Neutralization Assay

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Neutnet code: 4A and 4B

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A recombinant virus assay was initially developed to measure antiretroviral drug resistance during a single round of virus replication and has subsequently been adapted to measure virus-antibody neutralization. HIV genomic RNA is isolated from virus stocks or plasma using oligo-dT magnetic beads. First strand cDNA is synthesized in a standard RT reaction using an oligo-dT primer. Env DNA (gp160) is amplified by PCR using forward and reverse primers located immediately upstream and downstream of the env initiation and termination codons, respectively. The forward and reverse primers contain recognition sites for PinAI and MluI, respectively. Env PCR products are digested with PinAI and MluI and ligated to compatible ends in the pCXAS expression vector, which uses the CMV immediate-early promoter-enhancer to drive env insert expression in transfected cells. Ligation products are introduced into competent E. coli (Invitrogen) by transformation, and pCXAS-env plasmid DNA is purified from bacterial cultures (Qiagen). An aliquot of each transformation is plated onto agar, and colony counts are used to estimate the number of envelope sequences represented in each pCXAS-env library (generally, 500 to 5,000 clones). Sequence analysis of individual pCXAS-env clones (10-20) has been used to verify the heterogeneous composition (i.e. quasispecies) of pCXAS-env libraries. Virus particles containing patient virus envelope proteins are produced by co-transfecting HEK293 cells with pCXAS-env libraries plus an HIV genomic vector that contains a firefly luciferase indicator gene. pCXAS-env plasmid preparation and HEK293 cell transfection conditions have been optimized to insure consistent virus particle production. Recombinant viruses pseudotyped with patient virus envelope proteins are harvested 48 h post-transfection and incubated for 1 h at 37°C with serial 4-fold dilutions of heat-inactivated patient plasma samples (antibody). U87 cells that express CD4 plus the CCR5 and CXCR4 co-receptors are inoculated with virus-plasma (antibody) dilutions. Virus infectivity is determined 72 h post-inoculation by

measuring the amount of luciferase activity expressed in infected cells. Neutralizing activity is displayed as the percent inhibition of viral replication (luciferase activity) at each antibody dilution compared to an antibody negative control: % inhibition = {1 - [luciferase (+) Ab / luciferase (-) Ab]} x 100. Titers are calculated as the reciprocal of the plasma dilution conferring 50%, 80%, 90% and 95% inhibition ($IC_{50,}$ $IC_{80,}$ $IC_{90,}$ IC_{95}).

Reference:

Antimicrobial Agents and Chemotherapy, 2007: 566-575.
Development and Characterization of a Novel Single-Cycle Recombinant-Virus Assay To Determine Human Immunodeficiency Virus Type 1 Coreceptor Tropism
Jeannette M. Whitcomb, Wei Huang, Signe Fransen, Kay Limoli, Jonathan Toma, Terri Wrin, Colombe Chappey, Linda D. B. Kiss, Ellen E. Paxinos, and Christos J. Petropoulos*