

## POSTER 24

### UNIVERSAL PROTOCOL FOR SEQUENCING HIV-1 *POL* ACROSS CLADES FOR SUBTYPE IDENTIFICATION AND ANALYSIS OF DRUG RESISTANCE

Ting Nie, Mervi Detorio, and Raymond F. Schinazi\*

Center for AIDS Research, Department of Pediatrics, Laboratory of Biochemical Pharmacology, Emory University/VA Medical Center, Atlanta, Georgia, USA

Background: Increased access of anti-HIV-1 treatments to developing countries primarily infected by non-subtype B clades necessitates development of novel tools to assess susceptibility and resistance. HIV-1 genomes are highly polymorphic and present challenges in development of a universal protocol for simultaneous screenings across subtypes. Viral genotyping was previously reported using a series of primers designed to amplify HIV-1 *gag* (Lu W, et al, 1999), which is primarily useful for viral quantification, but is not applicable for mutation detection in highly polymorphic RT regions. Thus, a novel and universal protocol was developed which was applicable across all HIV-1 clades and subtypes for effective identity confirmation and simultaneous generation of sequence profiles of *pol* genes, useful for mutation detections.

Methods: Primer sets specifically targeting highly conserved regions in HIV-1 *pol* were designed. Using the universal primers, one-step reverse-transcription PCR was performed on supernatants from total 83 HIV-1 clades originally obtained from NIH AIDS Research and Reference Reagent Program, including A(9), B(12), C(19), D(13), E(9), F(3), G(4), A/C(2), A/E(7), A/D(1) of group M, group O(3) and N(1). One DNA fragment was universally amplified with a unique size of 1.7kb. Using another set of primers, the PCR products were sequenced and the results were analyzed with Vector NTI to generate cDNA sequence. We also performed a blast of the cDNA sequences in the Stanford HIV database for clade/subtype classification and mutation detection.

Results: The novel universal primers were used to amplify one unique band in all the tested clades/subtypes. The sequencing of this PCR product confirmed the identity of each HIV-1 isolate and covered *pol* regions, including RT, RNaseH, and IN, which provided sufficient data for clade profiling.

Conclusions: The universal protocol developed represents a novel and effective tool for evaluation and identification of HIV-1 genotypes. Its capacity for rapid profiling of HIV-1 sequences will be useful for analysis of drug resistance across HIV-1 populations. This protocol also provides a foundation for understanding and prediction of the effects of experimental and approved drugs on various HIV-1 clades/subtypes.