Evaluation of Recombinant Foot-and-Mouth Disease 2C3AB Protein for Use as an Antigen

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Abstract

Background: Foot-and-mouth disease (FMD) represents a major economic threat to global cattle production systems. While vaccination programs effectively control this highly contagious viral disease, endemic countries require robust surveillance systems to distinguish naturally infected animals from vaccinated populations. Nonstructural proteins (NSPs) are essential for viral replication within infected cells and serve as valuable diagnostic antigens since they are absent from inactivated vaccines but produced during natural infection.

Among these NSPs, the 3ABC polyprotein has emerged as serological diagnosis in vaccinated populations, offering high sensitivity and specificity for DIVA (Differentiating Infected from Vaccinated Animals) testing. However, recent evidence suggests that the 2C3AB polyprotein may provide comparable diagnostic performance while potentially offering additional advantages, such as avoiding the autocatalytic processing issues associated with 3C protease and incorporating broader epitope coverage through the inclusion of 2C domain sequences alongside 3AB components.

Objectives: Clone and express the 2C3AB gene from FMDV O/Mya-98 strain using the baculovirus expression system under the control of AcNPV promoters; develop and optimize an indirect ELISA protocol using the recombinant 2C3AB protein as coating antigen, and establish diagnostic performance parameters including optimal cutoff values, sensitivity, and specificity through ROC curve analysis for potential implementation in DIVA-based serological surveillance programs

Methods: A total of 96 bovine serum samples were utilized, comprising 64 positive and 32 negative samples as determined by commercial blocking ELISA. The gene encoding 2C3AB from FMDV serotype O/Mya-98 was amplified by RT-PCR and initially cloned into pGEM-T Easy vector. Subsequently, the 2C3AB gene was subcloned into the pFastBac1 donor plasmid and transformed into DH10Bac competent *E. coli* to generate recombinant bacmid through site-specific transposition. Following purification, the recombinant bacmid was transfected into Sf9 insect cells, and passage 3 (P3) recombinant baculovirus was harvested for protein expression in High Five cells. The recombinant 2C3AB protein, containing a C-terminal 6×His tag, was purified using Ni-NTA affinity chromatography, and protein concentration was quantified. SDS-PAGE and Western blot analyses were performed to confirm specific protein expression and molecular weight. ROC curve analysis was conducted using MedCalc software to determine optimal cutoff values, sensitivity, and specificity parameters.

Results: RT-PCR amplification successfully generated a 1.6 kb fragment corresponding to the 2C3AB gene. Positive clones were confirmed by double restriction enzyme digestion analysis. The recombinant r2C3AB protein was expressed with an apparent molecular weight of approximately 61 kDa without Histag, consistent with theoretical calculations. Western blot analysis using rabbit anti-6×His antibody demonstrated specific recognition of the recombinant protein, confirming successful expression and purification. Following optimization studies, the indirect ELISA protocol was established using 1 μg/mL of r2C3AB as coating antigen and bovine serum diluted at 1:20. ROC curve analysis revealed optimal diagnostic performance with a recommended cutoff value of OD $\geq$ 0.24. At this threshold, the assay demonstrated high sensitivity (96.87%) and specificity (93.73%) with 95% confidence intervals of 91.1% to 99.4%. The area under the curve (AUC) value of 0.969 indicates excellent diagnostic accuracy, confirming the potential of r2C3AB-based indirect ELISA for DIVA applications in FMD surveillance.

Conclusion: The 2C3AB gene from FMDV O/Mya-98 was successfully cloned and expressed using the baculovirus expression system, yielding a functionally active recombinant protein. The r2C3AB demonstrated strong potential as a diagnostic antigen, as evidenced by its specific recognition by sera from naturally FMDV-infected cattle. The developed indirect ELISA achieved excellent diagnostic performance (96.87% sensitivity, 93.73% specificity, AUC = 0.969), comparable to commercial blocking ELISA standards, confirming its capability for serological detection of FMDV infection in DIVA applications. These findings support the use of r2C3AB as an alternative diagnostic antigen that may offer advantages over conventional 3ABC-based assays, including avoidance of autocatalytic processing issues and broader epitope coverage. However, further validation studies using larger, more diverse sample populations across different geographical regions and serotypes are essential to establish the assay's reliability for routine diagnostic implementation in FMD surveillance programs.

Keywords: Foot-and-mouth disease, 2C3AB protein

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