Faculty of Women for, Arts, Science, and Education

Scientific Publishing Unit

Journal of Scientific Research in Science

Biological Sciences

Volume 41 , Issue 2 , 2024

ISSN 2356-8372 (Online) \ ISSN 2356-8364 (print)

Journal of Scientific Research in Science 2024, 41, (2), 167:194

Contents lists available at EKB

Journal of Scientific Research in Science

Journal homepage: https://jsrs.journals.ekb.eg/

Efficacy of Recombinant Genetic Diagnostic Tools for Foot-and-Mouth Disease Virus (FMDV)

Mai M. Abd El-Rahman[1*], Rokaya H. Shalaby[2], Alaa A. E. El-Kholy[1], Noura R. Mohamed[2] and Shereen H. B. Elwakeel[2]

[1]Veterinary Sera and Vaccines Research Institute, Agricultural Research Center, Abbasia, P.O. Box 131, Cairo, Egypt.
[2]Zoology Department, Faculty of Women for Arts, Science and Education, Ain Shams University, Asmaa Fahmy Street, Heliopolis, Cairo, Egypt

Abstract

The Foot and Mouth Disease Virus (FMDV) is one of the most fearful animal viruses infecting all cloven-footed species and causing significant economic losses in the global livestock industry. The virus's seven serotypes and numerous genetic subtypes complicate its diagnosis in both at-risk and endemic areas, facing any control programs. The non-structural polypeptide (NSP) 3ABC of FMDV is the most reliable for serotype-independent antibody detection, and an enzyme-linked immunosorbent assay (ELISA) based on this has proven reliable. To facilitate FMDV diagnosis in Egypt, the polyprotein 3ABC encoding nucleotide sequence of FMDV serotype O_1 was cloned, expressed in a baculovirus-insect cell system, and characterized. An indirect ELISA was developed using the secreted recombinant 3ABC protein (rec3ABC) as a coating antigen, derived from insect cells infected with the recombinant baculovirus recBac/3ABC. This ELISA demonstrated efficacy in detecting anti-FMDV antibodies in bovine sera, showing reasonable correlation ($R \geq 0.85$) with a commercial 3ABC-blocking ELISA. The developed assay emerges as a reliable and cost-effective tool for anti-FMD antibody detection in both control and experimental bovine sera. Its potential applications include routine monitoring of FMDV seroconversions in infected, carrier, and vaccinated animals, as well as screening animals during quarantine and quality control of FMDV vaccines.

Keywords: FMD, FMDV, NSP, 3ABC, Developed 3ABC-ELISA

***Corresponding author**: **Mai M. Abd El-Rahman,** Veterinary Sera and Vaccines Research Institute, Agricultural Research Center, Abbasia, P.O. Box 131, Cairo, Egypt.
Email: mai_seafy@yahoo.com
(Received 2 May 2024, revised 29 May 2024, accepted 30 May 2024)
https://doi.org/10.21608/JSRS.2024.286967.1128

1. Introduction

Foot and mouth disease (FMD) is a viral disease described as highly contagious and affects all species of cloven-hoofed animals, such as cattle, goats, sheep, and pigs. It poses a significant economic threat to the livestock industry and trade **[1, 2]**. FMD virus belongs to the Aphthovirus genus in the Picornaviridae family. The virus is present in seven distinct varieties that may be differentiated based on serological and genetic characteristics. These types include O, A, C, Asia 1, South African Territories (SAT) 1, 2, and 3. All serotypes occur in Africa, except Asia 1. Besides, there is a great antigenic diversity within each serotype, resulting in numerous subserotypes. These serotypes are clinically indistinguishable, and their pathogenicity depends on the virus strain / isolate, host, species, and age **[3, 4]**.

Moreover, FMDV is a small icosahedral non-enveloped virus with a positive-sense single-stranded (ss) ribonucleic acid (RNA). It is about 8.5 kbp in length with a single open reading frame, encoding for both structural proteins (SPs) and non-structural proteins (NSPs). The P1 region of the genome encodes the 4 genes of SPs (VP1 [1D], VP2 [1B], VP3 [1C], and VP4 [1A]), which make up the virus capsid. The P2 and P3 regions of the virus genome consist of 8 genes encoding for the NSPs (L, 2A, 2B, 2C, 3A, 3B, 3C and 3D), which together allow virus replication in an infected cell **[5, 6]**.

On the other hand, the use of anti-NSP antibody testing is part of the World Organization for Animal Health (WOAH) guidelines for declarations of freedom from FMD virus circulation and also to differentiate between vaccinated and infected animals, and moreover, for the detection of antibodies to NSPs in both clinically infected and carrier animals. Following an animal's infection, certain NSPs have been found to be capable of triggering an immune response, specifically 2C, 3A, 3D, and the polyprotein 3ABC. However, previous research suggests that the most accurate indication of infection is the detection of antibodies targeting the NSP-3ABC polypeptide. This is because it is not influenced by the specific serotype, allowing it to differentiate between vaccinated and infected animals **[7, 8]**.

Diagnosing FMDV poses challenges due to the error-prone nature of FMDV's RNA-dependent RNA polymerase, resulting in a lack of cross-reactivity among FMDV serotypes. Additionally, there is substantial intratypic antigenic divergence due to the FMDV quasispecies

structure. This complexity underscores the need for an effective diagnostic tool, and ELISA emerges as a valuable solution. ELISA offers fast, sensitive, and cost-effective antibody detection **[9]**. Its suitability for large-scale screening of test samples and its ability to use purified recombinant viral proteins as antigenic substrates, produced in large quantities without the need to handle live viruses, also helps reduce the risk of false-positive reactions **[10, 11]**.

Baculoviruses have been used successfully as vectors for the expression of foreign genes both *in vitro* and more recently *in vivo* **[12, 13]**, particularly in the development of pharmaceuticals, gene therapies, diagnostic reagents, and vaccines. The traditional method for the development of baculovirus expression vectors is based on the replacement of a very late, non-essential virus gene coding region with the gene of the interest via homologous recombinant. Target genes of recombinant baculoviruses expressed within insect cells undergo most eukaryotic posttranslational modification, as in an authentic manner, and have the same biological activity and immunological reactivity as the authentic protein **[14-16]**.

Thus, due to the huge economic loss caused by FMDV to the livestock industry nationally and internationally and the high cost of the commercial ELISA sample, the present study was designed to produce a recombinant protein useful as a coating antigen in a 3ABC-based ELISA. That could be a potential to develop a sensitive, rapid and reproducible FMDV diagnostic test capable of sero-monitoring any virus presence or circulation in both areas of FMD at risk and endemic areas (detection of subclinical infection and carriers) regardless the FMDV serotype, as well as after vaccination, for local and imported animals.

2. Materials and methods

2.1. Virus and cells

A reference vaccinal strain of FMDV serotype O_1 (Mannisa) was used in this study. It was obtained from the Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI). FMDV was cultured in Baby Hamster Kidney (BHK21) cells, which were cultivated and maintained in minimal essential medium with Earle's salts (MEME). The media was supplemented with 2-10% fetal calf serum (FCS; PAA Laboratories, Austria), 100 U/ml penicillin, 100 µg/ml streptomycin, and 25 iu/ml mycostatin. When the cytopathic effect (CPE) reached 100% (about 48 hours after infection), the culture supernatants of BHK21 cells infected with FMDV were collected and cleared by centrifugation at 9000 × g/4 ◦C for 20 minutes. Next, the purified FMDV

stocks were quantified and stored at a temperature of -80ºC for subsequent procedures. Before conducting the experiments, the BHK_{21} cells and FCS were examined for the presence of FMDV using indirect immunofluorescence, as described before **[17]**. The *Spodoptera frugiperda* (Sf 9) insect cells, obtained from Invitrogen in the UK, were cultivated and kept at a temperature of 27 ◦C in Grace's insect medium from Biochrom in Germany. The medium was supplemented with 10% fetal calf serum (FCS), Gentamycin at a concentration of 25 mg/L, and l-glutamine at a concentration of 0.6 g/L. Sf 9 cells were utilized throughout the entire process of baculovirus expression of FMDV/3ABC.

2.2. Sera

The experimental sera utilized in this study were kindly provided by the Central Laboratory for Evaluation of Veterinary Biologics (CLEVB) for the purpose of assessing the quality of FMDV vaccines. Additionally, we employed commercially available sera for our study.

2.2.1. Negative Control Sera

Negative control sera (n=21) were selected from various sources, including three samples from a commercial source (FCS - PAA), three from commercial newborn calf sera (NCS - Gibco), and fifteen samples from naive calves (confirmed to be free from anti-FMD antibodies) collected prior to vaccination (day zero) based on a previous study **[17]**.

2.2.2. Positive Control Sera

Control serum samples included mono-clonal anti-FMDV-3ABC ruminant antiserum (IDEXX Laboratories, USA). Titration of these antibodies at dilutions ranging from 1:10 to 1:10,000 was done. Besides, fifteen sera from calves experimentally infected with serotypes O, A, and SAT 2 (five for each serotype) and forty-five sera collected from calves experimentally vaccinated with the local inactivated polyvalent FMDV vaccine (O, A, and SAT2) four weeks' post-vaccination, were included.

2.3. Viral RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR)

The viral FMDV RNA was extracted using QIAamp DNA Blood Kit (Qiagen, Germany) following the manufacturer's instructions. The 3ABC encoding sequence full length was amplified by RT-PCR utilizing specifically designed primers: Forward: 5′- CGC CGG ATC CTT CAA GCC TCA ACC ACC C-3′- Reverse: 5′- GCC GCC CTG CAG TCG TAT CTC CCT TGT GTT TGG-3′. The cDNA segment was modified to include BamHI and PstI restriction sites at its 5′ and 3′ ends, respectively, in order to enable specific cloning into a baculovirus transfer vector. The RT-PCR amplification was performed in a single step using 0.3 µg (5µl) of viral RNA, 20µmole of both forward and reverse primers, 5µl of 4x One-step RT PCR reaction buffer (Enzynomics), and nuclease-free water in a total reaction volume of 20µl. Then, samples were subjected to optimized thermal cycles as follows: 1 cycle of 30 min at 50ºC and 10 min at 95˚C; 38 cycles each of 1 min at 95˚C, 1 min at 56˚C, and 2 min at 72˚C; one last cycle of 10 min at 72˚C, then hold at 4ºC until analysis. The resulting PCR product was analyzed through agarose gel electrophoresis (1%) with ethidium bromide staining, as described before **[18]**. The PCR product (amplicon) of the proper expected size (~ 1.67 kbp) was purified from agarose gel using BioSpin Gel extraction kit (BioFlux).

2.4. Construction of the recombinant baculovirus

The recombinant baculovirus expressing 3ABC of FMDV (rec Bac/3ABC) was constructed exploiting the homologous recombination phenomenon as illustrated (Figure 1).

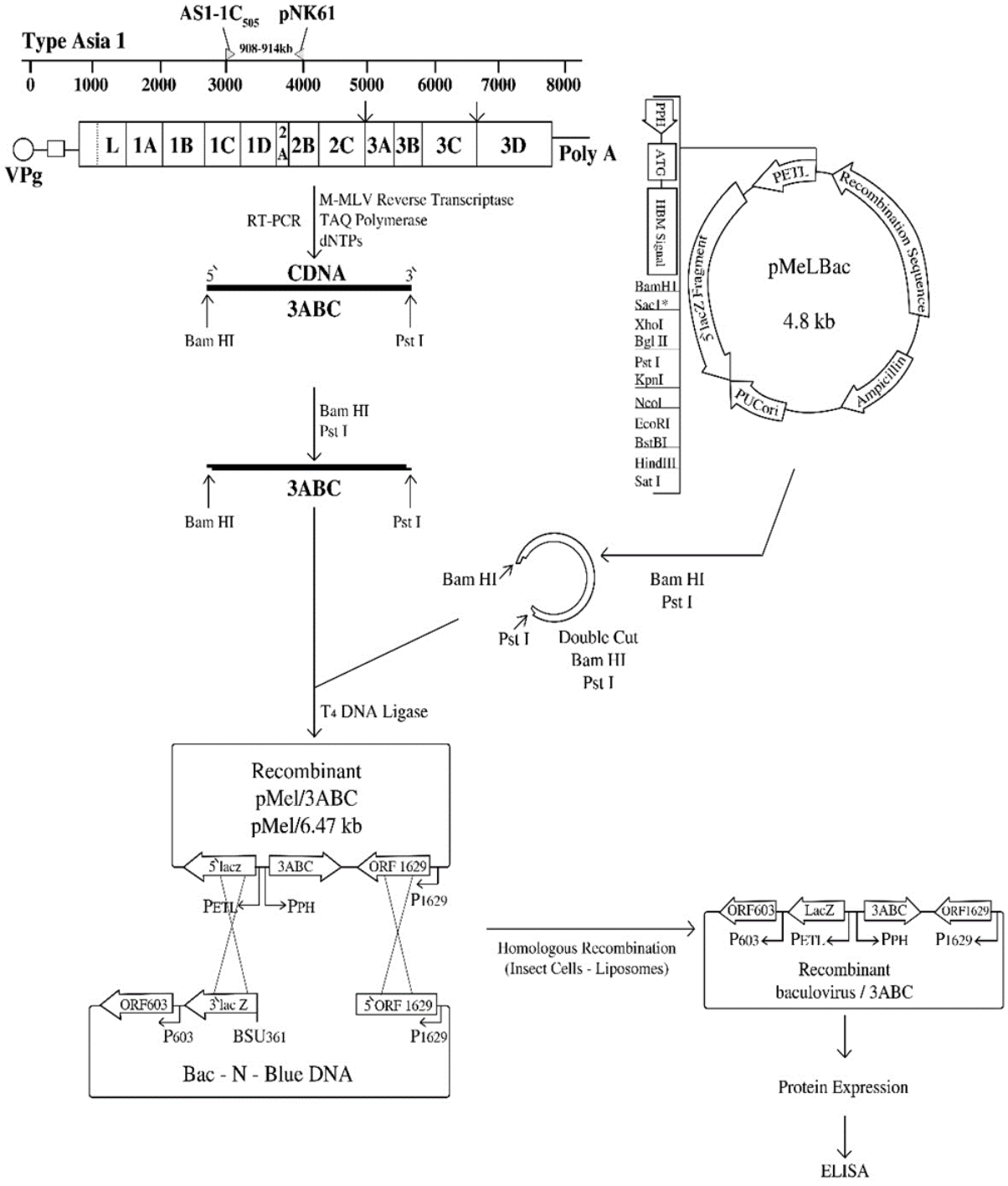


Figure (1) Strategy for Developing Recombinant Baculovirus Construct

2.4.1. Cloning of FMDV 3ABC encoding sequence

The plasmid pMelBac, manufactured by Invitrogen in the United Kingdom, is a baculovirus transfer vector measuring 4.821 kilobase pairs (kbp) in size. The process permits the incorporation of exogenous genes by positioning them alongside the honeybee melittin secretion signal and regulating their expression using the powerful polyhedrin (PH) promoter. In order to generate the recombinant plasmid, the 3ABC cDNA fragment and the pMelBac transfer vector were both digested using the BamHI and PstI enzymes from TOYOBO, Japan. Afterwards, the samples were purified from agarose gel as previously described and then connected through ligation using a DNA ligation kit (Fermentas, Canada), following the directions provided by the manufacturer. The ligation reaction was subsequently introduced into XL-1 Blue competent bacteria (Stratagene, USA). The genetically modified bacteria were cultivated in LB broth using a shaking incubator (N-BioTek, South Korea) for a duration of 2 hours. Subsequently, they were evenly distributed onto LB agar plates supplemented with ampicillin (50 µg/ml) and incubated overnight at 37°C. The ampicillin-resistant clones that emerged were individually chosen and examined for the existence of the recombinant plasmid (rec pMelB/3ABC) containing the 3ABC encoding sequence inserts. This assessment was performed by employing a double BamHI-PstI restriction digestion and PCR analysis with the aforementioned 3ABC-specific primers.

2.4.2. Generation and purification of the recombinant baculovirus construct

Log-phase Sf 9 cells were co-transfected in a 25 ml tissue culture flask (Greiner, Germany) using the Bac-N-Blue baculovirus transfection kit (Invitrogen). The co-transfection involved the use of wild-type baculovirus genomic DNA and purified rec pMelB/3ABC DNA. HiFect® transfection reagent (Lonza, USA) was used to facilitate the process, following the instructions provided by the manufacturer. The recombinant virus was obtained during a period of 3-6 days following transfection. To distinguish it from the non-recombinant virus, a selection procedure was utilized. This involved the use of the X-Gal-based plaque assay, which allowed for the identification of positive blue plaques. Every single one of these blue plaques was individually separated and moved to newly seeded log-phase Sf 9 cells in 12-well plates. After the cytopathic effects (CPE) became visible within 72 hours, the culture supernatants were collected to assess the purity of the recombinant baculovirus/3ABC (recBac/3ABC) using the following procedure. The partially purified recBac/3ABC harvests were subjected to multiple rounds of plaquing (4 rounds)

until a completely pure recBac/3ABC virus was obtained. The purity of the virus was then assessed to establish the P-1 stock.

2.5. Analyses of the recBac/3ABC.

2.5.1. PCR

In order to confirm the presence of the recombined 3ABC encoding sequence in the recBac/3ABC progeny virus and ensure its complete purification, viral DNA from the purified virus stock was extracted using the Jet Quick Blood and Cell Culture Spin Kit (Genomed, Germany). Afterwards, polymerase chain reaction (PCR) was conducted to amplify the 3ABC complementary DNA (cDNA) insert using the initial 3ABC primers. Subsequently, a second round of polymerase chain reaction (PCR) was performed to confirm the integrity of the recBac/3ABC by amplifying the 3ABC insert and the adjacent baculovirus sequences using the Polyhedrin (PH) PCR primers from Invitrogen, UK. Both amplification processes were carried out in accordance with the manufacturer's instructions. The PCR amplicons (20 µl each) obtained were examined using 1% agarose gel electrophoresis, following the previously reported method **[18]**.

2.5.2. Indirect immunofluorescence assay (IFA)

The IFA approach was implemented with several adaptations, adhering to the established protocol **[17]**. Concisely, log-phase Sf 9 cells were placed in Lab-Tek II 8-well Chamber slides (Nalge, USA) in pairs, with each well holding 0.05 ml of pure rec Bac/3ABC. The cells were subsequently placed in an incubator and kept at a temperature of 27°C for 48 hours. Each plate contained two rows that acted as controls for non-infected cells. After the incubation period, the Sf 9 cells were washed with phosphate-buffered saline (PBS, pH 7.6), fixed, and then washed again with PBS. Afterwards, they were exposed to a reference anti-FMD positive serum (VMRD, USA) at a temperature of 27°C for a duration of 1 hour. Following this, the Sf 9 cells were rinsed again with PBS (pH 7.6) and then exposed to fluorescein isothiocyanate (FITC) labeled anti-bovine IgG (KPL, USA) at a concentration of 1:50 in PBS. The slides underwent three rounds of washing with PBS, were then mounted with a glycerol solution (in a ratio of 4 parts glycerol to 1 part PBS), and subsequently examined using fluorescence microscopy.

2.5.3. Preparation of antigen

The log-phase Sf 9 cells (2.5×10^6 cells/ml) were inoculated with the recombinant baculovirus rec3ABC at a multiplicity of infection (m.o.i.) of 10 in a 75 cm^2 tissue culture flask. The flask was then incubated at 27°C until at least 90% of the cells showed signs of infection, which usually occurred within 2 to 3 days. The infected cell culture underwent three freeze-thaw cycles, and the resultant culture supernatant was collected using centrifugation at 10,000 xg for 10 minutes at 4°C. In order to prepare the culture supernatant for future use, it was diluted in PBS (pH 7.4) that contained 0.1% Triton X-100 (Sigma, USA), 3% BSA (Sigma), and 1% Protease inhibitor 100x mix (Serva, Germany). The solution was agitated for 1 hour at a temperature of 4°C and subsequently purified using cold centrifugation at a force of 14,000 times the acceleration due to gravity for a duration of 3 minutes. The cleared supernatant obtained was separated into smaller portions and preserved at a temperature of -70°C as crude recombinant antigen (rec3ABC). The ideal timing for protein expression and the protein content were established by collecting the rec3ABC virus from infected Sf 9 insect cells every 12 hours after infection.

2.5.4. Molecular characterization and biological reactivity of the expressed protein rec3ABC

2.5.4.1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot.

The identity and reactivity of the expressed recombinant protein (rec3ABC) were determined using SDS-PAGE and western blot analyses, following the methodology described before **[18]**. In brief, the protein constituents of clarified crude antigens, rec3ABC were separated by 10% SDS-PAGE gel electrophoresis in duplicates, then one gel was stained with 0.25% Coomassie brilliant blue R250 solution and examined on white fluorescent trans-illuminator. In contrast, the remaining gel was transferred onto a nitrocellulose (NC) membrane (Bio-Rad, Italy) and then electro-transferred using a semi-dry blotter (Biometra, Germany). Afterwards, the NC membrane was examined using an FMDV anti-3ABC antibody (IDEXX Laboratories, USA) for 2 hours at a temperature of 37°C. It was then washed in Tris-buffered saline with 0.05% Tween 20 (TTBS) and blocked with a 5% non-fat milk buffer (in TTBS) for 1 hour. Following additional TTBS washing procedures, the membrane was subjected to the horseradish peroxidase-labeled

recombinant protein G conjugate (Bio-Rad, Italy) for 1 hour at 37°C. After thorough washing, the resultant reaction was observed using the DAB solution substrate (Pierce, USA).

2.5.4.2. Enzyme linked- Immunosorbent Assays (ELISAs).

2.5.4.2.1. Indirect 3ABC-specific ELISA

The indirect 3ABC-ELISA was conducted to confirm the antigenic integrity and biological reactivity of the rec3ABC protein. This was done to initially assess its diagnostic potential for detecting FMDV anti-3ABC antibodies. The experiment utilized the previously indicated produced recombinant coated ELISA antigen (rec3ABC) using the previously reported approach **[19]** with certain changes. Various checker-board titrations were conducted to improve the ELISA and achieve a minimal background. These experiments involved manipulating the kinds, pH, volumes, and concentrations of buffers and reactants, serum dilutions, washing conditions, and cut-off values. Optimal conditions were obtained by diluting the rec3ABC antigen at a ratio of 1:200 in PBS solution containing 0.05% Triton X-100. 96-well microtitration plates (Nunc, USA) were coated with 100 µl of the diluted rec3ABC antigen per well and incubated at a temperature of 4 ◦C overnight. The plates were cleansed using PBS with 0.1% Tween 20 (PBST), then obstructed by adding 100 µl/well of blocking solution (PBS, pH 7.4 with 5% non-fat milk and 3% bovine serum albumin, BSA), and last incubated for 2 hours at 37 ◦C. The plates were poured out and cleaned three times as previously. The bovine serum samples were diluted at a ratio of 1:100 in the PBS solution containing 3% BSA. Then, 100 µl of each diluted sample was inoculated into four separate wells. Each plate consisted of positive and negative sera, along with a blank control. The plates were placed in an incubator at a temperature of 37 ◦C for a duration of 2 hours. Afterward, the liquid was poured off, and the plates were rinsed. The conjugate, horse radish peroxidase-labeled anti-bovine IgG (KPL, USA), was diluted to a concentration of 1:2000 in PBS solution containing 3% BSA. Then, 100 µl of this diluted conjugate was applied to each well. Following 1-hour incubation at a temperature of 37 ◦C, the plates were emptied, rinsed, and then 100 µl of the TMB-ELISA substrate (KPL, USA) was introduced into each well. The plates were then stirred until the color appeared which took around 10–15 minutes. The reaction was halted by introducing 100 µl per well of a stopping solution (KPL, USA). The plates were analyzed using a spectrophotometric computer-assisted microplate reader (Vmax kinetic microplate readers, Molecular Devices) at a wavelength of 450 nm. The ELISA cutoff point was determined to be an

Absorbance value (OD) of 0.2. Consequently, an OD value of 0.2 or more was considered positive for anti-3ABC antibodies, whereas a value below 0.2 was considered negative.

2.5.4.2.2. Blocking 3ABC-ELISA

The FMDV 3ABC antibody test ELISA kit, a commercial product from IDEXX Laboratories in the USA, was used in this study. The kit was employed according to the manufacturer's instructions for performing and interpreting the ELISA. As per the manufacturer's instructions, the anti-3ABC ELISA titers were reported as a percentage (%). A serum sample with a value of 30% or more was classified as positive, while a sample with a value below 30% was declared negative.

3. Results

3.1. Amplification and cloning of 3ABC encoding sequence from FMDV O_1.

The RT-PCR amplification of full-length 3ABC encoding RNA fragment of FMDV O_1 yielded a cDNA PCR product (amplicon) at the expected size of approximately 1.67 Kbp (Figure 2).

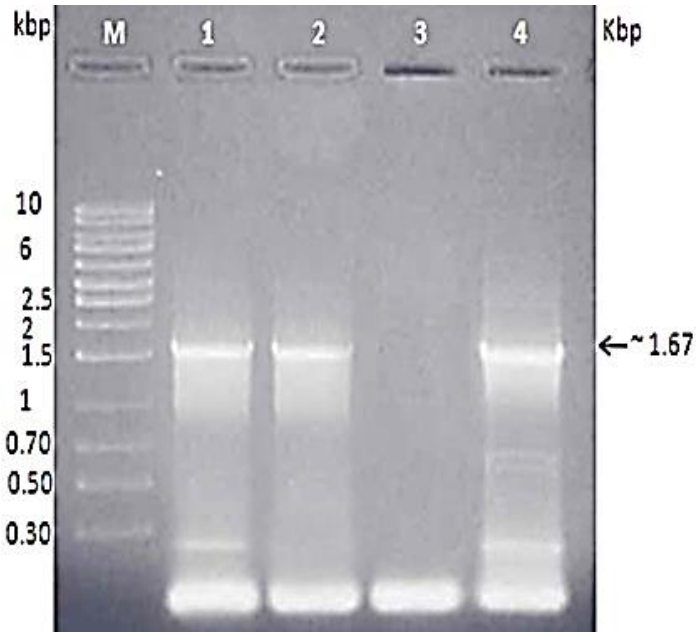


Figure (2). 1% Agarose gel electrophoresis of the RT-PCR amplicons derived from the 3ABC encoding sequence of FMDV O1 using specific 3ABC amplification primers. Lanes: (M) 1Kb DNA ladder (Axygen, USA); (1, 2 and 4) are FMDV O1 Samples; (3) is a control negative sample. Notice the size of the RT-PCR amplicons of about 1.67 Kbp.

After cloning steps of gel purified 3ABC cDNA amplicons, a 3ABC-based PCR analysis of recombinant plasmid (rec pMelB/3ABC) DNA, extracted from positive bacterial clones grown on selective LB-ampicillin agar plates, was done to verify presence of the 3ABC insert. The resulted PCR amplicons were approximately 1.67 kbp, matching the expected size of the 3ABC coding sequence (Figure 3). This result assured successful cloning of the entire 3ABC cDNA fragment into the pMelBac transfer plasmid.

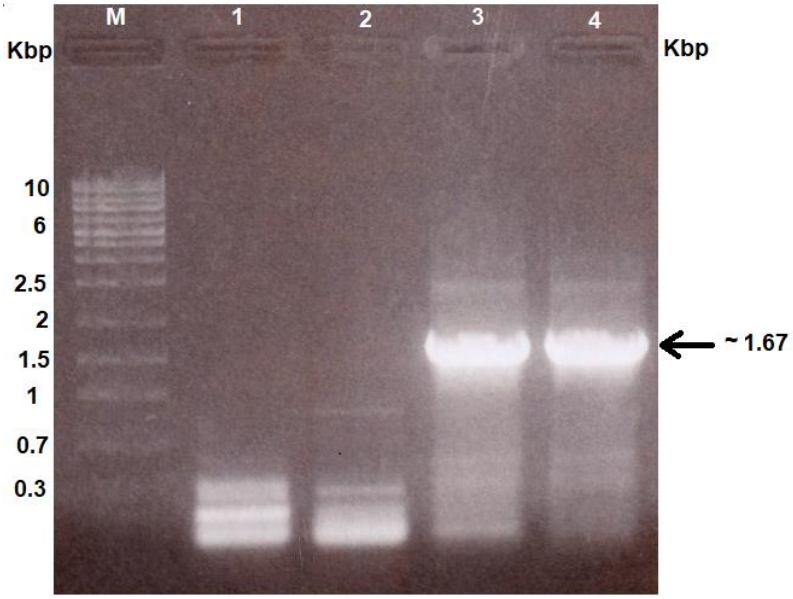


Figure (3): 3ABC-based PCR analysis of the recombinant plasmid (rec pMel B/3ABC) using 3ABC specific primers. 1% agarose gel stained with ethidium bromide. Lanes: (M) 1Kb DNA ladder (Axygen, USA); (1, 2) non-recombinant pMelBac DNA; (3, 4) rec pMel B/3ABC cDNA.

Furthermore, the recombinant plasmid DNAs isolated from positive clones was analyzed using double BamHI - PstI restriction enzyme digestion. This analysis revealed two distinct bands after digestion, correspondent to the expected sizes of 4.82 Kbp and ~ 1.67 Kbp for pMelBac vector DNA and 3ABC insert cDNA, respectively. Whereas, the non-recombinant pMelB plasmid showed only the 4.82 Kbp band with no insert (Figure 4).

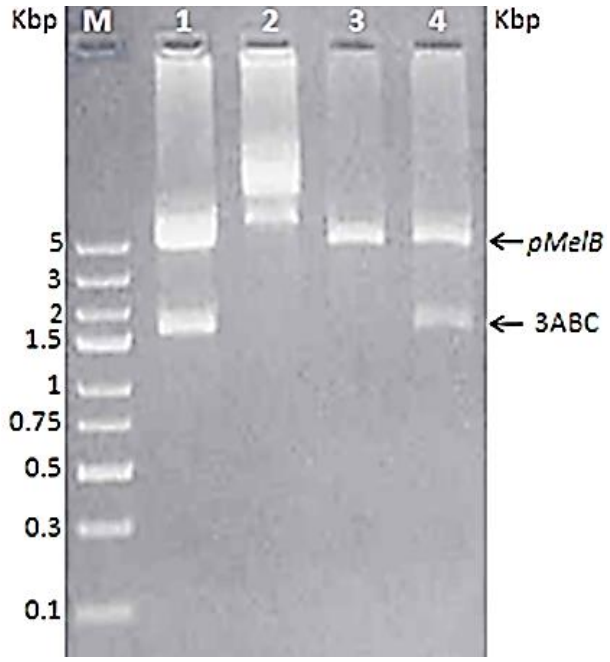


Figure (4). Restriction endonuclease analysis by double digestion of the recombinant plasmid (rec pMelB/3ABC) using BamH I and Pst I enzymes. 1% agarose gel stained with ethidium bromide. Lanes: (M) Gene Ruler Express DNA Ladder (Fermentas); (1, 4) double cut rec pMelB/3ABC; (2) uncut recpMelB/3ABC; (3) cut non-recombinant pMelB. Notice the sizes of the pMelB cut plasmid DNA (4.82Kbp) and the 3ABC cDNA insert (1.67 Kbp).

3.2. Generation and purification of the recombinant baculovirus construct

Monitoring insect Sf 9 cells transfected with the rec pMelB/3ABC, showed appearance of the cytopathic effect (CPE) signs within 24 hours' post-transfection (PT), manifested by increased cell width and nucleus size. That was followed by more cellular changes including growth inhibition, granular appearance, detachment, and eventually cell lysis, through 2 to 7 days PT (Figure 5A). In contrast, non-transfected control Sf 9 cells did not exhibit any sign of CPE (Figure 5B).

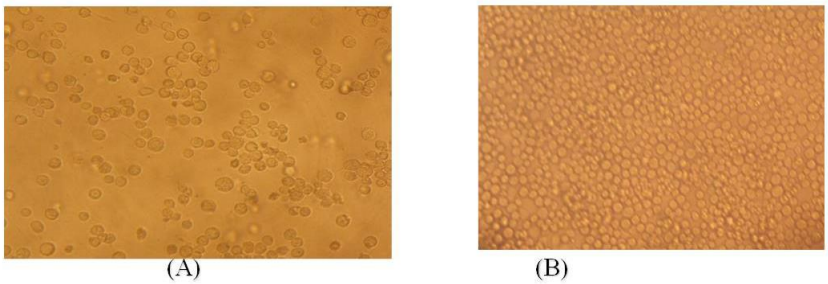


Figure (5). (A) The characteristic cytopathic changes appeared in the transfected Sf9 cells. (B) The normal non-infected Sf 9 cells showing no cytopathic changes under low power (100X).

PCR screening of recombinant baculovirus (recBac/3ABC) DNA from starting putative PT blue plaques, using polyhedrin (PH) PCR primers, revealed the presence of an 839 bp amplicon, representing a PCR PH fragment of wild-type (WT, non-recombinant) baculovirus, contaminating that of the recBac/3ABC amplicon (~ 1.98 kpb). After 5 successive rounds of plaque assay and 11 rounds of *In vitro* cloning, a 100% pure rec Bac/3ABC was successfully isolated as confirmed by PH- PCR analysis that revealed merely the 1.98 kpb amplicon of the recBac/3ABC DNA (Figure 6).

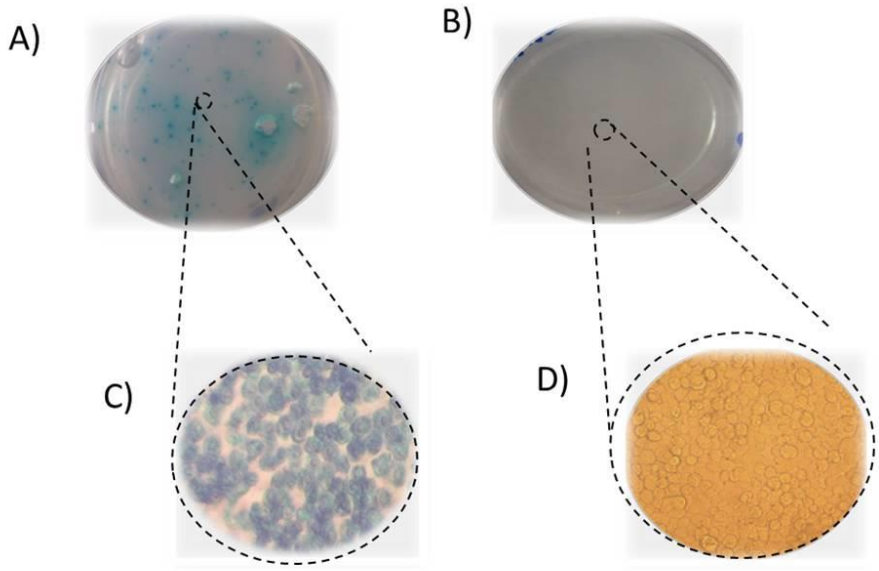
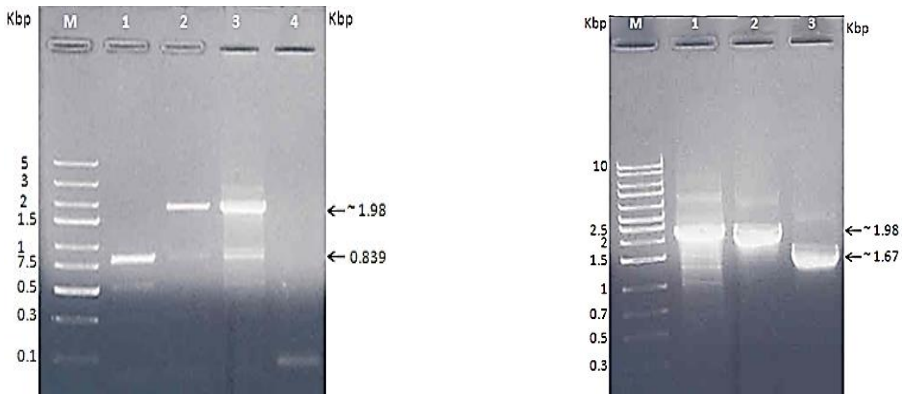


Figure (6): Plaque purification of the recombinant baculovirus (recBac/3ABC) in log-phase Sf 9 cells overlaid with baculovirus agarose containing X-Gal in 6-well tissue culture plates. Notice the microscopic appearance of recombinant blue plaques versus the non-recombinant unstained background of insect cells. (A) Transfected cells with rec Bac / 3ABC; (B) Control Sf 9 cells; (C) Microscopically examination of obtained plaques for recombinant baculovirus in Sf 9 cells, (D) Control Sf 9 cells at high power (300X).

Consequently, P-1 stock was obtained, titrated, and kept as 100% pure rec Bac/3ABC virus, with a titer of $10^{5.5}$ pfu/ml. Furthermore, the specific 3ABC-PCR of purified recBac/3ABC DNA was conducted that confirmed presence of the 3ABC insert intact and purity of P-1viral stock (Figures 7A and B). Additionally, integrity, identity, and proper orientation of the cloned 3ABC encoding sequence were verified by cDNA nucleotide sequence analysis, utilizing baculovirus PH sequencing primers.



A) **B)**

Figure (7): A) PCR screening of starting putative plaques of rec Bac / 3ABC DNA using polyhedrin (PH) PCR primers. 1% agarose gel stained with ethidium bromide. Lanes: (M) Gene Ruler Express DNA Ladder (Fermentas, USA); (1) non-recombinant baculovirus showing only the wild baculovirus DNA PCR fragment (839bp); (2, 3) Non-purified rec Bac / 3ABC fragment (~ 1.98 Kb) plus the baculovirus DNA PCR fragment (839bp); (4) non-infected Sf 9 cell culture supernatant control negative. B) PCR analysis of rec Bac / 3ABC DNA after several plaquing Lanes: (M) 1 Kb DNA Ladder (Axygen, USA); (1) Partially purified rec Bac / 3ABC; (2) Purified rec Bac / 3ABC viral stock using PH primers; (3) 3ABC PCR amplicon from purified rec Bac / 3ABC viral DNA using specific 3ABC amplification primers.

3.3. Authenticity and cellular localization of the recombinant polyprotein 3ABC (rec 3ABC)

The indirect immunofluorescence (IFA) staining of Sf 9 cells infected with the recombinant baculovirus (rec3ABC) exhibited robust greenish fluorescence on the cell surfaces and around the cell nuclei (Fig. 8). This distinct response indicated that the rec3ABC expressed protein was authentic as it was positively reacted to the anti-3ABC positive serum and properly localized within Sf 9 cells.

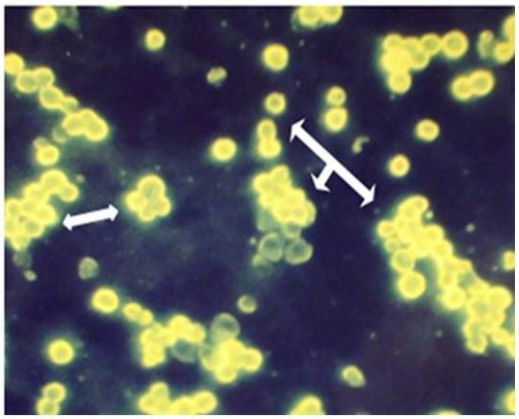


Figure (8): Indirect immunofluorescence staining of the recombinant 3ABC protein reacted with anti-FMDV-3ABC antibody (IDEXX) in Sf 9 cells infected with purified rec3ABC.

3.4. Characterization of the expressed recombinant protein (rec3ABC)

The recombinant polyprotein 3ABC (rec3ABC) produced in Sf 9 cells became visible on SDS-PAGE gel between 20 to 72 hours after infection, appearing at an approximate size of ~ 60 kDa (Figure 9).

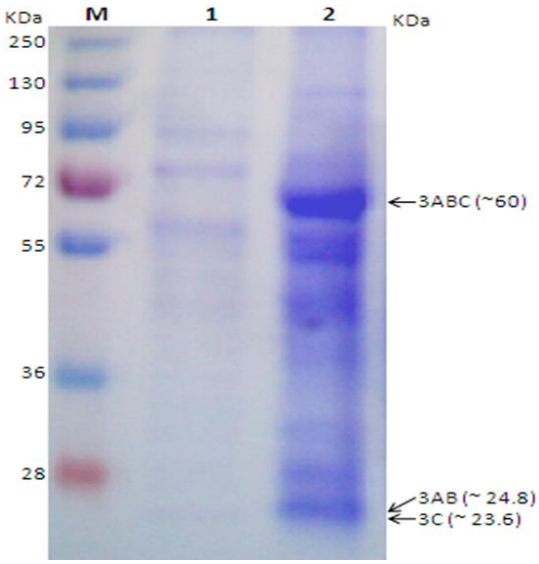


Figure (9): A) SDS-PAGE analysis of lysate recovered post-infection (PI) of Sf 9 cells infected with rec3ABC. Samples were electrophoresed through 10% SDS-PAGE and stained with 0.25% Coomassie brilliant blue R250 Lanes: (M) PageRuler prestained protein ladder (Fermentas, USA); (1) non-infected Sf 9 cells; (2) cell lysate harvested at 72 PI, respectively. (Size is ~ 60 KDa).

The optimal time for rec3ABC expression was determined to be 72 hours' post-infection (PI) in insect Sf 9 cells. In Western blot analysis, the antigenic preparation of rec3ABC evidenced a positive biological reactivity with the anti-FMDV-3ABC antibody, illustrating a molecular weight of around 60 kDa. Notably, there was no reaction observed between the anti-FMDV-3ABC antibody and the crude antigen derived from Sf 9 cells infected with the non-recombinant baculovirus (Figure 10).

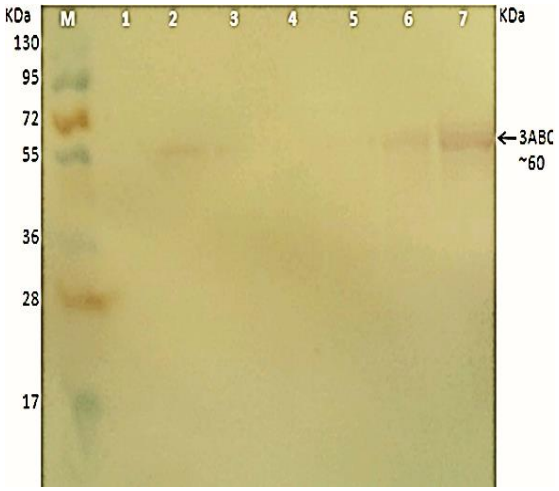


Figure (10): Western blot analysis of lysates recovered post-infection (PI) of Sf 9 cells infected with rec3ABC. Samples were electrophoresed through 10% SDS-PAGE, semi-dry electroblotted on NC membrane reacted with Anti-FMDV-3ABC Ab (IDEXX Labs). Lanes: (M) PageRuler prestained protein ladder (Fermentas, USA); (1) non-infected Sf 9 cells; (2-6) infected Sf 9 cell lysate harvested at 18, 24, 36, 48, 60 and 72 PI, respectively. (Size is ~ 60 KDa).

3.5. ELSA findings

As indicated in Table 1 and Fig. 11, there was a relative proportion of estimated anti-FMDV Ab expressed as OD values and ELISA % obtained by both 3ABC-indirect and 3ABC-commercial ELISAs, respectively. Sera collected from naive calves (zero days) and commercial control sera (FCS and NCS) tested negative for anti-FMDV Ab using both assays.

The anti-3ABC monoclonal antibody (mAb) dilutions $1:10^{-1}$ and $1:10^{-2}$ exhibited positive anti-FMDV Ab manifested as mean OD values of 1.03 and 0.92 by indirect 3ABC-ELISA as well as 346% and 100% by Commercial 3ABC-ELISA, respectively. Whereas its dilutions $1:10^{-3}$ and $1:10^{-4}$ tested negative by both ELISAs.

Sera from experimentally infected calves displayed consistent positive anti-FMDV Ab values.

That was determined as mean ELISA % of 34.4% by the commercial 3ABC-ELISA and mean OD value of 0.40 by the developed 3ABC-ELISA.

All serum samples from experimentally FMDV vaccinated cattle (4 WPV) also tested seropositive by both ELISAs, as the commercial 3ABC-ELISA estimated a mean ELISA percentage of 31.2%, while the indirect 3ABC-ELISA showed a mean OD value of 0.379.

Table (1). Summary of comparative mean reactivity of different bovine sera tested by commercial 3ABC-ELISA and developed 3ABC-ELISA

Serum Samples	**A. Positive Sera:**						**B- Negative Sera:**		
	1. Experimentally Infected sera© (n=15)	2. Anti-3ABC				3. Experimentally vaccinated sera¶ (n=45)	1- FCS€ (PAA) (n=3)	2- NCS₰ (GIBCO) (n=3)	3- Naïve sera¥ (control zero day) (n=15)
		1:10	1:100	1:1000	1:10000				
3ABC-Indirect ELISA IDEXX (Commercial Kit) Mean ELISA %**	34.4 ± 0.176%	346.0 ± 0.13%	100.0 ± 0.11%	6.90 ± 0.08 %	0.00 ± 0.05%	31.2 ± 0.17%	0.77 ± 0.07%	14.4 ± 0.07%	16.3 ± 0.04%
3ABC-Indirect ELISA (Developed) Mean OD value*	0.403 ± 0.13	1.030 ± 0.09	0.920 ± 0.101	0.088 ± 0.05	0.072 ± 0.018	0.379 ± 0.12	0.095 ± 0.06	0.105 ± 0.13	0.125 ± 0.05

* = A serum sample with a mean OD value of ≥ 0.2 was regarded as positive by the developed indirect 3ABC-ELISA.

**= A serum sample of an ELISA % with ≥ 30% was regarded as positive by the commercial 3ABC-ELISAs (IDEXX). The ELISA % for each sample was calculated as follows:

$$\text{ELISA (\%)} = \frac{OD_{samples} - OD_{negative}}{OD_{positive} - OD_{negative}} \times 100\ \%$$

© = Experimentally infected with serotypes O, A, and SAT 2, (n=15; 5 of each serotype).

¶ = Experimentally vaccinated calves 4 weeks' post-vaccination (n=45; 15 of each serotype).

€ = Fetal Bovine Serum (PAA)

³ = Newborn Calf Serum (Gibco)

¥ = Sera from naive calves (tested free from anti-FMD Ab) before vaccination (n=15; 5 of each serotype)

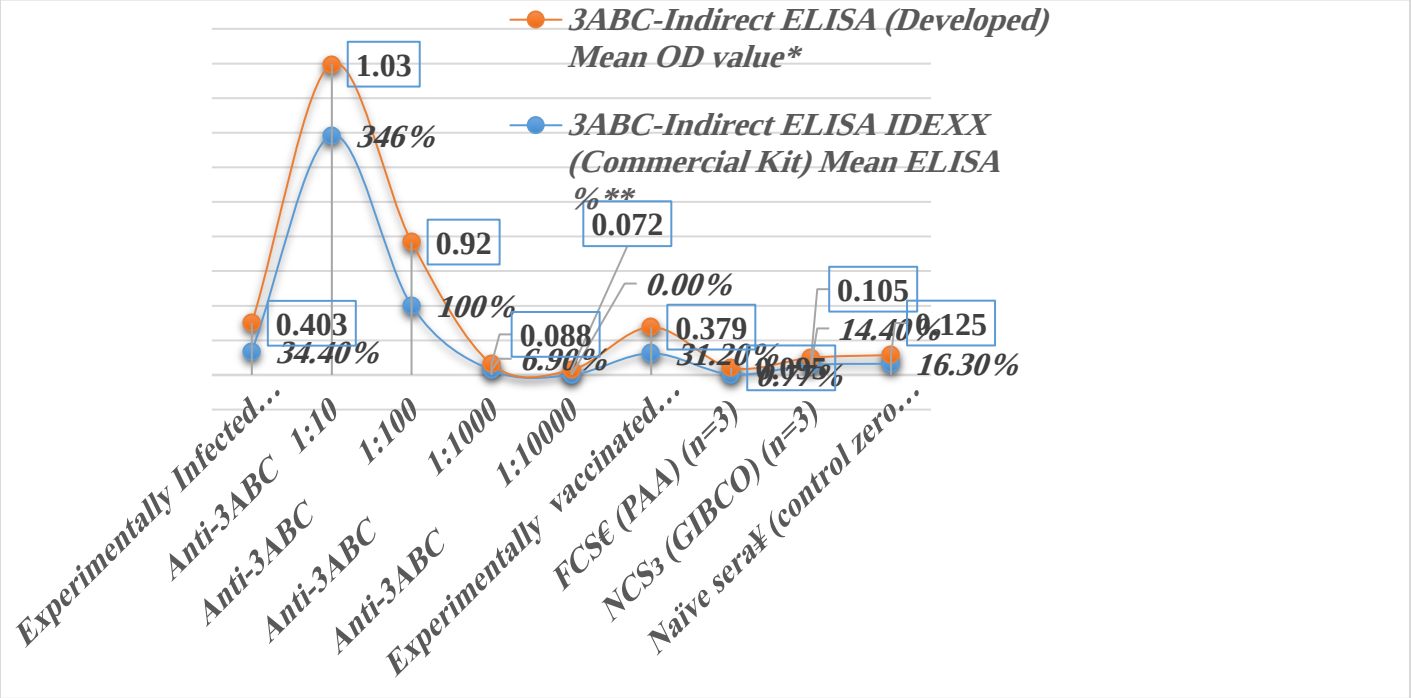


Figure (11): Comparison between the mean reactivities of bovine sera tested by commercial 3ABC-ELISA and developed 3ABC-ELISA

4. Discussion

Due to its extensive global distribution among all species of cloven-hoofed animals and its damaging influence on bovine trade and industry, Foot and Mouth Disease Virus (FMDV) has been recognized as one of the most terrifying livestock viral diseases. To assure the eradication of infected animals while keeping others safe, countries have developed a combination of vaccination programs together with test and slaughter rules. Therefore, routine serological herd testing should be used to identify such persistently infected, carrier and immune status of vaccinated animals **[20, 21]**.

FMDV serotypes are known for their significant variations in antigenic domains, which pose challenges for suitable serodiagnosis of FMDV. Therefore, there is a pressing need for a reliable tool capable of monitoring seroconversion to FMDV, irrespective of its serotype. This study's primary goal was to locally clone and express a recombinant NSP-3ABC of FMDV, a highly conserved polyprotein amongst all FMDV serotypes as antibodies (Ab) against the non-structural 3ABC protein are elicited early after infection and persist in circulation for a longer duration compared to other anti-NSP Abs **[21]**. That enables the detection of anti-3ABC antibodies for any FMDV serotype. This recombinant protein was generated using a baculovirus expression system, to be used as a coating antigen in a simple indirect ELISA for detecting anti-FMDV antibodies, regardless its serotype. In Egypt, the natural open geographical borders, importing animals from regions enzootic with diverse FMDV serotypes, and unregulated breeding practices, made control of FMD mostly challenging. Consequently, animals that are not vaccinated in a timely manner remain susceptible to diverse FMDV infections, complicating efforts to track efficacy of FMDV vaccination regarding immune status in these animals and distinguish infected from vaccinated animals based on seroconversion **[22-25]**.

For the expression of the FMDV polyprotein 3ABC, we initiated the process by amplifying the 3ABC encoding sequence from FMDV serotype O_1 (Mannisa, a vaccine strain). This amplification was achieved through RT-PCR, employing primers with a considered design that included two distinct restriction enzyme sites, namely BamHI and PstI, at the sequence's 5' and 3' ends. This strategic design facilitated the directional cloning process. The amplified 3ABC cDNA fragment was confirmed to be of the expected size, approximately 1.67 kbp. This fragment was successfully gel purified and cloned into the pMelBac transfer vector. Further, findings of

restriction endonucleases and PCR analyses of the resulted recombinant vector demonstrated reliability of the used strategy of directional cloning. This method effectively prevents the insert from self-replicating or self-ligating and ensures the correct orientation of our cloned insert in the chosen positive clones. This outcome aligns with the results reported before **[26-29]**.

The cloned 3ABC cDNA fragment was successfully expressed in baculovirus - insect cells system with high frequency. In that regard, Sf9 insect cell line was chosen to simplify post-transfection CPE recognition. Sf9 cells exhibit a round and uniform shape with distinct sizes, which enables clear detection of virus infection-related changes as shown in figures (6) and supported before **[30]**. Also, Bac-N-Blue with its triple-cut-linearized AcMNPV DNA was selected to avoid DNA self-ligation and to enhance its essential recombination between homologous DNA sequences of transfer vector ORF1629 (which is essential for baculovirus replication and assembly) and the 5' and 3' ORF603 fragments flanking the PH gene of DNA, with high frequency (up to 90%) of recombinant baculovirus progeny. That was in accordance with the findings reported before **[31-34]**.

PCR analysis of the plaque purified recombinant baculovirus (rec pMelB/3ABC) DNA assured that 3ABC encoding sequence was properly recombined integrally into the baculovirus genome, downstream the PH promoter and in-frame with the honeybee melittin secretion signal. This secretion signal has been reported to increase the secretion levels of heterogonous proteins five times more than the native signal, allowing better and correct protein expression. This result coincides with previous results obtained **[35]**.

The recombinant polyprotein rec3ABC had the expected molecular weight (MW) of approximately 60 kDa with reliable reactivity, as exhibited by immunological and biological analyses using SDS-PAGE and Western blot. Also, **Hosamani et al. [36]** and **Guliy et al. [37]** reported high reactivity of anti-FMDV Ab to the recombinant 3ABC as the most intact and biologically reactive expressed NSP. However, these analyses showed other specific protein fractions at the sizes of about 24.8 kDa and 23.6 kDa, which were referred to be posttranslational cleaved recombinant 3AB and 3C NSP, respectively. That was consistent with previous findings reported before by **Brychcy et al., [21]**, who showed that the 3C protein is a significant viral protease that can completely cleave the produced protein and cleaves in 10 to 13 sites across the

viral polyprotein, and confirmed that the size of polyprotein 3ABC is of about 60 kDa. Compatibly, the utilized baculovirus system expressed a high harvest of soluble rec3ABC. Whereas, other bacterial systems produced low yield and barely soluble recombinant 3ABC due to high concentrations of hydrophobic regions within expressed polypeptides causing protein instability, aggregation, with formation of inclusion bodies **[38]**. While, they were mostly hydrophilic when expressed in the baculovirus system **[24, 39]**.

Moreover, Result of the *in situ* IFA in infected Sf9 cells revealed that expressed rec3ABC was of an authentic and biologically active as it specifically reacted to the anti-FMDV Ab as revealed by peri-nuclear cytoplasmic staining, confirming its synthesis and translocation in insect cells as appropriate as it would be expected in its natural host cell. Similar findings have been reported that support our results **[40, 41]**. All that would strongly support our objectives and the strategy used to produce an authentic rec3ABC that is biologically reactive with high yield and superior solubility.

The potential diagnostic value of the virus supernatant from Sf9 infected cells containing the secreted recombinant protein (rec3ABC) was assessed by standard indirect ELISA. As the rec3ABC was used as a coating ELISA antigen, its 1:100 dilutions in PBS containing 0.001% Triton X-100, and an overnight blocking with 5% non-fat milk at 4^{o}C, were found to be the optimal conditions for detection of anti-FMDV Ab in bovine sera by indirect ELISA. Obtained data demonstrated that the developed rec3ABC-based indirect ELISA was effective in detecting anti-3ABC Ab, at various dilution levels, in all tested control negative and positive serum samples including those from animals experimentally infected and vaccinated with different FMDV serotypes (O, A, and SAT 2). Notably, results of locally developed 3A indirect ELISA were in harmony with that of the commercial ELISA. Also, variations in data obtained through our developed 3ABC-Indirect ELISA, compared to the Commercial 3ABC-blocking ELISA (IDEXX), were statistically significant (<0.05), with a reasonable correlation ($R \geq 0.85$), featuring the efficacy of our developed assay. Nevertheless, production of the rec3ABC was inexpensive. A 75 cm^2 flask containing 2.5x10^6 Sf 9 cells provided antigens adequate for coating 10 standard 96-well plates. This was sufficient for testing 440 serum samples in duplicate. The virus supernatant used for coating the ELISA plates apparently did not interfere with the ELISA test. Findings obtained by both local and commercial 3ABC-ELISAs came in convention with previous reports

that emphasized the importance of NSP/3ABC-ELISAs to distinguish seropositive animals with potential differentiation of vaccinated from those infected with FMDV regardless its serotype **[42]**.

Therefore, the baculovirus-insect cell produced recombinant 3ABC protein of FMDV was proved reliable to be utilized as a coating antigen in a simple local indirect ELISA for detection of antibodies against FMDV regardless its serotype. Furthermore, it provides a safe alternative to risky handling of live FMDV in diagnostic laboratories, for checking previous or current infection in non-vaccinated animals, to certify animals prior to movement (trade purposes), and sero-monitoring of FMDV vaccinated animals. That might be functional for the assessment of FMD vaccination programs and the quality control of FMDV vaccines.

5. Conclusion

Our research has produced a non-infectious antigen that allows the detection of FMDV antibodies, irrespective of the serotype, using ELISA. The developed 3ABC indirect-based ELISA exhibits significant potential for adaptation into a rapid, on-site test that can be conducted on farms or at animal quarantine facilities. This advancement would diminish the reliance on inactivated FMDV antigen in diagnostic tests, subsequently reducing biosecurity risks and broadening the applicability of assay reagents across different geographical regions. The rec3ABC, a notably conserved non-structural protein among various FMDV serotypes, serves as a suitable ELISA coating antigen for rapid serotype-independent screening of sera from both infected and vaccinated animals. This advance is particularly valuable for quality control assessments of polyvalent inactivated vaccines by enabling the quick examination of animals before and after vaccination. Furthermore, it substantially reduces the time and costs associated with detecting seroconversion against various FMDV serotypes, while also avoiding the risks associated with live virus use. Additionally, it emphasizes the efficacy of the FMDV 3ABC-based ELISA in identifying FMDV antibodies in sera from experimentally infected and vaccinated animals, regardless of the specific FMDV serotype. This is invaluable for confirming past or continuing infections in non-vaccinated herds or populations and certifying animals before their movement for trade purposes.

6. Authors' contributions

The authors participated equally in all parts of the research.

7. Conflict of interest

The authors declare that they have no conflict of interest.

Reference

[1] K. Li, C. Wang, F. Yang, W. Cao, Z. Zhu, H. Zheng, "Virus–host interactions in foot-and-mouth disease virus infection". Front Immunol 12: (2021) 571509.

[2] A. H. Bazid, H. M. Amer, M. Nayel, M. Attia, N. Maklad, M. Wasfy, Y. Badr, "Assessment of the potency and effectiveness of a heptavalent oil-adjuvanted (ISA 206) foot-and-mouth disease vaccine in Egypt". Arch Virol 168: (2023) 1-8.

[3] M. R. Herod, O. O. Adeyemi, J. Ward, K. Bentley, M. Harris, N. J. Stonehouse, S. J. Polyak, "The broad-spectrum antiviral drug arbidol inhibits foot-and-mouth disease virus genome replication". J General Virol 100: (2019) 1293-1302.

[4] S. A. Khulape, J. K. Biswal, C. Jana, S. Subramaniam, R. P. Singh, "Novel pan-lineage VP1 specific degenerate primers for precise genetic characterization of serotype O foot and mouth disease virus circulating in India". J Vet Sci 24: (2023) 3.

[5] H. Keck, B. Litz, B. Hoffmann, J. Sehl-Ewert, M. Beer, M. Eschbaumer, "Full-Length Genomic RNA of Foot-and-Mouth Disease Virus Is Infectious for Cattle by Injection". Viruses 14: (2022) 1924.

[6] S. Bimrew, M. Abera, "Review on Classification and Nomenclature of Viruses". Sciences 11: (2023) 11-23.

[7] M. A. Zia, S. J. Dobson, D. J. Rowlands, N. J. Stonehouse, M. S. Shah, M. Habib, "Development of an ELISA to distinguish between foot-and-mouth disease virus infected and vaccinated animals utilizing the viral non-structural protein 3ABC". J Med Microbiol 71: (2022a) 001516.

[8] T. Haynie, "Recombinant and Vaccine Design as a Potential Strategy Against Bovine Foot and Mouth Disease Virus (FMDV)". (2023) DOI https://doi.org/10.31390/gradschool_theses.5772

[9] M. A. Zia, M. S. Shah, R. S. A. Khan, U. Farooq, J. Shafi, M. Habib, "High level expression and purification of recombinant 3ABC non-structural protein of foot-and-mouth disease virus using SUMO fusion system". Protein Express Purif 191: (2022b) 106025.

[10] C. L. Wong, C. Y. Yong, H. K. Ong, K. L. Ho, W. S.Tan, "Advances in the diagnosis of foot-and-mouth disease". Front Vet Sci 7: (2020) 477.

[11] M. Gobiye, G. T. Fosgate, L. Heath, D. D. Lazarus, L. Seoke, P. A. Opperman, "Preliminary validation of a single-spot version of a solid-phase competition ELISA for the detection of southern African territories foot-and-mouth disease serotype exposure in goats". Small Rumin Res 224: (2023) 106982.

[12] Y. Tang, J. Saul, N. Nagaratnam, J. M. Martin-Garcia, P. Fromme, J. Qiu, J. LaBaer, "Construction of gateway-compatible baculovirus expression vectors for high-throughput protein expression and in vivo microcrystal screening". Sci Rep 10: (2020) 13323.

[13] D. Howard, "Development of a Novel Fluorescent Reporter Baculovirus Vector for Testing Promoters in Lepidopterans". https://tigerprints.clemson.edu/all_theses/4041 (2023)

[14] C. Grose, Z. Putman, D. Esposito, "A review of alternative promoters for optimal recombinant protein expression in baculovirus-infected insect cells". Protein Express Purific 186: (2021) 105924.

[15] S. Schaly, M. Ghebretatios, S. Prakash, "Baculoviruses in gene therapy and personalized medicine". Biologics: Targets and Therapy (2021)115-132.

[16] X. Zhang, A. He, Y. Zong, H. Tian, Z. Zhang, K. Zhao, H. Chen, "Improvement of protein production in baculovirus expression vector system by removing a total of 10 kb of nonessential fragments from Autographa californica multiple nucleopolyhedrovirus genome". Front Microbiol 14: (2023) 1171500.

[17] A. B. Ludi, V. Mioulet, L. B. Kassimi, D. J. Lefebvre, K. De Clercq, E. Chitsungo, D. P. King, "Selection and use of reference panels: A case study highlighting current gaps in the materials available for foot and mouth disease. Diagnostic test validation science: a key element for effective detection and control of infectious animal diseases (A. Colling & IA Gardner, eds)". Rev Sci Tech Off Int Epiz 40: (2021) 239-251.

[18] J. Sambrook, D. W. Russell, "The condensed protocols from molecular cloning: a laboratory manual". Cold Spring Harbor Laboratory Press. http://www.loc.gov/catdir/toc/ecip0517/2005022077.html (2006)

[19] A. A. EL-Kholy, R. R. Abdo, D. I. Rady, M. M. Elseafy, "Baculovirus expression and diagnostic utility of the glycoprotein E of bovine herpesvirus-1.1 Egyptian strain Abu Hammad". J Virol 96: (2013) 1747-1754.

[20] F. C. Mansilla, C. S. Turco, M. C. Miraglia, F. A. Bessone, R. Franco, M. Pérez-Filgueira, A. V. Capozzo, "The role of viral particle integrity in the serological assessment of foot-and-mouth disease virus vaccine-induced immunity in swine". Plos One 15: (2020) e0232782.

[21], M. Brychcy, A. Kokodynski, D. E. Lloyd, V. G. Godoy, "AspFlex: molecular tools to study gene expression and regulation in Acinetobacter baumannii". BioRxiv 2023: (2023) 3.

[22] A. Clavijo, P. Wright, P. Kitching, "Development in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease". Vet J 167: (2004) 9-22.

[23] M. L. Fabre, P. N. Arrías, T. Masson, M. L. Pidre, V. Romanowski, "Baculovirus-Derived Vectors for Immunization and Therapeutic Applications". Emerg Reemerg Viral Pathog 2020: (2020) 197–224.

[24] A. B. Ghisaidoobe, S. J. Chung, "Functionalized protein nanocages as a platform of targeted therapy and immunodetection". Nanomedicine 10: (2015) 3579-3595.

[25] M. R. Green, J. Sambrook, "Cloning and transformation with plasmid vectors". Cold Spring Harbor Protocols, 2021 (2021) 11.

[26] O. I. Guliy, S. S. Evstigneeva, V. A. Khanadeev, L. A. Dykman, "Antibody Phage Display Technology for Sensor-Based Virus Detection: Current Status and Future Prospects". Biosensors 13: (2023) 640.

[27] W. S. Gwak, S. N. Lee, J. B. Choi, H. S. Kim, B. K. Han, S. M. Bae, Y. H. Je, S. D. Woo, "Baculovirus entire ORF1629 is not essential for viral replication". PloS One 14: (2019) e0221594.

[28] X. He, L. Lu, P. Huang, B. Yu, L. Peng, L. Zou, Y. Ren, "Insect Cell-Based Models: Cell Line Establishment and Application in Insecticide Screening and Toxicology Research". Insects 14: (2023) 104.

[29] M. Hosamani, S. H. Basagoudanavar, R. P. Tamil Selvan, P. Varsha Das, B. P. Sreenivasa, H. Raveendra, R. Venkataramanan, "A multi-species indirect ELISA for detection of non-structural protein 3ABC specific antibodies to foot-and-mouth disease virus". Arch Virol 160: (2015) 937–944.

[30] C. Kielkopf, W. Bauer, I. Urbatsch, "Expression of Cloned Genes Using the Baculovirus Expression System. Cold Spring Harbor protocols. 2020". https://doi.org/10.1101/pdb.prot102152 (2020).

[31] Q. Li, A. K. Wubshet, Y. Wang, L. Heath, J. B. Zhang, "T Cell Epitopes of the Incursionary Foot-and-Mouth Disease Virus Serotype SAT2 for Vaccine Development". Viruses 15: (2023) 797.

[32] Q. Ma, L. Zhou, L. Ma, K. Huo, "Directional and direct cloning strategy for high-throughput generation of recombinant baculoviruses:. https://doi.org/10.2144/000112258 (2006).

[33] A. P. Rodríguez-Hernández, D. Martínez-Flores, A. Cruz-Reséndiz, T. Padilla-Flores, R. González-Flores, K. Estrada, L. Vaca, "Baculovirus Display of Peptides and Proteins for Medical Applications". Viruses 15: (2023) 411.

[34] R. Salem, A. A. El-Kholy, F. R. Waly, R. Khaled, W. Elmenofy, "Removal of 3C protease from the 3ABC improves expression, solubility, and purification of the recombinant 3AB of foot-and-mouth disease virus". Virus Genes 57: (2021a) 72-82.

[35] S. A. Salem, A. H. I. Bazid, D. J. Abo El-Hassan, "Molecular and serological typing of foot-and-mouth disease virus serotypes currently circulating in Egypt". Iraqi J Vet Sci 35: (2021b) 581-588.

[36] K. J. Sorensen, K. de Stricker, K. C. Dyrting, S. Grazioli, B. Haas, "Differentiation of foot-and-mouth disease virus infected animals from vaccinated animals using a blocking ELISA based on baculovirus expressed FMDV 3ABC antigen and a 3ABC monoclonal antibody". Arch Virol 150: (2005) 805–814.

[37] D.C. Tessier, D.Y. Thomas, H.E. Kouri, F. Labierte, T. Vernet, "Enhanced secretion from insect cells of a foreign protein fused to the Honeybee Melittin Signal Peptide". Vet Microbiol 128: (1991) 261-8.

[38] M. Ullah, Y. Li, K. Munib, H. U. Rahman, Z. Zhang, "Sero-Epidemiology and Associated Risk Factors of Foot-and-Mouth Disease (FMD) in the Northern Border Regions of Pakistan". Vet Sci 10: (2023) 356.

[39] X. Wu, L. Chen, C. Sui, Y. Hu, D. Jiang, F. Yang, J. Qi, "3Cpro of FMDV inhibits type II interferon-stimulated JAK-STAT signaling pathway by blocking STAT1 nuclear translocation". Virologica Sinica 38: (2023) 387-397.

[40] Y. S. Wungak, B. O. Olugasa, O. O. Ishola, D. D. Lazarus, G. H. Ularamu, "Foot-and-mouth disease (FMD) prevalence and exposure factors associated with seropositivity of cattle in north-central, Nigeria". Afri J Biotechnol 15: (2016) 1224-1232.

[41] M. Zhu, J. Wang, R. Deng, X. Wang, "Functional Regulation of an Autographa californica Nucleopolyhedrovirus-Encoded MicroRNA, AcMNPV-miR-1, in Baculovirus Replication". J Virol 90: (2016) 6526-6537.

[42] M. A. Zia, M. S. Shah, M. Habib, "Enhanced Solubilization and Purification of 3ABC Non-structural Protein of Foot-and-Mouth Disease Virus from Bacterial Inclusion Bodies". Pak Vet J 42: (2022c) 1.

الملخص العربي

كفاءة أدوات تشخيصية مركبة وراثيا لفيروس الحمى القلاعية

مي محمد عبدالرحمن١, رقية حسين أحمد شلبي٢, علاء عبدالمنعم أحمد الخولي١, نورا راضي محمد ابراهيم٢, شيرين حسن بدوي الوكيل٢

[١]معهد بحوث الامصال واللقاحات البيطرية بالعباسية، مركز البحوث الزراعية، العباسية، القاهرة، جمهورية مصر العربية
[٢] قسم علم الحيوان، كلية البنات للآداب والعلوم والتربية، جامعة عين شمس، هليوبوليس، القاهرة، جمهورية مصر العربية

الملخص العربى:

يعد فيروس مرض الحمى القلاعية أحد أكثر الفيروسات الحيوانية إثارة للفزع حيث يصيب جميع أنواع الحيوانات ذات الظلف المشقوق ويسبب خسائر اقتصادية كبيرة في صناعة الماشية العالمية. إن الأنماط المصلية السبعة للفيروس والأنواع الفرعية الجينية العديدة تجعل من الصعب تشخيصه في كل من المناطق المعرضة للخطر والموبوءة، والتي تواجه أي برامج مكافحة. يعد عديد الببتيد غير الهيكلي "٣أب س" لفيروس مرض الحمى القلاعية هو الأكثر موثوقية للكشف عن الأجسام المضادة المستقلة عن النمط المصلي، وقد أثبتت تقنية الاليزا موثوقيته. لتسهيل تشخيص فيروس الحمى القلاعية في مصر، تم استنساخ تسلسل النيوكليوتيدات متعدد البروتين "٣أب س" للنمط المصلي "و١" من فيروس الحمى القلاعية، وتم التعبير عنه في خلايا الحشرات، و قد تم تشخيصه كما تم تطوير تقنية الاليزا غير المباشرة باستخدام بروتين "٣أب س" المؤتلف و المشتق من خلايا الحشرات المصابة بالفيروس. أظهر اختبار الاليزا فعاليته في اكتشاف الأجسام المضادة لفيروس مرض الحمى القلاعية في الأمصال البقرية، مما أظهر ارتباطًا معقولًا مع الاليزا التجاري. يظهر الاختبار المطور كأداة موثوقة وفعالة من حيث التكلفة للكشف عن الأجسام المضادة لمرض الحمى القلاعية في كل من الأمصال البقرية الخاضعة للرقابة. وتشمل تطبيقاته المحتملة المراقبة الروتينية للتحولات المصلية لفيروس مرض الحمى القلاعية في الحيوانات المصابة والناقلة والمحصنة، بالإضافة إلى فحص الحيوانات أثناء الحجر الصحي ومراقبة جودة لقاحات فيروس مرض الحمى القلاعية.