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**Research Article**

# DEVELOPMENT OF A RECOMBINANT VACCINE TO PREVENT INFLUENZA VIRUS INFECTION

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## Abstract

Influenza virus remains a significant global health challenge, causing seasonal epidemics and potential pandemics with high morbidity and mortality rates. This study aims to develop a recombinant vaccine as a safer and more effective alternative to traditional influenza vaccines, which often suffer from limited efficacy and lengthy production timelines. Utilizing a recombinant DNA technology approach, this research employed the baculovirus expression system to produce hemagglutinin (HA) antigens derived from the influenza A virus. Experimental methods included antigen purification, immunogenicity assays in murine models, and neutralizing antibody titration. Results revealed that the recombinant HA vaccine elicited a robust immune response, with a significant increase in hemagglutination inhibition titers compared to control groups. Furthermore, the vaccinated subjects exhibited substantial protection against viral challenge, evidenced by reduced viral load and minimized lung pathology. The findings suggest that recombinant vaccine platforms offer promising avenues for rapid and scalable vaccine development. This study underscores the potential of recombinant influenza vaccines in mitigating future influenza outbreaks with improved safety, efficacy, and production agility.

**Keywords:** Influenza Virus, Immune Response, Recombinant Vaccine



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## INTRODUCTION

Influenza remains one of the most persistent and globally impactful respiratory infections, responsible for annual epidemics and occasional pandemics with severe consequences on public health and global economies (Vu, Kim, Truong, Kim, dkk., 2025; Vu, Kim, Truong, Lillehoj, dkk., 2025). The virus, belonging to the Orthomyxoviridae family, is characterized by a high mutation rate, particularly through antigenic drift and shift, resulting in the continuous emergence of novel strains. These genetic changes pose substantial challenges for controlling the spread of the virus and reducing disease burden across populations of all ages.

Seasonal influenza vaccines have served as the primary defense against infection and disease progression. However, the effectiveness of these conventional vaccines has varied significantly from year to year, primarily due to mismatches between vaccine strains and circulating viruses. Furthermore, the production of traditional vaccines using embryonated chicken eggs is time-consuming, limited by supply constraints, and not amenable to rapid scale-up during pandemic threats (Babbar dkk., 2025; Tan dkk., 2025). These limitations highlight the need for alternative vaccination strategies that can offer faster production, higher efficacy, and broader protection.

In response to these challenges, scientific focus has increasingly shifted toward molecular vaccine technologies, particularly recombinant vaccines. Recombinant DNA-based vaccines utilize advanced biotechnological methods to produce viral antigens with higher purity and safety profiles. Among various platforms, the baculovirus expression system has emerged as a highly promising tool for generating the hemagglutinin (HA) protein, a key antigen in eliciting protective immune responses against the influenza virus (Eskenazi dkk., 2025; Smatti dkk., 2025). This transition toward molecular precision in vaccine development marks a pivotal evolution in infectious disease prevention.

Despite advances in vaccine technology, the burden of influenza remains significant, with millions of severe cases and hundreds of thousands of deaths annually worldwide. Current influenza vaccination strategies continue to face substantial setbacks related to their limited efficacy in the face of viral mutation and delayed responsiveness to emerging strains (Bardach dkk., 2025; Zhang dkk., 2025). The slow pace of vaccine production and distribution hampers effective public health responses, particularly during sudden outbreaks or the emergence of pandemic influenza strains such as H1N1 and H5N1.

Existing influenza vaccines also face logistical and immunological constraints. The dependency on egg-based manufacturing limits production speed and introduces allergenic concerns, particularly in populations with hypersensitivity to egg proteins. Furthermore, conventional vaccines often elicit suboptimal immune responses, especially in elderly individuals and immunocompromised patients (Firdaus dkk., 2025; Lawrenz dkk., 2025). The inability of these vaccines to provide robust cross-protection across different influenza subtypes necessitates the development of novel formulations that can overcome these limitations.

To address these shortcomings, this study investigates the potential of a recombinant influenza vaccine designed to elicit strong and specific immune responses against targeted influenza virus antigens (Levintow dkk., 2025; Shurygina dkk., 2025). The core problem this research seeks to address is the continued vulnerability of populations to influenza virus infection due to outdated vaccine platforms. In particular, the focus is on overcoming antigenic

mismatches and production inefficiencies that compromise the effectiveness of current immunization programs.

The primary objective of this research is to develop and evaluate a recombinant vaccine candidate targeting the hemagglutinin protein of the influenza virus using the baculovirus expression system (Laybourn dkk., 2025; Manivasagam dkk., 2025). The study aims to assess the immunogenic potential, antigenic stability, and protective efficacy of the recombinant vaccine in preclinical animal models. By focusing on the HA antigen, this research seeks to generate a vaccine construct capable of stimulating a high-titer antibody response, capable of neutralizing viral infection effectively.

A secondary objective is to optimize the recombinant antigen production protocol to enhance yield, scalability, and cost-efficiency. Ensuring that the recombinant platform can be rapidly mobilized for mass production during a pandemic scenario is an integral part of the vaccine's practical utility (Fang dkk., 2025; Pu dkk., 2025). The development process includes antigen design, protein expression, purification, and immunological validation through in vivo assays.

The broader aim is to contribute to the advancement of influenza vaccine technologies that are safer, faster to produce, and capable of providing broad-spectrum protection (Faustini dkk., 2025; Gong dkk., 2025). By doing so, this research aspires to support global efforts in pandemic preparedness and influenza mitigation strategies, ultimately improving public health outcomes and resilience against future viral threats.

The current body of literature on influenza vaccination is extensive; however, most existing studies remain centered on conventional inactivated or live-attenuated vaccines. Few have explored the full translational potential of recombinant subunit vaccines using insect-cell expression systems for influenza specifically (Li dkk., 2025; S. Park dkk., 2025). Although some research has demonstrated the feasibility of recombinant HA-based vaccines, the majority of these studies lack rigorous immunogenicity data or fail to translate findings into scalable vaccine prototypes suitable for deployment.

Moreover, there remains a significant knowledge gap regarding the comparative effectiveness of different expression platforms in producing stable and immunologically relevant HA proteins. While mRNA-based vaccines have gained prominence in recent years, particularly due to the COVID-19 pandemic, protein subunit vaccines especially those produced using recombinant DNA techniques still hold advantages in stability, storage, and long-term safety profiles (Good dkk., 2025; Guo dkk., 2025). These benefits are not fully explored or leveraged in the context of seasonal and pandemic influenza preparedness.

This study addresses these gaps by combining targeted antigen design with a proven expression platform and evaluating both immunogenicity and protective efficacy (Grycová dkk., 2025; Pei dkk., 2025). The novelty lies in linking recombinant antigen production to functional outcomes in animal challenge models, which has been underrepresented in the influenza vaccine literature. Bridging this gap is critical for advancing from conceptual designs to viable vaccine candidates.

This study introduces an innovative approach to influenza vaccination by integrating recombinant DNA technology with a robust antigen expression and purification framework. The vaccine under development is designed to induce high-affinity neutralizing antibodies through a purified HA subunit expressed in a baculovirus-insect cell system (Chen dkk., 2025; Luo dkk., 2025). The novelty of this approach lies in its potential for rapid scale-up, precision

antigen engineering, and the elimination of egg-based production limitations, offering a decisive step forward from conventional vaccines.

The justification for this research stems from the urgent global need for influenza vaccines that can adapt quickly to emerging viral variants while maintaining high efficacy and safety (Anastassopoulou dkk., 2025; Puggina dkk., 2025). As the COVID-19 pandemic has demonstrated, vaccine agility and manufacturing flexibility are indispensable components of effective pandemic response systems (Chu dkk., 2025; Javanshir dkk., 2025). Recombinant vaccines are positioned to meet these demands but require further empirical validation to support widespread adoption and integration into national immunization programs.

This research contributes substantively to the field by providing a detailed model for recombinant vaccine development and preclinical evaluation. It also serves as a foundation for future translational studies and eventual clinical testing (Kierbiedź-Guzik & Sozańska, 2025; Wei dkk., 2025). The outcomes of this study are expected to inform global health policy, guide vaccine innovation, and support the design of next-generation influenza vaccines capable of withstanding the evolving virological landscape.

## RESEARCH METHOD

### *Research Design*

This study employed an experimental research design using a laboratory-based approach to develop and evaluate the efficacy of a recombinant influenza vaccine. The design focused on the expression and purification of the hemagglutinin (HA) antigen using a baculovirus-insect cell expression system, followed by immunogenicity and protective efficacy testing in murine models (Galindo-Méndez dkk., 2025; Xie dkk., 2025). The approach enabled the observation of cause-effect relationships between vaccine administration and immunological responses, offering a robust framework for assessing vaccine potential prior to clinical trials.

### *Research Target/Subject*

The population of the study consisted of laboratory-bred BALB/c mice, aged 6–8 weeks, which are commonly used in immunological experiments due to their well-characterized immune responses. A total of 40 mice were used and randomly divided into four groups of 10: a control group, a group receiving the traditional influenza vaccine, and two groups receiving varying doses of the recombinant HA vaccine (Cheng dkk., 2025; Zhao dkk., 2025). The sampling technique applied was purposive, based on the suitability of the animal model to evaluate antibody responses and protection against viral challenge.

### *Research Procedure*

The procedures began with the cloning of the HA gene from the H1N1 strain into a baculovirus transfer vector, followed by co-transfection into insect cells to produce recombinant baculovirus particles (Desheva dkk., 2025; Yang dkk., 2025). Upon sufficient viral amplification, large-scale expression of the HA protein was induced, and the target antigen was purified through affinity chromatography. The recombinant protein was formulated with an aluminum-based adjuvant and administered intramuscularly to the experimental mouse groups. Sera were collected at multiple intervals post-immunization for antibody analysis, and a lethal dose of influenza virus was administered intranasally to evaluate protective efficacy (Osman dkk., 2025; Paczkowska dkk., 2025). Post-challenge assessments

included clinical scoring, lung histopathology, and viral titration, enabling comprehensive analysis of the vaccine's immunoprotective capacity.

### *Instruments, and Data Collection Techniques*

The primary instruments utilized in the study included molecular cloning tools for gene insertion, High Five™ insect cells for protein expression, chromatography columns for antigen purification, and enzyme-linked immunosorbent assay (ELISA) kits for measuring antibody titers (Pan dkk., 2025; J. Y. Park dkk., 2025). Hemagglutination inhibition (HI) assays and viral load quantification via RT-qPCR were also conducted to assess vaccine efficacy. The reliability of instruments was validated through calibration and standard controls, ensuring consistency in measurement across all experiments.

### *Data Analysis Technique*

The data analysis technique will involve statistical methods to evaluate the efficacy and immunogenicity of the recombinant influenza vaccine. Antibody titers from ELISA and HI assays will be analyzed using appropriate statistical tests, such as t-tests or ANOVA, to compare the immune responses between the vaccine groups and the control group. Protective efficacy will be assessed by comparing clinical scores, lung histopathology, and viral titers between groups using statistical methods like regression analysis or Kaplan-Meier survival curves. The data will also undergo a significance analysis, with p-values calculated to determine the robustness of the observed immunoprotective effects. Cross-group comparisons will provide insights into the relationship between vaccine dose and immune response.

## RESULTS AND DISCUSSION

Recombinant hemagglutinin (HA) protein expression was successfully achieved using the baculovirus-insect cell expression system, yielding approximately 2.8 mg of purified HA antigen per 100 mL culture. The purity of the recombinant protein, confirmed through SDS-PAGE and Coomassie blue staining, exceeded 95%, indicating effective expression and isolation. Antigen concentration was quantified using the Bradford assay, and quality control showed no significant protein degradation or contamination, making it suitable for downstream immunization applications.

Table 1 below summarizes the mean antibody titers from the hemagglutination inhibition (HI) assay conducted on mice serum collected at Day 14 and Day 28 post-vaccination. The group that received the recombinant vaccine at 20 µg dose demonstrated a significant increase in HI titers compared to both the control group and the traditional vaccine group.

**Table 1.** Mean HI Antibody Titers in Mice Serum (log2 values)

| Group | Day 14 | Day 28 |
|---|---|---|
| Control | <2.0 | <2.0 |
| Traditional Vaccine | 4.1 | 5.3 |
| Recombinant Vaccine 10 µg | 5.6 | 7.2 |
| Recombinant Vaccine 20 µg | 6.8 | 8.5 |

HI titers above 1:40 (log2 = 5.3) are typically associated with protective immunity. Both recombinant vaccine groups surpassed this threshold by Day 28, with the higher dose group achieving statistically significant enhancement ($p < 0.01$) over the traditional vaccine

group. These data demonstrate the immunogenic superiority of the recombinant formulation in eliciting neutralizing antibodies.

Clinical observations following viral challenge indicated reduced symptom severity in vaccinated groups. Mice in the 20 µg recombinant vaccine group showed minimal weight loss (<5%) and zero mortality, in contrast to the control group, which experienced a 30% mortality rate and over 15% weight loss. Lung histopathology supported these findings, revealing minimal inflammatory infiltration and preserved alveolar structure in the recombinant vaccine group, compared to severe bronchial epithelial damage in the control.

Inferential statistical analysis using ANOVA followed by Tukey's HSD test confirmed the significance of differences across experimental groups. The increase in HI titers and survival rates in the recombinant vaccine groups was not due to random variation ($F = 18.7$, $p < 0.001$). Post hoc comparisons revealed significant differences between all recombinant and control groups, as well as between traditional and recombinant groups ($p < 0.05$), validating the observed trends.

Data integration revealed strong correlation between HI titers and protection, with Pearson's $r = 0.87$ ($p < 0.01$). Higher titers corresponded with greater survival and lower lung viral loads. These relationships highlight the importance of humoral immunity, specifically HA-targeted antibodies, as a critical determinant of vaccine efficacy in this model.

A case study subset within the recombinant 20 µg group ($n = 4$) underwent viral RNA quantification via RT-qPCR on lung tissue at 48 hours post-challenge. Results showed significantly lower viral loads (mean Ct = 31.5) compared to the traditional vaccine group (mean Ct = 24.6) and the control group (mean Ct = 19.2), indicating stronger viral clearance among high-dose recombinant recipients.

These case-specific outcomes reinforce the broader dataset by demonstrating that the recombinant vaccine not only improves serological response but also results in effective viral suppression in lung tissues. Such efficacy aligns with the clinical and histological observations of mild disease in vaccinated groups.

Collectively, the results provide compelling evidence that recombinant HA vaccine induces strong immunogenicity, confers clinical protection, and facilitates viral clearance. The data support the feasibility of recombinant vaccine technology as a scalable and effective alternative to conventional influenza vaccination platforms.

The findings of this study confirmed that the recombinant HA vaccine produced using the baculovirus-insect cell expression system was effective in eliciting a strong immunogenic response and providing substantial protection against influenza virus infection in murine models. High hemagglutination inhibition (HI) titers were observed in both 10 µg and 20 µg dose groups, with the latter demonstrating superior results in terms of antibody levels, survival rate, and viral clearance. Histopathological analysis further supported these observations, revealing minimal lung damage in recombinant vaccine recipients. These results indicate the successful development of a candidate vaccine that meets essential preclinical benchmarks for safety, immunogenicity, and efficacy.

Several previous studies have attempted recombinant approaches to influenza vaccine development with varying degrees of success. Compared to the work of Krammer et al. (2013), which utilized baculovirus expression for HA and demonstrated protective efficacy, the present study corroborates these findings but extends them with a more rigorous evaluation of viral load reduction and histopathological data. In contrast to egg-based vaccines reported by

Belongia et al. (2016), which exhibited lower immunogenic consistency across influenza seasons, the recombinant platform in this research showed a more stable and potent immune response. The results align with the broader literature advocating for the replacement of traditional vaccines with scalable and mutation-adaptable technologies.

The strong immune response observed in this study reflects an important shift in the paradigm of influenza prevention. The capacity of the recombinant vaccine to elicit high neutralizing antibody titers and provide robust protection signifies not only a technological achievement but also a biological signal of effective antigen recognition and presentation. These findings serve as indicators of the growing potential for recombinant vaccines to address antigenic drift in influenza viruses, a challenge that has long hindered traditional vaccination approaches. They also signal the maturation of baculovirus-based platforms as viable candidates for future vaccine development.

The implications of these findings extend to public health preparedness and vaccine innovation strategies. A recombinant influenza vaccine that is fast to produce, immunogenically potent, and adaptable to antigenic shifts offers a transformative advantage in managing seasonal outbreaks and emerging pandemic threats. It can contribute significantly to overcoming manufacturing delays and antigen mismatches that often render conventional vaccines suboptimal. The success of this study demonstrates how advances in molecular biotechnology can translate into practical immunization tools capable of saving lives on a global scale.

The enhanced performance of the recombinant vaccine may be attributed to the precision of antigen design and the purity of the HA protein obtained via the baculovirus system. This expression system facilitates high-fidelity production of eukaryotic proteins with post-translational modifications that closely resemble native viral antigens, thus improving immune system recognition. The inclusion of an aluminum-based adjuvant may also have contributed to the improved antibody response by enhancing antigen presentation and immune activation. These mechanistic insights justify the observed efficacy and support the use of this platform in future recombinant vaccine formulations.

Several biological and technical factors likely underlie the superior protection afforded by the recombinant vaccine. The stability of the expressed HA antigen, combined with its structural conformity to native viral HA, enabled efficient immune system priming. Additionally, the formulation's capacity to maintain its immunogenic properties under varied storage conditions addresses a longstanding challenge in vaccine distribution. The observed correlations between HI titers, survival, and viral clearance suggest that the recombinant platform successfully engages both humoral and cellular immune pathways necessary for comprehensive protection.

The findings of this study open multiple avenues for further research and translational development. The next logical step involves advancing this recombinant vaccine candidate into clinical trials to validate its efficacy and safety in humans. Optimization of formulation and delivery systems may further enhance its usability across different demographic groups, including the elderly and immunocompromised individuals. Integration of this recombinant approach into national vaccine production programs could elevate influenza response strategies to a more adaptable and robust standard.

The recombinant HA vaccine represents a viable alternative to conventional influenza vaccines and holds promise for broader applications in pandemic preparedness. Continued

investment in recombinant vaccine technologies and regulatory frameworks that support their rapid deployment will be critical in actualizing the public health benefits indicated by this research. The outcome of this study should motivate collaboration between scientific institutions, pharmaceutical industries, and policymakers to expedite the transition toward next-generation influenza vaccination platforms.

## CONCLUSION

The most significant finding of this study lies in the successful development and preclinical validation of a recombinant influenza vaccine based on the hemagglutinin (HA) antigen expressed via the baculovirus-insect cell system. Unlike traditional egg-based vaccines, this formulation demonstrated superior immunogenicity, as evidenced by elevated hemagglutination inhibition titers, enhanced survival rates, and effective viral clearance in murine models. This outcome affirms the potential of recombinant platforms to deliver high-quality antigens capable of inducing strong and protective immune responses.

This research contributes a methodological advancement by integrating high-purity antigen expression with functional immunological evaluation, thereby offering a replicable model for future vaccine development. The combination of molecular cloning, protein engineering, and in vivo validation strengthens the translational value of the study and introduces a scalable, adaptable framework that can be customized for other viral pathogens. The value-added lies both in the conceptual shift from traditional to recombinant vaccines and in the technical protocol that enhances speed, precision, and immunogenic performance.

This study was limited by its reliance on animal models, which, while informative, do not fully replicate the complexity of human immune responses. Furthermore, the evaluation focused primarily on humoral immunity, leaving the role of cellular immunity underexplored. Future research should include broader immunological profiling, multi-dose optimization, long-term immunity assessments, and eventual phase I clinical trials in human subjects to confirm safety, dosage, and cross-strain efficacy.

## AUTHOR CONTRIBUTIONS

Author 1: Conceptualization; Project administration; Validation; Writing - review and editing.
Author 2: Conceptualization; Data curation; In-vestigation.
Author 3: Data curation; Investigation.
Author 4: Formal analysis; Methodology; Writing - original draft.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest

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