Reproducibility of Dengue RVP Assays

Reproducibility of Dengue Virus Assays

The clinical development of Dengue vaccines relies on efficient and reproducible methods for screening large sample sets of human serum for antibody immunoreactivity and neutralization. The current gold standard assay for measuring the neutralizing ability of serum samples is the PRNT (Plaque Reduction Neutralization) assay. However, the PRNT assay can be time-consuming and variable due to requirements for live virus, manual manipulations, and subjective visual assessments. Automated and more reproducible screening methodologies for Dengue virus infectivity and immunoreactivity would enable higher quality data to be collected.

The Dengue Reporter Virus Particle

Integral Molecular has developed Dengue Reporter Virus Particles (RVPs) to rapidly and reproducibly screen human serum samples for Dengue neutralizing and enhancing antibodies. Dengue RVPs correspond to serotypes 1, 2, 3, or 4 and retain the antigenic determinants of wild type virions including envelope, pre-membrane and membrane proteins. To enable convenient and reproducible detection, Dengue RVPs express a luciferase reporter gene upon infection of permissive target cells, which provides a quantitative assessment of levels of Dengue infection. Assays using Dengue RVPs are optimized for standard luminescent microplate readers, are amenable to automation, and are ideal for screening large numbers of serum samples from vaccine trials or epidemiological surveys.

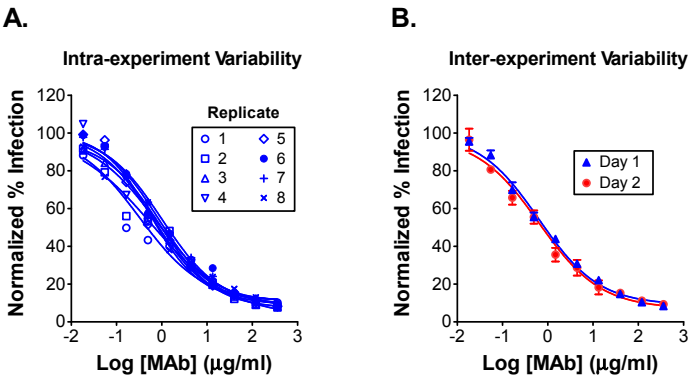

Figure 1. A. Dengue RVPs were preincubated with increasing concentrations of the neutralizing antibody 4G2 prior to addition of Raji DC-SIGNR cells. After 48 hours, infected cells were quantified by luminescence. Data from eight replicate experiments done on the same day are shown. **B**. Dose response curves constructed from data gathered on separate days show the same inhibition profile and indistinguishable IC_{50} values.

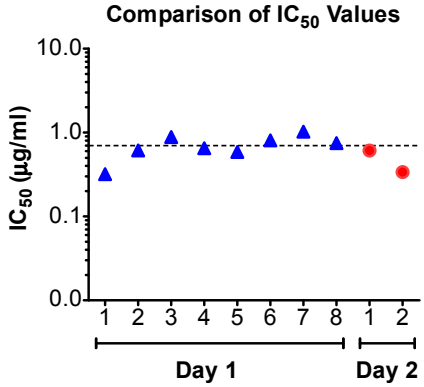

Figure 2. Individual IC_{50} values from replicate Dengue RVP neutralization experiments performed on two different days were calculated. Values ranged from 0.3-1.0 ug/ml. The mean IC_{50} of 0.7 ug/ml for all experiments is denoted by a dashed line.

Technical Description

The reproducibility of the Dengue RVP neutralization assay was assessed using the monoclonal antibody (MAb) 4G2 with Dengue RVPs. Equivalent amounts of Dengue RVPs (DENV2) were used to infect permissive Raji DC-SIGNR target cells in the presence of increasing amounts of neutralizing antibody. To assess *intra*-experimental variability, dose response curves measuring concentration-dependent neutralization of RVP infection were conducted for eight replicate assays within a single experiment (Figure 1A). The individual IC_{50} values from this experiment showed no statistically significant differences. To measure *inter*-experimental variability, identical dose-response experiments were conducted independently on separate days. The dose-response curves from both days showed the same antibody dependent inhibition profile, with IC_{50} values that were indistinguishable (Figure 1B). Individual IC_{50} values calculated from all of these dose response curves ranged from 0.3-1.0 ug/ml, with a mean value of 0.7 ug/ml (Figure 2). These results demonstrate that Dengue RVPs can be used in replicate experiments to obtain reproducible values of infection and neutralization.

Contact Us

Quality controlled Dengue Reporter Viruses are commercially available from Integral Molecular with defined strains of DENV serotypes 1, 2, 3, or 4 structural proteins, and with convenient luminescent reporters. For more information contact us at:

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