4 negative controls, was 0.17. Based on obtained results, when the recombinant scFv-mAb coated in bottom of plate, it was able to show OD of $0.3 \leq X \leq 1.5$ at 450 nm wavelength in different positive control treatments.

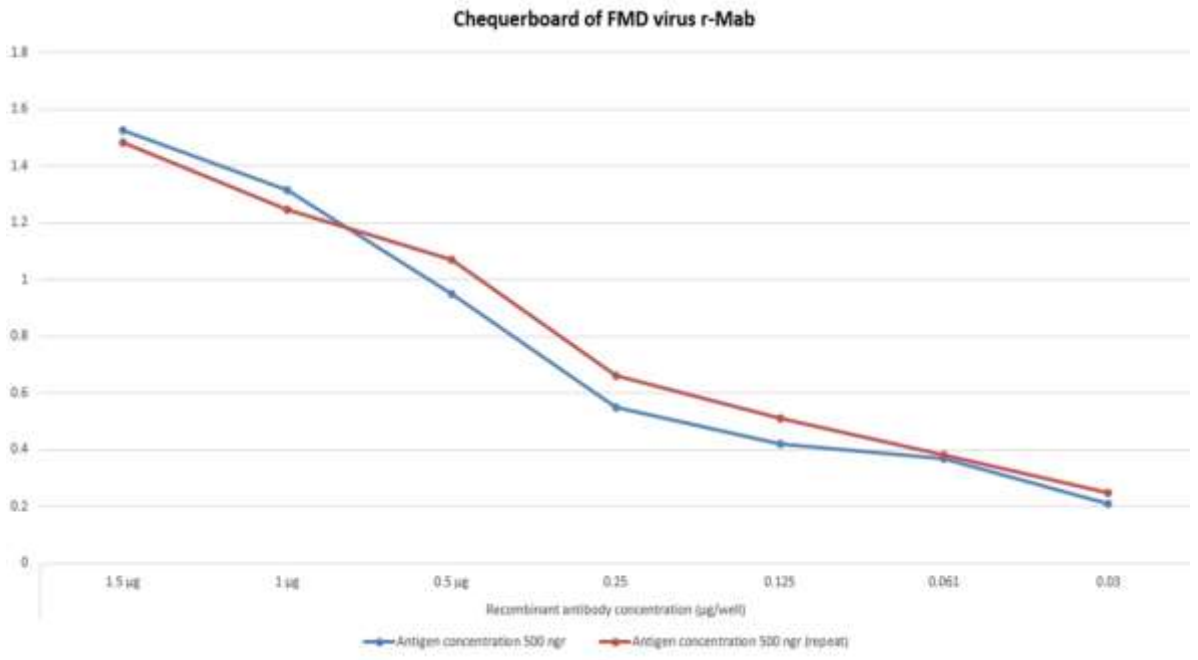

Figure3. ELISA absorbance results in a chequerboard pattern with different concentrations of recombinant antibody (X axis) and fixed virus antigen 0.5 µg/well (Y axis), 1/10 dilution of secondary BRS antibody and also 1:10000 dilution of Hrp-conjugated tertiary antibody were used. Finally, Optimum concentration of 0.4 µg/well of r-mAb antibody was selected for next Capture ELISA tests.

There was also at least 0.4 OD difference between purified protein and crude or lysate protein. Altogether, present results indicated good sensitivity of synthetic recombinant scFv-mAb for detection of FMDV serotype O whole particles (16,17).

3. Discussion

This study describes development of scFv-mAb against VP1 capsid of FMDV in prokaryotic host for detection of FMDV serotype O. Although it may use for detection of another FMDV serotypes such as A, C, Asia 1, SAT 1, 2 and 3, preparation of antigenic panel (especially in OIE reference countries) and for use in ELISA, diagnostic kits, lateral flow test, virus neutralization test and as a positive serum control.

Recombinant antibodies have important roles in treatment, research and diagnosis. While full-length antibodies require mammalian expression systems due to their complex folding and post translation modification. Most antibody fragments and antibody-like molecules are non-glycosylated and can be more conveniently prepared in *E.coli* based expression system. Some commercial recombinant antibodies produced in *E.coli* and used for treatment are Certolizumabpegol (CIMZIA®), Ranibizumab, Brolucizumab, Caplacizumab (trade name Cablivi®) Moxetumabpasudotox (MxP) (18).

In research performance, 1F10 (O UK) used as Pan-FMDV mAb (13,19). Also, Ochao in 2000 reported crystallographic determinations for complexes of 4C4 or SD6 mAb with AnSA peptides and revealed important conserved RGD motif structural characteristics (15,20). Both studies showed after binding of viral peptide to both 4C4 and SD6 mAbs and have a similar pattern of interactions and declared Asp143 and Leu144 residues were structurally conserved and also part of the cell receptor recognition motif (21-23).

Ochoa, in 2000, registered neutralizing monoclonal antibody 4C4 in the PDB database (3).

Its corresponding peptide adopted a compact fold with a nearly cyclic conformation and a disposition of the receptor-recognition motif Arg-Gly-Asp (RGD). It was complexed with Fab fragment of the neutralizing monoclonal antibody 4C4. Although various studies have been conducted on antibody response against FMDV, determination of antibody crystallographic structure and its registration is rare and limited to this structure.

Up to now, there are three hundred and twenty-four mAbs introduced for O, A, C and Asia 1 serotypes of FMDV. In details; 130 mAbs for serotype O, 108 mAbs for serotype A, 53 mAbs for serotype Asia 1 and 33 mAbs for serotype C (11,12,19,24).

Initial approach consist of using indirect sandwich ELISA with low specificity and sensitivity employing polyclonal immune sera as both capture (rabbit) and detecting conjugate antibody (guinea pig). Then, it updated with using polyclonal and mAb as a capture and conjugate antibody respectively (25,26).

Grazioli and colleagues in 2020, developed and validated a simplified serotyping ELISA based on monoclonal antibodies for detection of FMDV serotypes such as O, A, C and Asia 1 (12). They employed

Pan-FMDV mAb (1F10) as a detector conjugate in multiplex ELISA with 79% sensitivity compared to 72% sensitivity of polyclonal ELISA. This multiplex ELISA is simple, rapid and stable. So, it could replace existing polyclonal ELISAs for FMDV detection and serotyping. These Pan-FMD antibodies showed valuable results in LFIAs, also a sandwich-type immunoassay combined with a set of well-characterized mAbs. One LFIA antibody work as detecting and identifying O, A and Asia-1 serotypes, the second antibody enable to detection and differentiation of the SAT1 and SAT2 serotypes (2).

Also, Another mAb, which is actually a nanobody (M170 Nab), produced in Lama glama, a member of the camelid family. (PDB ID:7DST) These antibodies are produced specificly against VP3 protein of FMDV serotype O (15).

The production of recombinant antibodies in *E.coli* was first described in 1988 for Fv and scFv fragments. In present study, we successfully expressed recombinant scFv with relatively high efficiency and performance in *E.coli* BL21(DE3) Rosetta strain. Many types of research are in line with our study (16,27,28). Expression of scFv for detection of different serotypes of FMDV has very effective results. (17,29) scFv-mAb against 3ABC effectively produced in *E.coli* in 2014. In another study in 2003, expressed a known mAb against FMDV serotype O in *E.coli* (30). Commercial recombinant antibodies that produced in prokaryotic and eukaryotic hosts for human and animal diseases include some products from Creative biolabs, Biocompare, Sinobiological and etc.

Further studies could further demonstrate the value of this recombinant antibody in the future, and thus their commercial applications will expand dramatically.

It should be noted the cost of scFv production after purification estimated as 2.00 mg/ml 1 $ that significantly lower than other expressing platforms such as hybridoma technology and mammalian expression systems.

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Authors' Contribution

Not applicable

Ethics

Not applicable

Conflict of Interest

No conflict of interest is declared

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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