



MOL2NET, International Conference Series on Multidisciplinary Sciences
02.USINEWS-03: US-IN-EU Worldwide Science Workshop Series,UMN,
Duluth, USA, 2019



UNIVERSITY OF MINNESOTA DULUTH
Driven to Discover™



Development of a serological test, ELISA, to evaluate the immunogenic activity of diphtheria vaccines

Leidi Hernández

lhsuarez96@gmail.com

*Department of Pharmacology, Faculty of Medicine, University of the Basque Country UPV/EHU,
48940, Leioa, Basque Country, Spain*

Abstract. (mandatory)

Diphtheria is an infectious disease prevented by vaccination. To ensure the safety and immunogenicity of these vaccines, the regulatory entities demand *in*

vitro and *in vivo* techniques. *In vitro* techniques, such as ELISA, reduce the number of animals, are more sensitive, faster and cheaper, then, currently work is focused on its implementation. Therefore, the purpose of this work was to develop a serological assay, ELISA in curiel, that allow the determination of the immunogenic activity of this type of vaccines. The batches of reference materials (RM) were prepared: Diphtheria coating toxoid, curiel serum, calibration curve and positive control. The optimal conditions of the assay were established, the interval and linearity of the curve were defined, as well as the intra- and inter-assay precision and the specificity. The results of the serological test, ELISA in curiel, and the *in vivo* seroneutralization test dose were compared. The calibration curve was generated with a standard serum and a good linear adjustment was achieved with a $R_2 \geq 0,98$; stablishing 10Lf/mL as the optimal coating concentration, 1/12000 as the dilution of conjugate and an incubation temperature of 37°C. The variation coefficients in the intra- and inter assay precision tests in the intervals established for each one were $\leq 10\%$ and $\leq 20\%$ respectively, showing an adequate correlation between both tests. The results obtained endorse the employment of this quantitative ELISA for the evaluation of the immunogenic activity of antidiphtheric vaccines.

KEYWORDS: diphtheria; ELISA, immunogenic activity

Bibliographic references: Grasse M, Meryk A, Schirmer M, Grubeck-Loebenstein B, Weinberger B. Booster vaccination against tetanus and diphtheria: insufficient protection against diphtheria in young and elderly adults. *Immun Ageing*. 2016; 13:26.

Kreeftenberg JG. Consistency Testing of Diphtheria and Tetanus to replace Potency Testing for lot release. *Development in Biologicals*. 2002; 111: 291-298.

Russell W, Burch R. The principles of humane experimental technique.
http://altweb.jhsph.edu/pubs/books/humane_exp/references.