A Web-based Method for Computing Endpoint Titer and Concentration of Anti body/Antigen

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Abstract

In this report, we describe a web server for calculating the endpoint titers and concentrations of antibody/antigen (Ab/Ag) from the optical density (00) taken from ELISA data. The server utilizes a graphical method (Raghava et al 1992) for determining the concen

tration of either the antibody or the antigen. In order to calculate the endpoint titer, we first fit the OD versus concentration of control data using a least-squares curve-fitting method. Then we fit the OD versus concentration of the standard sample using a graphical method. Finally, we determine the intersection or nearest point of the two curves, which we have called the endpoint titer. In order to calculate concentrations of the antibody/antigen of un known samples, we have to first fit OD versus the concentration of the known samples using a graphical method and to determine the linear interpolation and hyperbolic formulas. Then we calculate the concentration of the unknown samples from their OD using these formulas. This web server utilizes a ceI program written in Perl and Javascript, which makes the server live and interactive (imtech.res.in/ raghava/ abagj).

Description

The endpoint titer is routinely used in immunology to measure the secretion of antibodies. In order to compute the titer of an antibody, Peterfy et al. (1983) used the low endpoint titer (10% of maximum OD). Caulfield and Shaffer (1984) developed a pro gram and calculated the endpoint titer using an OD of 0.1. They fitted the standard curve using a local method. Recently, Tremain (1993) developed a program for calculating endpoint titer of antibody from ELISA data. The standard curve was fitted using an iterative simplex algorithm (NeIder and Meed 1965, Tijssen 1985). This, method allowed the user to select the cut-off point for calculating endpoint titer. However, 10% of the maximum OD was recommended in this method.

In these methods, the authors use the different ODs for endpoint titer. None of them has taken into consideration the effect of antibody concentration on the OD in the absence of any interaction (control data). The background OD varies with the concentration of the antibody, in addition to different antibodies producing different background levels. In order to consider the effect of concentration of an antibody on the background OD and other factors, a new method has been developed to compute the endpoint titer of the antibody.

The method described in this report utilizes the OD versus anti body concentration of known samples using a graphical method that is more accurate and sensitive (Raghava et al. 1992). The graphical method combines the power of local and global fitting methods. The OD versus log concentration of antibody of the control data was fitted using the least

squares curve fitting method. We then calculated either the intersection of the standard curve and the control curve, or nearest point of the two curves. This allows the method to incorporate the effect of background aD due to non-specific noise induced by the antibody.

In the past, a number of computer programs have been developed for calculating the Ab/Ag concentrations (Slezak et al. 1983, Caulfield et al. 1984, Studnicka 1987, Studnicka 1991). In the ELISA procedure, an equation is derived using standards to measure the Ab/Ag concentration of unknown samples. This is done by a series of dilutions of known standards to derive an equation, by fitting a standard curve. This serves as an internal calibration for the unknown samples on the plate. The equation of the standards is used to measure the Ab/Ag concentration of the unknown samples.

Previously, we have developed a computer program called ELISAeq (Raghava et al. 1992) which was designed to determine the concentration of Ab/Ag using ELISA data. In ELISAeq, the graphical method was used which utilizes both the linear regression and hyperbolic regression methods for calculating Ab/ Ag concentration (Raghava et al. 1992, Raghava et al. 1994). The linear regression method used in this program works only in the semi-logarithmic linear range (sl-range) but it is more sensitive than the hyperbolic regression method. We have also earlier shown that our graphical method is more sensitive than the previously published methods (Raghava et al. 1992).

In order to provide the service world-wide, we have developed a web server, which allows a user to compute the endpoint, titer, and concentration of antibody/antigen from ELISA data. In case the concentration is not known, it then allows the user to compute endpoint titer and quantification of antibody in terms of the dilution factor.

H a rdwarejSoftwa re Requirement

Use of the server (imtech.res.in/raghava/abag) requires access to the Internet and a web browser. These web pages can be loaded onto any computer that can run a web server and have the Perl language interpreter. The web pages use Javascript and CGI scripting and are written in Perl.

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