Abstract

Up to the present, the antivenin is prepared and purified from blood of horses that immunized with detoxified venom in CDC. It is known that aggregation of proteins during antivenin production can result in complement mediated side effects、serum sickness and anaphylactic shock upon administration to human beings. The production of yolk immunoglobulin from avian has been found low cost and side effects. The aim of this study is to develop production process of cobra antivenin from the egg yolk of immunized ducks. The first step of purification used ammonium sulfate precipitation and the purity of IgY ΔFc can reach 96%. IgY ΔFc also had good minimal lethal dose (MLD) after the second purification step by using affinity column method. The specificity of IgY was confirmed by Ouchterlony double diffusion test and Western blotting. In order to quickly screen the neutralization titer of IgY ΔFc, we also developed ELISA system. The results showed that the neutralizing response of IgY ΔFc reach the peak at the 12^{th}-14^{th} week, then it started to decline after the 19^{th} week. According to the experimental data, the proper immunization program was to administrate duck with 1mg、2mg、3mg、5mg and 10mg every two weeks separately, then it started to collect egg at the 12^{th} week after first immunization. The main type of IgY after purification process is IgY ΔFc. In order to reach 60MLD, the results implied that it required at least 30mg of IgY ΔFc protein purified by ammonium sulfate precipitation, but the concentration of antibody purified by ammonium sulfate precipitation and affinity column method only need 20mg.

Keywords: cobra；IgY；Duck；ammonium sulfate；affinity