Abstract

Title: Evaluation of efficacy of Dried Blood Spots (DBS) as compared to plasma samples for detection of HIV-1 drug resistance mutations

Department: Clinical Microbiology

Name of candidate: Priyanka Sabu

Degree and Subject: MD Microbiology (Branch IV)

Name of guide: Dr. Rajesh Kannangai

Introduction:

India has a large number of people living with HIV/AIDS. The infected individuals are associated with significant morbidity and mortality. The initiation of antiretroviral therapy (ART) has led to improvement among HIV infected individuals, however the advent of drug resistance can negate the benefits of ART. HIV-1 genotyping has become vital in ART-experienced persons who are failing their current regimen. The challenges involved in collection, storage and transport of plasma samples to reference laboratories for drug resistance testing has led to the need for alternative samples.

Aim:

This study aims to compare the efficacy of Dried Blood Spots (DBS) stored at 25^0-30^0C to plasma for the detection of HIV-1 resistance mutations and to confirm the origin of mutations by testing Peripheral blood mononuclear cells (PBMC).

Objectives:

1) To sequence HIV-1 *pol* gene from plasma, DBS and PBMC to assess drug resistance mutations in the reverse transcriptase and protease region in individuals showing treatment failure.
2) To compare the frequency of HIV-1 drug resistance mutations detected in plasma, DBS and PBMC to confirm the origin of mutations.
3) To evaluate the efficacy of DBS for detection of HIV-1 drug resistance mutations in samples stored at 25^0-30^0C for 10 days.

Materials and Methods:

Whole blood was collected from 29 individuals in clinical, immunological and or virological failure referred for HIV-1 viral load estimation and genotyping. 80 µl of blood was applied onto Whatman 903 filter paper card on five spots each and stored at 25^0-30^0C for 10 days before resistance testing.

From the remaining whole blood, plasma and PBMC were separated and genotyped by sequencing *pol* gene. HIV-1 drug resistance testing was carried out for all plasma, DBS and PBMC samples by amplifying and sequencing the *pol* gene. The frequency of drug resistance mutations detected was compared between the 3 sample types.

Results:

Of the 29 samples, 26 (89.7%) had a viral load (VL) >1000 copies/ml from which 25 (96.2%) amplified from plasma and 19 (73.1%) from DBS. From the remaining 3 samples, 1 (33.3%) amplified from plasma and 1 (33.3%) from DBS. All 29 (100%) samples amplified from PBMC.
From the total 29 samples, between plasma, DBS and PBMC, 18 samples amplified in all three sample types. Ten samples successfully amplified in any 2 sample types and one sample amplified only from one sample type which was PBMC. The overall sensitivity of DBS and PBMC with respect to plasma was 69.2% and 100%, respectively.
The mean number of mutations in plasma, DBS and PBMC were 5.885, 4.550 and 4.621, respectively. The difference between the means was not statistically significant. The agreement between plasma and DBS, and plasma and PBMC were 0.742 and 0.703, respectively. And the agreement between DBS and PBMC was 0.816.
In the 20 DBS samples which amplified and were sequenced, mutations against PI, NRTI and NNRTI were seen in 5%, 80% and 85% of the samples, respectively. Among all the PBMC samples, PI, NRTI and NNRTI mutations were seen in 6.9%, 72.4% and 79.3% of the samples, respectively. And among the 26 plasma samples, mutations against PI, NRTI and NNRTI were seen in 15.4%, 80.8% and 84.6% of the samples, respectively.

Discussion and Conclusion:

Studies have shown the amplification success rate of HIV-1 genotyping from DBS stored at ambient temperature ranges from 63-90% when VL >1000 copies/ml, depending on the duration of storage and our study shows 73.1%. Additionally PBMC showed 100% amplification making it a possibility in low viral load samples and complimentary to plasma. DBS is a promising sample for HIV-1 genotyping in resource limited settings due to transportation ease, however amplification success from DBS stored at ambient temperature is still wanting and larger sample size needs to be studied for better valuation.

Keywords: HIV-1, treatment failure, genotyping, drug resistance, dried blood spots, peripheral blood mononuclear cells