

VI2 - Development of an alternative method for quantification of neutralizing antibody against yellow fever virus

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Introduction:

Enzyme immunoassays have become the method of choice for quick evaluation of vaccine response by quantification of specific IgG antibodies. However, the plaque reduction neutralization test (PRNT) remains the gold standard for the detection of serologic immune responses to yellow fever (YF) virus, but due to the utilization of cell culture, PRNT is hard-working and time consuming, therefore difficulting its utilization in large scale. Further, it does not characterize the immunoglobulin isotype.

Objective:

The aim of this study was to develop an alternative method for evaluation of antibodies response against YF virus.

Methodology:

Thirty sera of individuals before and 30 days after 17DD YF virus vaccination and ten sera from control groups were used (Research Ethics Committee of the Evandro Chagas Clinical Research Institute, n° 0038.0.009.000-08 and of the Oswaldo Cruz Foundation, n° 145/01). An ELISA inhibition, called as ViBI (Virus Binding Inhibition) method was developed based on an adaptation of the Toxin binding inhibition test principle. The PRNT was used as standard method to compare YF antibody titration. Statistical analysis of results was done using GraphPad Prism 5.

Results:

PRNT is generally regarded as the standard assay method, so we considered the success of the ViBI method against this reference. The result of standard curve indicated a high sensibility and reliability of ViBI from 8 to 1000 mUI/mL with quantification limit of 125 mUI/mL. All of results were compared by a non-parametric, paired-sample Wilcoxon test, which showed there was no

significant difference ($P = 0.296$) and was observed an excellent positive correlation (Spearman test, $R^2=0.822$, $P < 0.0001$) between two assay methods.

Conclusion:

It is known that PRNT presented many variables, such as virus passage, cell lines and remains timeconsuming, labor-intensive, with low throughput. To overcome these deficiencies, alternative tests assessing YF virus neutralizing may be invested. The ViBI shows a potential as alternative method for screening YF antibodies, prior to use PRNT confirmation.

Keywords: PRNT, 17DD yellow fever vaccination, ELISA inhibition method - ViBI