Short Communication

ELISA Analyzer°, a Software to Analyze and Optimize ELISA Data

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Abstract

ELISA data is usually handled in the MS Excel worksheets through different methods and different formulas. Accurate and precise calculations of the data is time consuming and tedious. ELISA Analyzer was developed with a user friendly interface to streamline titration and analysis of the data obtained from ELISA and improve rapid and lucid presentation of the results. The software can also help finding appropriate reagent dilutions for the ELISA reaction. Users can determine better dilution of the antibody and the conjugate that was extracted from blood of animal for other ELISA tests. Overall it can assist researchers to speed up data analysis, reduce computational errors, improve the presentation of analysis by creating charts and distinguishing positive samples shown in red color from the negative samples that appear in blue cells of plate, calculate the threshold and ultimately determine the most economic proportions of antibody and conjugate in the mixture considering the antibody and conjugate prices.

Keywords: ELISA Analyzer; data analysis; ELISA software; antibody titration.

°A copy of the software can be obtained upon request via email from the author(s). The user(s) of ELISA Analyzer agree to acknowledge the software by citing this paper, provided any publication of results obtained from the software.
Introduction

Enzyme-linked immuno sorbent assay (ELISA) is a method of choice for detecting and quantifying an antigen immobilized on a solid surface. ELISA uses a specific antibody with a covalently linked enzyme. The amount of antibody that binds the antigen is proportional to the amount of antigen present which is determined by spectrophotometric measurement of the conversion of a clear substrate to a colored product by the coupled enzyme. The ELISA has been used as a diagnostic tool in medicine (1), forensic toxicology for initial drug identification purposes (2) and plant pathology (3), as well as a quality-control check in various industries (4).

Calculation and evaluation of ELISA data is usually carried out in the Microsoft Excel worksheets or by using manual calculators through different methods and different formulas. ELISA plate has 96 positions for samples which makes accurate and precise calculations tedious.

ELISA Analyzer was developed to streamline the analysis of the data obtained from ELISA tests and improve lucid presentation of the results.

Development of ELISA Analyzer algorithm

User interfaces were designed in NetBeans 6.9 (an Integrated Development Environment or IDE for developing primarily with Java) with an ELISA virtual plate similar to the real one and also for obtaining the data from MS Excel files utilizing a library of Apache POI (Poor Obfuscation Implementation). Apache POI is well-known in the Java field as a library for reading and writing MS Office file formats such as Excel, PowerPoint, Visio and Word. The new OOXML (Office Open XML or Extensible Markup Language) formats introduced in Microsoft Office 2007 have been supported since POI 3.5 version.

Users can insert the data directly or by importing the MS Excel files. Users can also

![Suggested map for dilution of the antibody (row A) and the conjugate (rows B to H), A = antibody dilutions, B = blank, C = infected, D = healthy (uninfected), E = (1⁄500) × C, F = (1⁄500) × D, G = (1⁄2000) × C, H = (1⁄2000) × D.](image-url)
edit the data as they show in the virtual form and also adjust other settings such as blank and negative sample positions as well as the parameters related to the method of pouring samples. The software subtracts the mean of the blank readings from every reading of samples and calculates threshold criterion value based on the estimates of the mean (µ) and standard deviation (σ) of negative samples:

$$Threshold = \mu + 3 \times \sigma + 10\%$$  \hspace{1cm} (1)

A yet more conservative equation for calculation of the cutoff point is:

$$Threshold = 3(\mu + 3 \times \sigma)$$  \hspace{1cm} (2)

An algorithm was developed to compare the absorbance of samples adjusted for blank with the threshold value computed based on healthy samples and decide on weather a sample is diseased or healthy.

Infected samples appear in red color and ratio of infection is also shown for sample. The software uses JFreeChart, a free chart library for the Java(tm) platform, to draw a diagram for samples. It runs on the Java 2 Platform (JDK= Java Development Kit 1.3 or later) and uses the Java 2D API (Java 2 Dimensional Application Programming Interface) for drawing charts. The result is output and saved in JPEG (Joint Photographic Experts Group) format.

Another task that the software can perform is finding appropriate reagent dilutions for the ELISA reaction. Users can determine better dilution of the antibody and the conjugate that was extracted from blood of animal for other ELISA tests. Different methods of analysis are offered on the way the dilution of the reagents is determined, although we suggest a map for determining position of the dilutions (Fig. 1).

A user manual with illustrative information is provided to help the users to activate and use this ELISA Analysis. The package utilizes the new tools to provide a favorable environment for Java programming language and combine them to enter data (browse), edit them and also better visualize them. This software can assist researchers to expedite ELISA data analysis, reduce computational errors, facilitate computation, improve the presentation of analysis by creating charts and distinguishing positive samples shown in red color from the negative samples that appear in blue cells of...
plate, calculate the threshold and ultimately determine the most economic proportions of antibody and conjugate in the mixture considering the antibody and conjugate prices.

**Running ELISA Analyzer**

In the main dialogue box of ELISA (Fig. 2(a)), the user has two choices: "Analyze" (to run the diagnostic form) and "Titration" (to calculate the optimal concentration). Analysis mode is invoked if the "Analyze" button is pushed and subsequently the data entry form is displayed (Fig. 2(b)). In this form the user can enter the information manually (in the textboxes) or alternatively the data may be imported directly from the "xls" Excel file by choosing "Open" option of the "File" menu. The user must specify the location of the negative and blank samples. Average of blank is subtracted from all the data and the negative samples are used to determine the threshold (calculated as described above). Now the user can proceed to "diagnosis" and calculate the severity (relative antigen) by pressing "Analyze" button.

After invoking the "Analyze", output is displayed in two different forms: an output a matrix similar to the entry form and a bar chart shown in Fig. 2(c) and Fig. 2(d) respectively. The Figs in each cell indicate the severity (relative antigen content) of the cell which is obtained by dividing the amount of cell absorption by the threshold value. In order to better display the results, graphical display is used in Fig. 2(d). By moving the mouse over each bar in the graph, the severity of the cell is displayed.

If the user invoke the "Titration" mode by pushing the "Titration" button from the main menu, an entry form for determining the concentrations shows up (Fig. 2(a)).
Appropriate dilution of antibodies or conjugates is calculated as intuitive or experimentally and there is no specific rules to follow. Any researcher or company uses a different formula for calculation of proper antibody and conjugate dilutions which may be subject to error. It appears that development of a general formula could facilitate data handling and add to uniformity in calculations is helpful. An appropriate dilution for use in ELISA can be inferred based on the economic value of antibody which is used as a multiplier in the equation and may be determined by entering a proper value in the "Antibody/Conjugate" in "Titration" mode of the software. The output of this section of the software is shown in Fig 3. The red bars are the dilutions calculated using threshold equation and proposed by the ELISA. The bars that are shorter than the others are considered economically optimal.

References