ASSESSMENT OF THE ELISA TO DETECT NS1, AS A NEW TOOL OF EARLY DIAGNOSTICS OF DENGUE INFECTION IN PANAMA

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INTRODUCTION

Dengue is the mosquito-transmitted disease with the major relevance in terms of morbidity, mortality and economic impact. As the quantity of infections by Dengue virus (DENV) increases, the necessity to make efficient decisions for its diagnostic and treatment is crucial. A sensitive and specific detection of dengue acute infection in the early diagnostic reduces the risk of complications, as dengue hemorrhagic fever and the dengue shock syndrome.

The detection of DENV antigen NS1 by ELISA allows the early detection of primary and secondary dengue infection. The NS1 antigen is a non-structural glycoprotein that is expressed by the four DENV serotypes and that can be detected in the serum one to nine days after the beginning of fever, whereas the IgM antibodies can be detected only 5-8 days after the manifestation of the symptoms. This technique is easier, faster, less expensive and do not require special or sophisticated equipment like PCR or viral isolation attempt.

MATERIALS AND METHODS

This study compared and analyzed the results of NS1 and IgM ELISA (Dengue Early ELISA and Dengue IgM Capture ELISA, Panbio, Australia) from 138 sera of febrile patients with suspicion of DENV infection that were seen in Hospital San Fernando in Panama, with results obtained using other techniques performed at the laboratory of the Department of Research in Virology and Biotechnology, Gorgas Memorial Institute of Health Studies (ICGES). We tested those sera with viral isolation and RT-PCR. The viral isolation was done using Vero E6 cells and the positivity of the samples were detected with inmunofluorescence using monoclonal antibodies (CDC, Ft. Collins, USA), to identify the serotype. For the RT-PCR, the viral RNA was extracted with the commercial kit QIAamp Viral RNA Mini (QIAGEN, USA), according to manufacturer's instructions and Dengue RNA was detected using SuperScript III Platinum kit (Applied Biosystem, S.A., Life Technologies, USA). The primers and probes used were published by Leparc-Goffart *et al*., 2009 and the machine used was the Applied Biosystems 7500 Fast.

RESULTS

Of the 138 serum samples suspected of acute Dengue, 50 were positive for NS1 ELISA (36.2%), 41 for RT-PCR (29.7%), 26 for viral isolation (18.8%), and 54 for IgM (36.2%). In total, 90/138 sera were positive for one or more of those methods. Secondary Dengue infections were excluded using the IgG specific ELISA kit from Panbio..

Thus we found that the sensitivity for each method was: 55.5% (50/90) for NS1, 45.5% for RT-PCR, and 60% for IgM. When both NS1 and IgM were used for diagnosis of acute Dengue infection, 82 sera were positives given a sensitivity of 91.1%. Additionally, the combination of RT-PCR and IgM showed the highest sensitivity (95.5%).

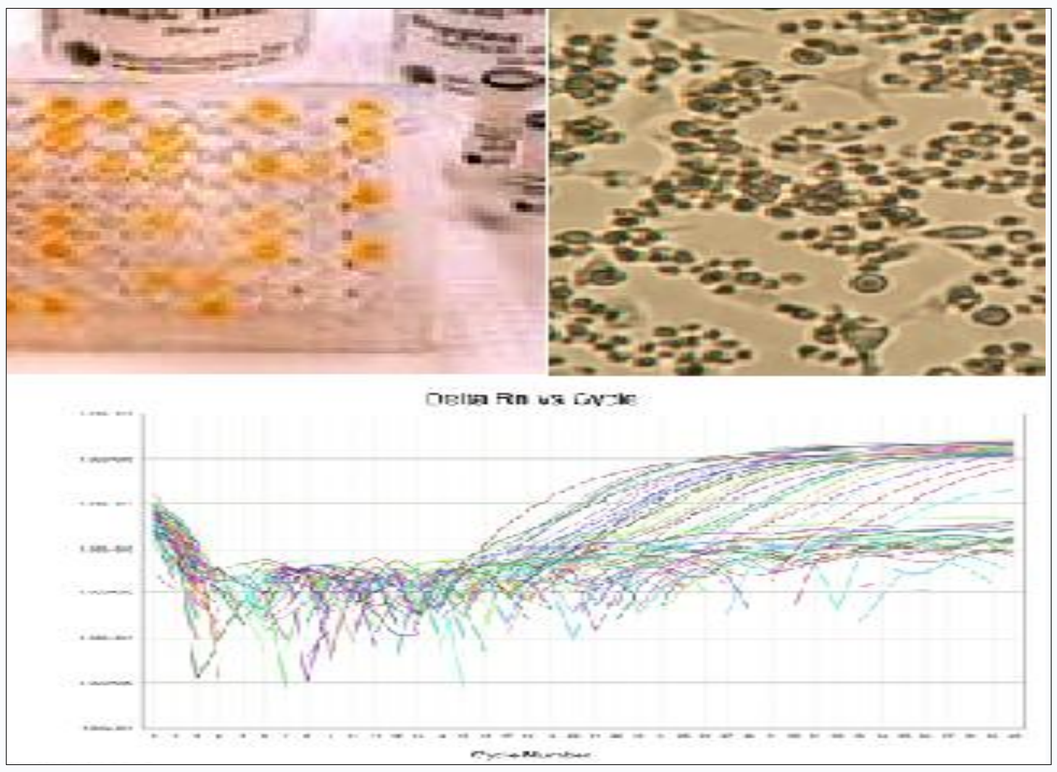

Figure1. Methods compared: Dengue Early ELISA and Dengue IgM Capture ELISA, viral isolation and RT-PCR .

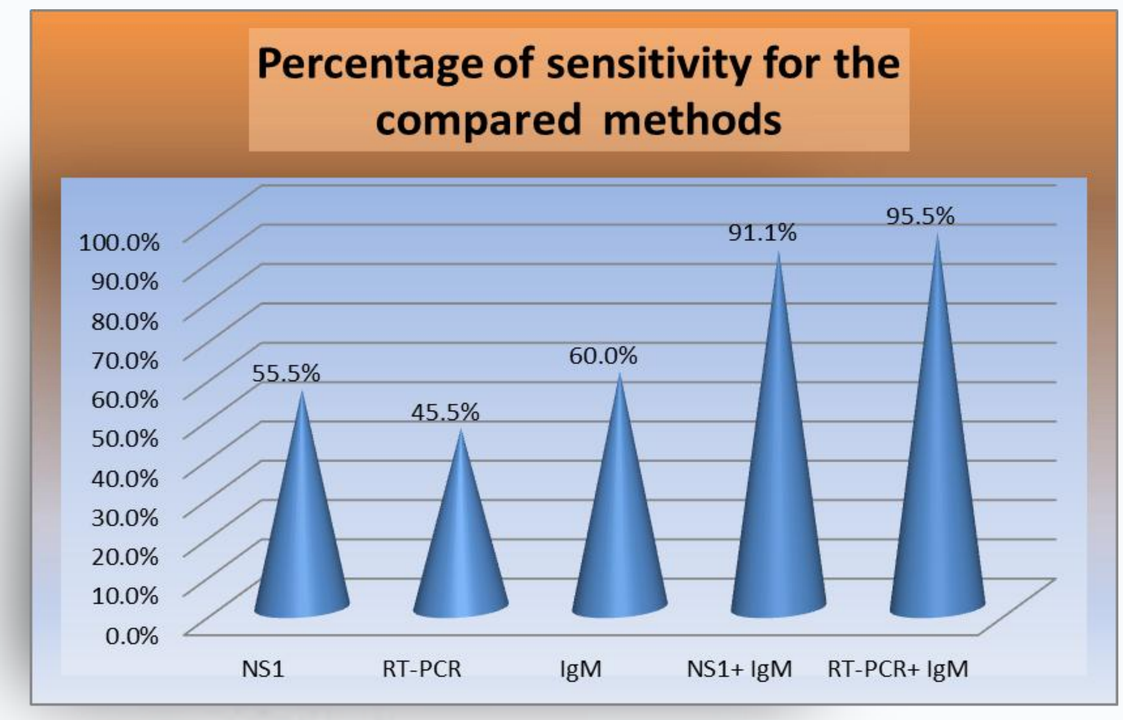

Figure 2. Sensitivity of each mehtod tested.

CONCLUSIONS

Most of the positive results obtained for either NS1 or IgM ELISA or for both, were also positive by viral isolation and/or DENV specific RT-PCR performed at ICGES. We observed the best sensitivity for the combination of RT-PCR + IgM, which are the methods used at ICGES for surveillance, as National Reference Center for Dengue. However, because RT-PCR is an expensive method, we suggest the use NS1+IgM in the health installations. The use of both NS1 and IgM ELISAs could reduce the probability to lose the detection of some acute positive cases, and could permit the diagnosis of dengue cases earlier. In conclusion, NS1 ELISA is designed to offer an early diagnostic of DENV infection as a complement of IgM detection.

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