Detecting Neutralizing Antibodies using Dengue Reporter Viruses

Quantification of Dengue Neutralization

Measuring the occurrence of a neutralizing antibody (NAb) response is critical for characterizing the efficacy of potential Dengue vaccines, and for epidemiological monitoring of natural epidemics. The current gold standard for quantification of NAbs is the PRNT (Plaque Reduction Neutralization) assay, in which live Dengue virus is pre-incubated with test serum, and residual infectivity is measured in cell culture by counting plaques. In addition to being technically-challenging, the PRNT assay is labor-intensive and slow, and requires BSL2/3 conditions. The analysis of large numbers of serum samples in vaccine trials or epidemiological studies requires a quality-controlled, automated method to test for neutralizing antibodies specific for each Dengue virus serotype.

The Dengue Reporter Virus

Integral Molecular has developed the Dengue Reporter Virus Particle (RVP) for rapidly screening sera for neutralizing antibodies in a standardized, high-throughput format. RVPs are serotype-specific Dengue viral particles that carry a gene for an optical reporter (such as GFP, luciferase, or β-galactosidase) (Figure 1A). When RVPs are added to permissive cells, infectivity can be determined by expression of the reporter gene (Figure 1B). RVPs retain the antigenic determinants of wild-type virions, so can be used to monitor humoral protection against all four Dengue virus serotypes. Importantly, RVPs are safe, lacking the viral machinery required for viral replication, allowing their use within standard cell culture facilities. The assay format is optimized for standard fluorescent or luminescent-based microplate readers, providing an easy and objective measure of infection that can be obtained within 48 hours. The RVP neutralization assay is designed to permit automated, high-throughput screening of sera from vaccine trials or epidemiological surveys.

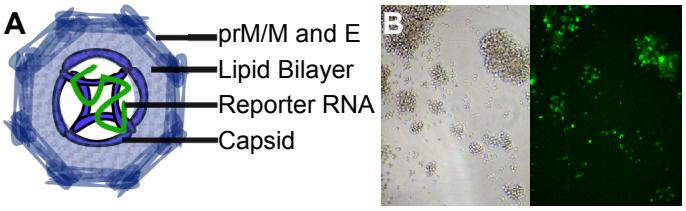

Figure 1. Dengue Reporter Virus composition and reporter expression. (A) Schematic representation of RVPs containing the viral structural proteins envelope (E), capsid, and pre-membrane/membrane proteins (prM/M), a lipid envelope, and a reporter RNA. The Reporter RNA encodes GFP or Luciferase and DENV non-structural proteins responsible for replication of the RNA. **(B)** Target Raji DC-SIGNR cells infected by RVPs expressing GFP are easily visualized by microscopy or quantified by flow cytometry.

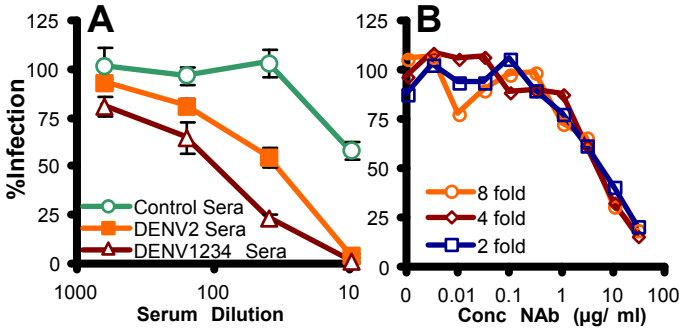

Figure 2. Detection of NAbs using Dengue Reporter Viruses. RVPs (serotype 2) were incubated with dilutions of human sera (A) or a purified monoclonal antibody (B) before the addition of target Raji DC-SIGNR cells. Approximately 48 hours later, infected cells were quantified by cytometry. RVPs were used either at a 2 fold dilution (A) or as indicated (B).

Technical Description

Dengue reporter viruses were used to quantify neutralizing antibodies present in a panel of WHO reference standards, comprising antiserum against Dengue serotype 2, antiserum raised against all four Dengue virus serotypes (D1234), and a naïve serum sample. RVPs carrying a GFP reporter gene were produced from Dengue serotype 2 (strain 16803, PDK50), and used to infect permissive Raji DC-SIGNR cells in the presence of each serum. Concentration-dependent neutralization of RVP infection was observed for all specific antiserum standards, and RVPs were not affected by naïve serum until very high concentrations were used (Figure 2A). Neutralization results were fully consistent with results obtained by conventional PRNT assays using the same sera. Dilution of reporter viruses from 2-fold to 8-fold did not affect the IC_{50} values derived from neutralization curves (Figure 2B), indicating that calculated NAb titers are quantitatively meaningful. These data demonstrate the functional and antigenic authenticity of RVPs, and their utility as a tool for rapid screening of human serum for the presence of NAbs. RVPs can also be used for additional applications, including the detection of antibody-dependent enhancement (ADE) and drug discovery.

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:

215.966.6061
info@integralmolecular.com
www.integralmolecular.com

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