RESEARCH ARTICLE

Bulk Production of Baculovirus Expressed Stable Virus Like Particles of FMD Virus: Concentration, Purification and Semi-Quantification

Das Adwitiya,Tilak Chandan S., Samriddhi Dubey, Lakshmikanth Katrapati, Pankaj Dhakarwal, Pallab Chaudhuri, Paramasivam Saravanan*

Abstract

The Virus-like Particles (VLPs) have the same epitopes as the native virus offering a great alternative to conventional vaccines due to the lack of genome along with eliciting both arms of immunity. The thermostabilized Foot and Mouth Disease (FMD) VLP-based antigen with inherent DIVA compatibility requires minimum biosafety and cold chain maintenance for the production and transit process. It can potentially replace conventional virus handling during antigen production for prophylactic as well as diagnostic use. Preparation of baculovirus expressed VLPs for vaccine formulation can be achieved by bulk production of VLPs in insect cell culture like Tn5 and Sf21 grown in a shaker incubator at standard conditions followed by concentration of the harvested cell culture supernatant and purification by discontinuous sucrose density gradient with nine different gradients giving high yield. We standardized the bulk production of VLPs in a refrigerated shaker incubator maintaining a temperature of 26°C, concentrated and purified it in simplified sucrose density gradient ultracentrifugation, that could prove to serve as a potential vaccine candidate attributing to its thermostable properties.

Keywords: Bulk production, Foot-and-mouth disease virus, Insect cells, Purification, Sucrose gradient, Virus-like particles.

Ind J Vet Sci and Biotech (2025): 10.48165/ijvsbt.21.3.11

Introduction

Foot and Mouth Disease is an extremely contagious viral infection that affects cloven-hoofed animals, leading to severe economic losses in the agricultural sector. Therefore, the persistence of this disease is closely linked to a country's economy and trade. The causative agent of the disease, Foot-and-Mouth Disease Virus (FMDV), is a single-stranded positive-sense RNA virus classified under the genus *Aphthovirus* within the family *Picornaviridae* (Sharma *et al.,* 2018) with seven serotypes O, A, C, Asia-1, SAT-1, SAT-2 and SAT-3. The FMDV genome consists of a single open reading frame (ORF) that encodes a large polyprotein, which is then cleaved by viral proteases into distinct mature viral proteins. The 3C protease, encoded by the virus, carries out most of the cleavages, producing the structural proteins necessary for assembling the FMDV capsid within infected cells (Belsham, 1993). The FMDV capsid has an icosahedral shape and consists of 60 copies of subunits called protomers. Each protomer includes a single copy of each structural proteins VP0 (which includes VP4 and VP2), VP3, and VP1. The formation of complete capsid bounding the viral RNA requires an appropriate formation of 12 pentamers putting together from the assembly of 5 protomers (Grubman *et al.,* 1995). The RNA is translated into a polyprotein from a single open reading frame that is primarily cleaved by the 3C protease to generate both structural and non-structural proteins that are required for virus replication and assembly (Grubman *et al.,* 2008).

Currently, conventional vaccines are used to control the spread of the disease. These traditional vaccines often rely on live or inactivated viruses, which can present risks and require careful handling. However, factors such as the failure to maintain the cold chain and improper inactivation of the antigen have led to several outbreaks in the country. The potential for accidental virus release from manufacturing facilities highlights the need for alternative vaccine options. Virus-like particles (VLPs) present a promising alternative. VLPs consist of the structural proteins that form the capsid but lack the infectious genetic material, meaning they can stimulate an immune response without causing the disease itself. Additionally, they have benefits over conventional vaccinations, as they display the same surface structures as the virus, helping the immune system recognize and respond to the actual pathogen without the risks associated with live viruses.

However, the development of VLP-based vaccines requires further research into their structural and functional

ICAR-Indian Veterinary Research Institute, Hebbal, Bengaluru-560024, Karnataka, India

***Corresponding author:** Dr. Saravanan Paramasivam, Principal Scientist, Indian Veterinary Research Institute, Hebbal, Bengaluru-560024, India. E-mail: drsaravana72@gmail.com 9449986834

How to cite this article: Adwitiya, A., Chandan, T. S., Dubey, S., Katrapati, L., Dhakarwal, P., Chaudhuri, P., & Saravanan, P. (2025). Bulk Production of Baculovirus Expressed Stable Virus Like Particles of FMD Virus: Concentration, Purification and Semi-Quantification. Ind J Vet Sci and Biotech, 21(3), 53-57.

Source of support: Nil

Conflict of interest: The author(s) declare that there is no conflict of interest.

Submitted 07/02/2025 **Accepted** 17/03/2025 **Published** 10/05/2025

characteristics. While various expression systems are being investigated for VLP production, many reports depict the challenges with purification, as the VLPs often remain trapped in the cytoplasm (Dong *et al*., 2014; Madhavan *et al*.,2021). Since vaccine production requires large quantities of the antigen, methods that can increase antigen productivity in a shorter period are crucial. This study explores the strategies for efficiently producing and purifying FMD VLPs in bulk for use as a potential vaccine candidate. Semi-quantification of VLPs yields a better-standardized amount for accurate dosage of the vaccine in animals.

Materials and Methods

The insect cell lines *Spodoptera frugiperda* (Sf-21) and *Trichoplusia ni* (Tn5) maintained with Sf-900™ II SFM serum-free medium (Cat no.10902088, Gibco, USA) supplemented with 100 U/mL penicillin and 100 mg/mL streptomycin were used for baculovirus expression. In-house recombinant baculovirus expressing gene P1-2A of FMDV serotype O/IND/R2/72, A/IND/42/00 and Asia1/IND/63/72 were used to produce recombinant thermostable capsid proteins of respective serotypes separately (Ganji *et al.,*2018; Deepak, 2022; Madhavan *et al.,*2024).

Expression and Confirmation of Recombinant Baculovirus

The Tn5 insect cells in a 500 mL Erlenmeyer flask (MAXOME, MLCEFPC500) containing 100 mL media with a cell density of 2.5 million cells/mL were infected with thermostable baculoviruses with a moi (multiplicity of infection) of 5 to express the mutant FMDV capsid proteins individually. After 72 h of incubation, the infected cells were monitored for viability and harvested when live cell counts decreased below 10%. The harvested culture was subjected to centrifugation at 4500 x g for 20 min at 4 °C and the supernatant was collected and stored at 4°C for further methodologies.

ELISA for Testing Immunoreactivity of the VLPs

The specificity of the processed supernatant containing the VLPs was confirmed with serotype-specific sandwich ELISA (Bhat *et al*., 2013). Antibodies raised against 146S FMDV antigen in rabbit and guinea pig were used as coating and tracing antibody, respectively, in s-ELISA with the following dilutions (Table 1).

Table 1: Dilutions of coating serum and tracing serum used in Sandwich ELISA

Serotypes	Dilution of coating serum (Rabbit serum)	Dilution of tracing serum (Guinea pig serum)
O/IND/R2/72	1:4000	1:4000
A/IND/42/00	1:2000	1:2000
Asia1/IND/63/72	1:2000	1:4000

Concentration and Purification of VLPs

The processed supernatant (VLPs) after confirming their immunoreactivity was subjected to Tangential Flow Filtration using a membrane pore size of 100 kDa. The concentrated material was further subjected to purification by discontinuous sucrose density gradient ultracentrifugation as described with modifications (Ruiz *et al.,* 2018). Briefly, the nine sucrose gradients were prepared in Tris NaCl buffer (20 mM Tris, 150 mM NaCl, pH: 7.6), layered sequentially starting from the bottom 55% to the top layer (15%) and concentrated supernatant containing VLPs was layered above the top layer of sucrose (15%). The samples were centrifuged at 1,50,000 x g for 8 h using an Optima XPN-100 ultracentrifuge (Beckman Coulter, USA). After the centrifugation, the gradients were fractionated (1 mL) from the bottom using a siphoning tube and each fraction was tested with serotype-specific sandwich ELISA. The fractions corresponding to the highest peak OD value were pooled and dialyzed against Tris NaCl (pH: 7.6) at 4°C for 6 h. These dialyzed fractions were processed for TEM analysis and further methods. The quantification of protein was performed by Thermo Scientific PIERCE® BCA (biocinchoninic acid) assay as per the protocol (Cat. No 23225).

Transmission Electron Microscopy

A copper grid was utilized to adsorb purified recombinant VLPs for a few minutes at 25°C or room temperature. The grids containing the VLPs were dried gently and stained with 1% uranyl acetate for 5 min. With the help of filter paper, excessive staining was removed, and the samples were examined under a TEM at 80 kV (TECNAI, T12, USA) after incubating for an h at 37°C.

Results and Discussion

Expression of Capsid Proteins of VLPs

In this research, we aimed to produce FMD VLPs in bulk by growing them in suspension culture using a baculovirus expression system and proposed a less time-consuming purification method that combines filtration and sucrose density ultracentrifugation.

Recombinant baculovirus were produced using healthy Sf21 cells (Fig. 1a) which were harvested after 5-6 days of Cytopathic Effect (CPE) (Fig. 1b) on infection with baculovirus stock. On the other hand, infection of healthy Tn-5 cells (Fig. 1c) with the recombinant baculovirus at a high m.o.i. (multiplicity of infection) of 5 produced good CPE by 3 days which was marked by a declined live cell count below 10% (Fig.1d). The infected cells showed characteristic CPE with minimal cell viability on the day of harvest.

Immunoreactivity of the Expressed VLPs

Analysis of the infected lysate in a serotype-specific sandwich ELISA developed good immunoreactivity (Fig. 2) confirming the expression of serotype-specific capsid proteins , thereby demonstrating that the VLPs exhibited immunoreactivity similar to that of the original FMDV virions.

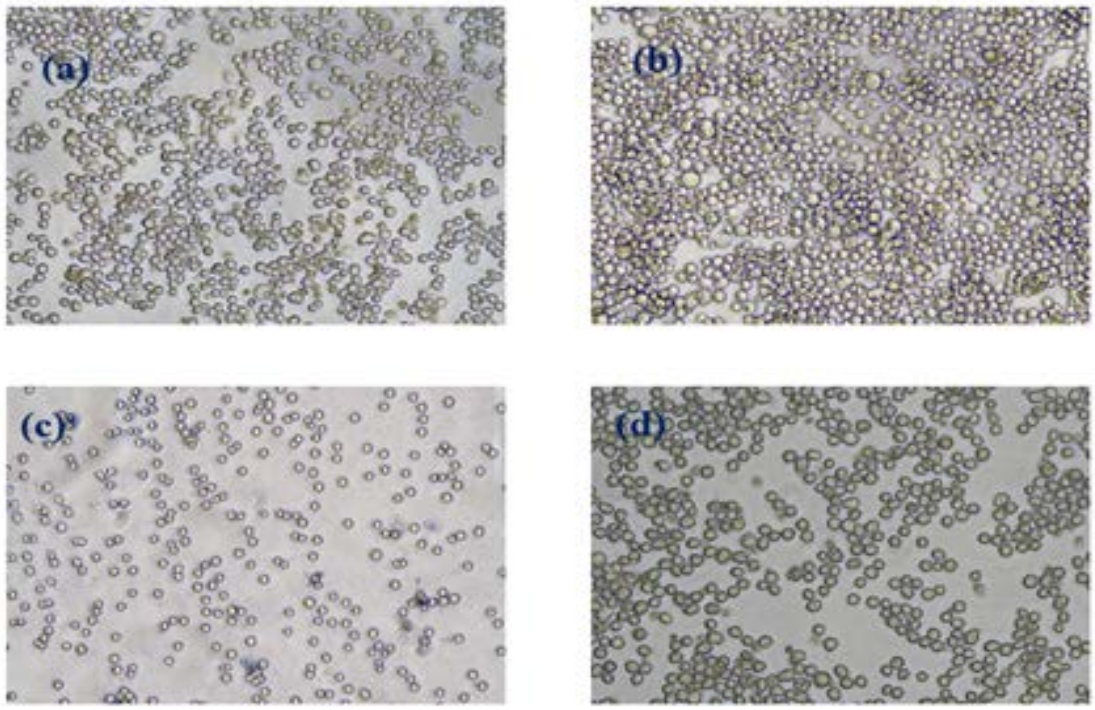

Fig. 1: (a) Sf21 healthy cells, (b) Sf21 cells showing CPE, (c) Tn5 healthy cells, (d) Tn5 cells showing CPE with decreased cell viability

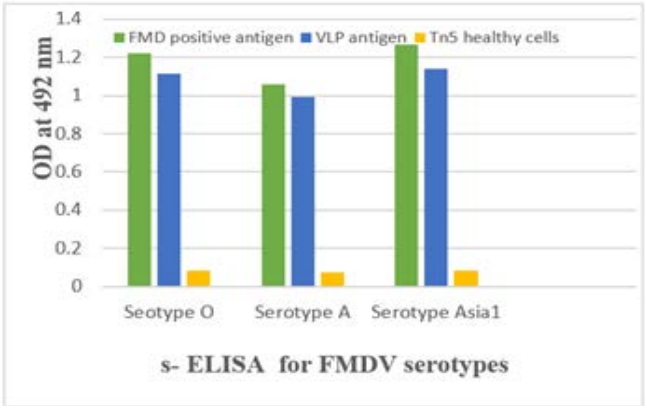

Fig. 2: Screening of VLPs in s-ELISA

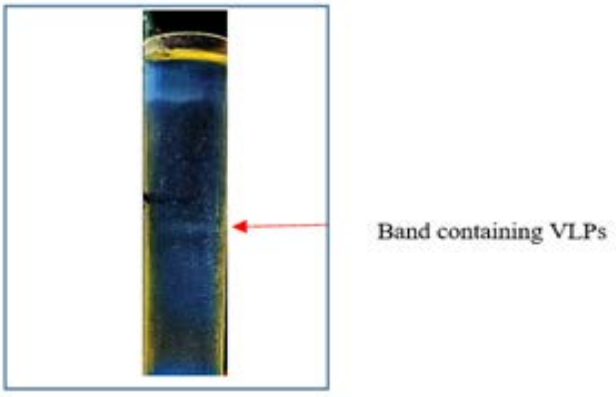

Fig. 3: Purification of VLPs by discontinuous sucrose gradient

Concentration, Semi-Quantification and Purification of the Assembled VLPs

The lysed cells from the infected flasks were centrifuged thoroughly to collect the supernatant and concentrated with Tangential Flow Filtration before loading on a discontinuous gradient sucrose. This resulted in development of a clear white band slightly above the middle of the gradient indicating the separation of VLPs from other cellular components and proteins (Fig. 3).

Purification Efficiency

The purification process successfully produced VLPs that were highly pure and suitable for further testing. The TEM images (Fig. 4) confirmed that this expression system enables the proper assembly of capsid proteins into a three-dimensional capsid structure, preserving the antigenic epitopes necessary for inducing neutralizing antibody responses. Further confirmation of these epitopes, along with crystallographic studies elucidating the structure of FMDV capsids, would offer a better comprehension of these mechanisms. However,

maintaining optimal buffer conditions, particularly pH (around 7.6) and temperature (ice-cold condition), is critical during the VLP purification process, as even small changes in these factors can lead to dissociation of the VLPs (Madhavan *et al,*. 2021).

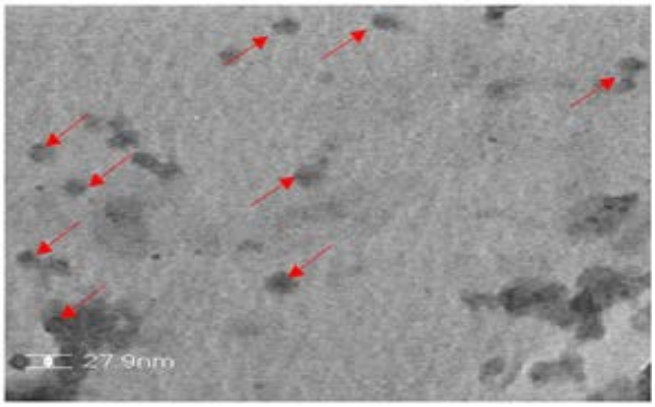

Fig. 4: TEM images showing capsids (Virus Like Particles)

Sandwich ELISA results of the VLP fractions, using a polyclonal antibody, detected concentrated VLPs just above the middle of the gradient (fractions 9, 10 and 11 from the bottom), corresponding to the location of the white band observed. Fractions collected beyond this white band (13, 14, and 17) also showed higher OD values, indicating the possibility of smaller and nonspecific proteins present in those fractions (Fig. 5).

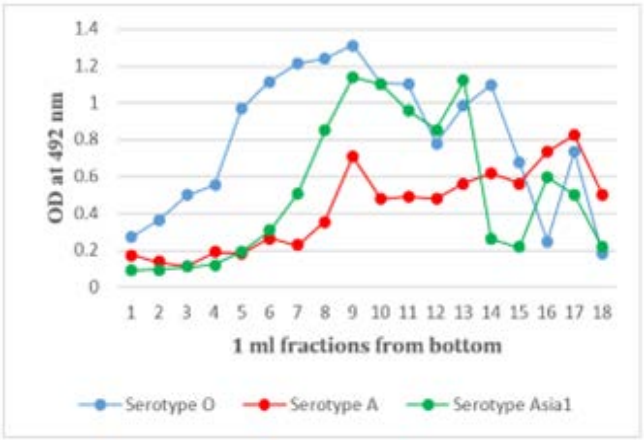

Fig. 5: Specific reactivity of the fractions in sandwich ELISA with serotype-specific polyclonal antibodies

Further, BCA and spectrophotometer analysis revealed that the protein concentration of the purified VLPs was decreased compared to the protein content of the crude VLPs after density gradient ultracentrifugation which indicates elimination of small proteins and cellular debris resulting in purified protein of the VLPs (Table. 2). Thereby, this method ensures a smooth separation of the VLPs, which concentrate at a specific point corresponding to their density and provides pure, concentrated VLPs for structural and functional analysis.

Table 2: BCA and spectrophotometer analysis

Sample ID	Protein Estimation (BCA Assay) - Whole Lysates (µg/mL)	Spectrophotometer Reading - Whole Lysates (mg/mL)	Protein Estimation (BCA Assay) - Ultracentrifuge Fraction with Peak OD Value (µg/mL)	Spectrophotometer Reading - Ultracentrifuge Fraction(mg/mL)
OT7	2154.846	2.32	619.85	0.59
OT8	1761.769	1.99	553.30	0.54
AT3	1854.462	1.92	584.08	0.61
AT4	774.4615	0.7	394.84	0.4
XT1	2159.846	2.2	684.85	0.7
XT6	1120.615	1.3	240.61	0.2

Additionally, this purification method avoids a pelleting step, reducing the risk of structural changes, time loss due to pellet resuspension, and protein loss. It allows for faster production of more concentrated and purer VLPs suitable for laboratory studies. Further optimization using techniques like affinity tags or size-exclusion chromatography will be necessary for large-scale vaccine production to make this bio-safe vaccine platform a viable solution for mass vaccination (Madhavan *et al.,* 2021).

This research established the fact that VLPs can be produced and purified efficiently in a laboratory setting. The advantages of using VLPs include increased safety with no risk of infection and avoiding the use of expensive biocontainment facilities making them more accessible in controlling and diagnosing the disease, especially in developing countries like India.

Conclusion

The present study established a reliable method for the bulk production and purification of thermostabilized VLPs for Foot and Mouth Disease. These VLPs hold great promise as safe and effective alternatives to traditional vaccines, potentially improving FMD control in livestock.

The optimized purification protocol outlined in this study effectively eliminates host cellular debris and smaller proteins co-expressed with FMDV VLPs in the baculovirus expression system, resulting in pure and concentrated VLPs. Semi-quantification of VLPs yields a better-standardized amount for accurate dosage of the vaccine in animals.

Acknowledgement

The authors acknowledge the Joint Director, ICAR-IVRI, Bengaluru for providing the necessary facilities to conduct the research. This research work was a part of the PhD program of the first author.

References

Belsham, G.J. (1993). Distinctive features of foot-and-mouth disease virus, a member of the Picornavirus family: Aspects of virus protein synthesis, protein processing, and structure. *Progress in Biophysics and Molecular Biology, 60,* 241-260.

Bhat, S.A., Saravanan, P., Hosamani, M., Basagoudanavar, S.H., Sreenivasa, B.P., Tamilselvan, R.P., & Venkataramanan, R. (2013). Novel immunogenic baculovirus expressed virus-like particles of foot-and-mouth disease (FMD) virus protect guinea pigs against challenge. *Research in Veterinary Science, 95,* 1217-1223.

Deepak, P. R. S. (2022). Structure-based prediction to generate stable virus-like particles of foot-and-mouth disease virus serotype A and their characterization. *PhD Thesis.* Indian Veterinary Research Institute, Izatnagar, UP, India.

Dong, H., Guo, H.C., & Sun, S.Q. (2014). Virus-like particles in picornavirus vaccine development. *Applied Microbiology and Biotechnology, 98*(10), 4321-4329.

Ganji, V.K., Biswal, J.K., Lalzampuia, H., Basagoudanavar, S.H., Saravanan, P., Tamil Selvan, R.P., Umapathi, V., Reddy, G.R., Sanyal, A., & Dechamma, H.J. (2018). Mutation in the VP2 gene of P1-2A capsid protein increases the thermostability of virus-like particles of foot-and-mouth disease virus serotype O. *Applied Microbiology and Biotechnology, 102*(20), 8883-8893.

Grubman, M.J., Moraes, M.P., Diaz-San Segundo, F., Pena, L., & de los Santos, T. (2008). Evading the host immune response: How foot-and-mouth disease virus has become an effective pathogen. *FEMS Immunology and Medical Microbiology, 53,* 8-17.

Grubman, M.J., Zellner, M., Bablanian, G., Mason, P.W., & Piccone, M.E. (1995). Identification of the active-site residues of the 3C proteinase of foot-and-mouth disease virus. *Virology, 213,* 581-589.

Madhavan, A., Dhanesh, V.V., Selvaraj, D.P.R., Gopinath, S., Sreenivasa, B.P., Tamil Selvan, R.P., Sanyal, A., Thiyagarajan, S., & Saravanan, P. (2021). An optimized protocol for purification of virus-like particles of foot-and-mouth disease virus produced in the baculovirus expression system. *The Pharma Innovation Journal, 10*(10S), 33-37.

Madhavan, A., Paramasivam, S., Dhanesh, V.V., Selvaraj, D.P.R., Shreya, G., Adwitiya, D., Madhusudan, H., Sreenivasa, B.P., Tamilselvan, R.P., Sanyal, A., Goyal, S., Thiyagarajan, S., & Chaudhuri, P. (2024). Diagnostic and prophylactic potential of a stabilized foot-and-mouth disease serotype asia1 virus-like particles designed through a structure guided approach. *International Journal of Biological Macromolecules, 277*(4), 134366.

Ruiz, V., Baztarrica, J., Rybicki, E.P., Meyers, A.E., &Wigdorovitz, A. (2018). Minimally processed crude leaf extracts of *Nicotiana benthamiana* containing recombinant foot and mouth disease virus-like particles are immunogenic in mice. *Biotechnology Reports, 20,* e00283.

Sharma, A.K., Bhatt, M., Sankar, M., Mohapatra, J.K., Dash, B.B., Gowane, G.R., Subramaniam, S., Ranjan, R., & Pattnaik, B. (2018). Kinetics of interferon gamma and interleukin-21 response following foot and mouth disease virus infection. *Microbial Pathogenesis, 125,* 20-25.