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Extended Abstract
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Epidemiology & Public Health 2020: A high-throughput and multiplex microsphere immunoassay based on non-structural protein 1discriminates three flavi-virus infections

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Abstract:

The four serotypes of dengue virus (DENV) are the leading cause of arbo-virus infections in humans. The recent outbreaks of Zika virus (ZIKV) and associated complications in DENV-endemic regions highlight the critical need for sensitive and specific sero-diagnostic tests. Most of these viruses are primarily transmitted by the bite from an infected arthropod (mosquito or tick), and hence are classified as arbo-viruses. Human infections with most of these arbo-viruses are incidental, as humans are unable to replicate the virus to high enough titers to re-infect the arthropods needed to continue the virus lifecycle – humans are then a dead end host. The exceptions to this are the yellow fever, dengue, and zika viruses. These three viruses still require mosquito vectors, but are well-enough adapted to humans as to not necessarily depend upon animal hosts. ZIKV and DENV belong to the genus Flavi-virus of the family Flaviviridae. Other virus transmission routes for arbo-viruses include handling infected animal carcasses, blood transfusion, sex, child birth and consumption of unpasteurised milk products. Cellular RNA cap structures are formed via the action of an RNA triphosphatase, with guanylyltransferase, N7-methyltransferase and 2′-O methyltransferase. The virus encodes these activities in its non-structural proteins. The NS3 protein encodes a RNA triphosphatase within its helicase domain. It uses the helicase ATP hydrolysis site to remove the γ-phosphate from the 5′ end of the RNA. Once translated, the polyprotein is cleaved by a combination of viral and host proteases to release mature polypeptide products.[11] Nevertheless, cellular post-translational modification is dependent on the presence of a poly-A tail; therefore this process is not host-dependent. Instead, the polyprotein contains an autocatalytic feature which automatically releases the first peptide, a virus specific enzyme. This enzyme is then able to cleave the remaining polyprotein into the individual products. One of the products cleaved is a polymerase, responsible for the synthesis of a (-) sense RNA molecule. Consequently, this molecule acts as the template for the synthesis of the genomic progeny RNA.

Flavivirus genomic RNA replication occurs on rough endoplasmic reticulum membranes in membranous compartments. New viral particles are subsequently assembled. This occurs during the budding process which is also responsible for the accumulation of the envelope and cell lysis. The N-terminal domain of the non-structural protein 5 (NS5) has both the N7-methyltransferase and guanylyltransferase activities necessary for forming mature RNA cap structures. RNA binding affinity is reduced by the presence of ATP or GTP and enhanced by S-adenosyl methionine.[10] This protein also encodes a 2′-O methyltransferase. Transmission from nonhuman vertebrates to humans without an intermediate vector arthropod however mostly occurs with low probability. For example, early tests with yellow fever showed that the disease is not contagiousThe Flaviviridae are a family of positive, single-stranded, enveloped RNA viruses. They are found in arthropods, (primarily ticks and mosquitoes), and can occasionally infect humans. Members of this

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family belong to a single genus, Flavivirus, and cause widespread morbidity and mortality throughout the world. Traditional envelope protein-based serological tests for flavivirus infections have been hampered by extensive cross-reactivity among different flaviviruses. In this study, we developed a high-throughput and multiplex IgG microsphere immunoassay (MIA) using the NS1 proteins of DENV1-DENV4, ZIKV and West Nile virus (WNV) to test samples from reverse-transcription-polymerase-chain reaction-confirmed cases,including primary DENV1, DENV2, DENV3, WNV and ZIKV infections, secondary DENV infection, and ZIKV infection with previous DENV infection.Combination of four DENV NS1 IgG MIAs revealed a sensitivity of 94.3% and specificity of 97.2% to detect DENV infection. The ZIKV and WNV NS1 IgG MIAs had a sensitivity/specificity of 100%/87.9% and 86.1%/78.4%, respectively. A positive correlation was found between the readouts of enzyme-linked immunosorbent assay and MIA for different NS1 tested. Consistent with our previous reports (Tsai et al. Clin Infect Dis 2017, 65:1829-1836; Emerg Infect Dis 2018, 24: 1355-1359), secondary DENV infection panel cross-reacted to ZIKV NS1 in IgG MIA, and ZIKV infection with previous DENV infection panel recognized both DENV and ZIKV NS1. Based on the ratio of relative median fluorescence intensity of ZIKV NS1 to DENV1 NS1, the IgG MIA can distinguish ZIKV infection with previous DENV infection and secondary DENV infection with a sensitivity of 88.9–90.0% and specificity of 91.7–100.0%. The multiplex and high-throughput assay could be applied to serodiagnosis and serosurveillance of DENV, ZIKV and WNV infections in endemic regions. The flaviviruses can be divided into 2 clades: one with the vector borne viruses and the other with no known vector. The vector clade in turn can be subdivided into a mosquito-borne clade and a tick-borne clade. These groups can be divided again. The mosquito group can be divided into two branches: one branch contains the neurotropic viruses, often associated with encephalitic disease in humans or livestock. This branch tends to be spread by Culex species and to have bird reservoirs. The second branch is the non-neurotropic viruses which are associated with haemorrhagic disease in humans. These tend to have Aedes species as vectors and primate hosts. The tick-borne viruses also form two distinct groups: one is associated with seabirds and the other - the tick-borne encephalitis complex viruses - is associated primarily with rodents. The viruses that lack a known vector can be divided into three groups: one closely related to the mosquito-borne viruses which is associated with bats; a second, genetically more distant, is also associated with bats; and a third group is associated with rodents. It seems likely that tick transmission may have been derived from a mosquito-borne group. Estimates of divergence times have been made for several of these viruses.[34] The origin of these viruses appears to be at least 9400 to 14,000 years ago. The Old World and New World dengue strains diverged between 150 and 450 years ago. The European and Far Eastern tick-borne encephalitis strains diverged about 1087 (1610–649) years ago. European tick-borne encephalitis and louping ill viruses diverged about 572 (844–328) years ago. This latter estimate is consistent with historical records. Kunjin virus diverged from West Nile virus approximately 277 (475–137) years ago. This time corresponds to the settlement of Australia from Europe. The Japanese encephalitis group appears to have evolved in Africa 2000–3000 years ago and then spread initially to South East Asia before migrating to the rest of Asia.

Phylogenetic studies of the West Nile Virus have shown that it emerged as a distinct virus around 1000 years ago. This initial virus developed into two distinct lineages, lineage 1 and its multiple

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profiles is the source of the epidemic transmission in Africa and throughout the world. Lineage 2 was considered an Africa zoonosis. However, in 2008, lineage 2, previously only seen in horses in sub-Saharan Africa and Madagascar, began to appear in horses in Europe, where the first known outbreak affected 18 animals in Hungary in 2008.[36] Lineage 1 West Nile virus was detected in South Africa in 2010 in a mare and her aborted fetus; previously, only lineage 2 West Nile virus had been detected in horses and humans in South Africa. A 2007 fatal case in a killer whale in Texas broadened the known host range of West Nile virus to include cetaceans.

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