ImmunoTools *multiplex* Award 2015

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Determination of dengue NS1 in antibody array

Dengue hemorrhagic fever (DHF), caused by dengue viruses (DV), is a major public health problem causing mortality and morbidity mainly to children in tropical and sub-tropical countries. The virus consists of four serotypes which co-circulate in endemic areas. DHF occurs in areas where more than one viral serotype co-circulates, and DHF is frequently found in those who respond secondarily to heterotypic (different serotype) viral infections. Sequential infections by some serotypes may contribute more to the outbreaks than others. Existing technologies to identify DV serotypes include laborious virus isolation and serotype-specific RT-PCR.

According to the finding that the viral non-structural protein 1 (NS1) of dengue virus is secreted into patients' circulation at the early stage of infection, several diagnostic tests to detect NS1 protein in plasma based on ELISA and immunochromatographic test (ICT) have been developed and commercialized. In comparison to a standard RT-PCR method, the specificity of NS1 assays is very high (95%), whereas the sensitivity is rather moderate (65-80%). In our laboratory, we have generated our own hybridoma clones producing monoclonal antibodies to dengue NS1 protein which are reactive either across all four serotypes or are specific to each serotype. We thus developed two in-house dengue NS1 ELISA tests, one could detect dengue NS1 across four serotypes, the other could detect NS1 and identify dengue serotypes simultaneously (the feature of which has not yet been available on the market), namely Serotyping-NS1-ELISA. The sensitivity and specificity of both assays are comparable to the commercial kits. To increase the sensitivity of detecting dengue NS1 in patient specimens, the assay should be sensitive enough to detect nanogram levels of NS1 protein by using the most reactive anti-NS1 antibodies to all four serotypes.

The antibody microarray, comprising more than one thousand known antibodies spotted on a chip, is the ideal tool for many kinds of protein discovery or

screening such as target identification, protein profiling and biomarker discovery. It would be the platform of our choice since there is free space provided for exploring any specific antibodies to the target antigens as requested and very small amounts of antibody and tested antigen are required. As we have a number of monoclonal antibodies to dengue NS1, those antibodies can be spotted on the free space and reacted with patients' sera containing dengue NS1 at the same time. We propose to use dengue NS1 of known concentration and serotype for setting up the dengue NS1 antibody array in order to analyze serotype specificity of the antibody as well as the limit of detection of the assay. Once the dengue NS1 antibody array is set up, it would be further tested with a panel of dengue patients' sera to detect dengue NS1 and serotypes and analyzed for sensitivity and specificity compared to a standard RT-PCR method.

ImmunoTools *multiplex* AWARD for **Chunya Puttikhunt**

includes free analysis of samples on several antibody arrays with large range of antibodies against human CDs, human cytokines, and others.