IVD.22 - Standardization of Plaque Reduction Neutralization Test on 96-well Plates (micro-PRNT) for Zika Virus

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Introduction: Plaque Reduction Neutralization Test (PRNT) is considered the "gold standard" by the World Health Organization for the confirmatory diagnosis of *Flavivirus* infection from the determination of neutralizing antibodies. Zika virus (ZIKV), a *Flavivirus* widely circulating in Brazil has caused considerable epidemic impact. Although 80% of cases are asymptomatic, virus infection in symptomatic cases causes headache, low fever, mild joint pain, red spots on the skin, itching and redness in the eyes. Other less common symptoms are swelling in the body, sore throat, coughing and vomiting. Infection by ZIKV has great importance especially for pregnant women, since the virus is a potential cause for the birth of children with a congenital malformation, in which the brain does not develop properly, called microcephaly. The Virological Technology Laboratory (LATEV, Bio-Manguinhos/Fiocruz) performs PRNT assays for different *Flavivirus* species, not only as confirmatory differential diagnosis, but mainly in the evaluation of the immunogenicity of commercial vaccines in development. Bio-Manguinhos currently participates in the development of three different vaccine proposals for the Zika virus. Therefore, considering potential increase in the demand for neutralizing antibody titers for diagnostic and vaccine evaluation, the standardization of a neutralization test for the Zika virus with high sample processing capacity meets the needs of LATEV and, consequently, of Institution and public health.

Objective: The objective of this work was to standardize the micro-PRNT for ZIKV, methodology with greater capacity of sample processing, performed in 96-well plates.

Methodology: The methodology of this work was based on the determination of the protocol of execution of the micro-PRNT for ZIKV. Although the rationale and test steps have already been determined for other flaviviruses, it was necessary to evaluate the variables of the micro-PRNT methodology and their different conditions specifically for the determination of neutralizing antibodies to ZIKV, ie standardize the test for ZIKV. Therefore, the standardization process involved the main steps/variables of the test: cell density, final incubation time, ideal virus dilution and concentration of semisolid medium.

Results: Monolayers prepared at 2.0 x 10^5 cells / ml 24 hours before virus infection and incubated for 4 days with 2.0% semisolid medium resulted in the best PFU profile for ZIKV in 96-well plates. The ideal virus dilution to obtain on average 80 PFU / well was previously determined.

Conclusion: As a result of the standardization of this gold-standard methodology in 96-well plates, LATEV becomes capable of increasing its sample processing capacity and, consequently, efficiently meet the increasing demand for the determination of neutralizing antibody titers for the ZIKV. Thus, the next step will be to validate the test according to Anvisa's regulatory standards.

Keywords: PRNT; Zika Virus; Standardization