

# [Technical summary](https://assignbuster.com/technical-summary/)

Technical Summary Objective The main objective of the research is to increase production capa for pandemic and seasonal influenza vaccines through a better technique other than the traditional one. The technique used is baculovirus-insect larvae system (the system consists of small living biofactories) to enhance the generation of recombinant influenza virus H1N1 hemagglutinin (HA). The traditional technique (embryonated hen’s egg technology) has its flaws, and this makes an ineffective method of addressing pandemic and seasonal influenza (Gomez-Casado 35).
Approach
The researchers used baculovirus-insect larvae system to enhance the generation of recombinant influenza virus H1N1 HA. Mouse-adapted H1N1 A/PR/8/34 strains were used for the study. Genomic RNA (full-length) was acquired by infecting MDCK cells; the procedure was done under biosafety level-2 conditions. pFBHAhisKDEL and pFBMelHAhis plasmids were used to produce the recombinant baculoviruses BacHAhisKDEL and BacMelHAhis using Bac-to-Bac baculovirus expression system. The instructions provided by the manufacturer of the system were strictly followed. Control used was baculovirus vector BacNI (this is a baculovirus, which has no foreign gene). There was propagation and amplification of the recombinant baculoviruses in Sf21 insect cells in order to attain infective titers of around 108 plaque-forming units (pfu) (Gomez-Casado 36).
Sf21 insect cells and the insect larvae (Trichoplusia ni) were infected using recombinant baculovirus dilution to attain the amount of pfu per dose required for each selection. Total soluble and non-denatured proteins (TSNDPs) were obtained through homogenization; these proteins were from baculoviruses infected T. ni larvae (Gomez-Casado 36). The proteins were prepared using various techniques for western blot (WB) assays and protein size determination. Recombinant HAhisKDEL protein purification from the infected larvae was done using Co2+-based immobilized metal affinity chromatography (IMAC) resins (Gomez-Casado 37). The response of HA-specific IgG (immunoglobin G) was measured using ELISA tests. Inhibition tests of hemagglutination were done for each of the duplicate based on instruction from the World Organization for Animal Health. Female mice (6 to 8 weeks old) were immunized and tested for virus challenge. The first group of mice (4) was immunized with TSNDP extract (containing HAhisKDEL protein) from the infected larvae. The second group (4 mice) was immunized with purified HAhisKDEL protein. Control group (3 mice) were immunized using TSNDP extract from the infected larvae containing the control baculovirus BacNI. The second control group (6 mice) was immunized using PBS (phosphate-buffered saline) (Gomez-Casado 37).
Results
A comparison of HA protein generation yields for the insect larvae cultures and the recombinant baculoviruses in T. ni larvae were done. The results indicate that, in both production systems (Sf21 cells and HA in larvae), HA productivity was increased by the KDEL sequence. There was a 198 percent increase in larvae and a 298 percent increase in Sf21 cells in comparison with similar HA sequence lacking the KDEL retention signal. The comparison results indicate that insects can enhance HA productivity by around 400, and 600 percent compared with “ Sf21 cells for HAhisKDEL and MelHAhis proteins respectively, per biomass unit (1 g of fresh larvae)” (Gomez-Casado 39). The first group of mice immunized developed particular antibodies after the first dose of antigen; however, ELISA tests done after 365 days indicated a considerable decrease in the antibody titers after the last dose of immunization. The second group of mice also developed specific antibodies, but after three doses of immunizations, antibody titers were still high. All the control mice immunized lacked specific cross-reactive immune responses (Gomez-Casado 39).
Works Cited
Gomez-Casado, E., Gomez-Sebastian, S., Nunez, M. C., Lasa-Covarrubias, R., Martinez-Pulgarin, S. & Escribano, J. M. “ Insect Larvae Biofactories as A Platform for Influenza Vaccine Production.” Protein Expression and Purification 79 (2011): 35-43. Print.