# Evaluation of Dried Blood Spots (DBS) for Human Immunodeficiency Virus (HIV-1) Drug Resistance Testing

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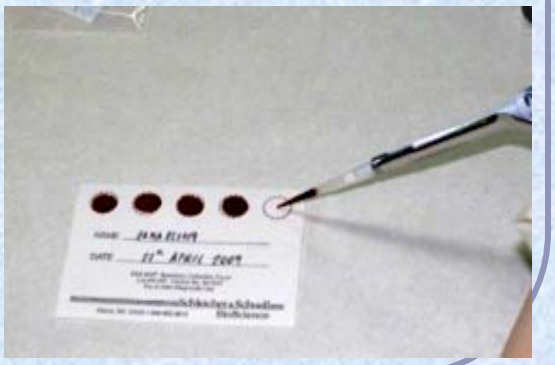
# BACKGROUND


- Free nationwide antiretroviral treatment in Ethiopia was launched in 2005,
- The number of patients on ART has been on a rise
- On average 4500/month pt are estimated to start newly ART
- Sites grown from 3 in 2005 to 517 by 2009
- Total 517 facilities
  - Hospital 135
  - Health centers 382

- With increasing availability of ART, the potential for the emergence and transmission of drug-resistant HIV strains increases.
- Thus, simple and inexpensive procedures are required to monitor the prevalence of HIV drug resistance
- DBS has been extensively used for
  - ✓ HIV-antibody testing,
  - ✓ molecular diagnostics,
  - ✓ viral RNA quantification (viral load) and
  - ✓ $CD4^+$ lymphocyte enumeration

# DBS

- Do not require venipuncture
- Do not require centrifugation
- Not biohazard for shipping
- Do not require dry ice for shipping
- Lower volume required (50 µl to 100 µl)



# OBJECTIVE

**To evaluate the efficiency of amplification and genotyping of HIV-1 subtype C from DBS and assess the similarity between *pol* sequences from paired plasma and DBS specimens stored at -20°C with desiccant for 40.7±5.4 months**

# METHODS

## Study populations

- Blood specimens were consecutively collected with venipuncture using EDTA vacutainer tubes from patients who were treatment naïve but eligible to start treatment at the St. Paul's Generalized Specialized Hospital, Addis Ababa, from April 2005 to July 2005, for base line HIV drug resistance survey
- DBS were prepared as shown below
- 32 of the DBS had corresponding plasma

  ➡ Used for evaluation of DBS for the HIV drug resistance testing

# DBS preparation

- 50µl of whole blood was pipetted onto each circles on S&S 903 filter paper cards using micropipette
- Dried overnight at RT in a vertical position on a rack
- Individually packed into in a gas-impermeable bag, containing silica gel pack and humidity indicator
- Individual bags were put in a larger bags and then stored at -20$^{o}$C for an average of 40.7 ± 5.4 (mean,SD) months

# HIV drug resistance from DBS

- **Nucleic acid extraction**

  Nuclisens-Silica based method

- **Amplification**
  - A 1,023 base pair fragment of HIV-1 *pol* amplified using an in-house nested RT-PCR method
  - CDC in-house assay in origin
  - Validated for HIV-1 subtype B & C

# Sequencing and Genotype Interpretation

- ABI 3100 automated genetic analyzer (Applied Biosystems, CA,USA) was used for sequencing
- Sequence data were manually edited by using ChromasPro version 1.42 software
- Corresponding plasma genotypes were obtained using both the ViroSeq™ HIV-1 genotyping v2.0 (Celera diagnostics, CA, USA) and the in-house nested RT-PCR
- The *pol* sequence were analyzed and compared using the Stanford Genotyping Resistance Interpretation algorithm (http://hivdb.stanford.edu)
- Vector NTI 10.3.1 program was used to calculate nucleotide similarities of the paired sequences

# Result

# Efficiency of amplification from DBS

- A 1023 base pair fragment of the HIV-1 *pol* comprising amino acid 13-99 of the PR and 1-254 of the RT were amplified and sequenced in 62 of the DBS stored at -20 $^{0}$c
- ⇒ Amplification efficiency of 98.4%

## Comparison of sequence similarity between DBS and plasma

- Excellent agreement between HIV drug resistance report generated by the DBS and plasma

⇒ 99.1 % complete concordance HIV drug resistance report using Stanford HIV drug resistance database

- Discordance result was found to be 0.38%, and

⇒ It corresponds to polymorphism.

- All (100%) HIV-1 drug resistance-associated mutations detected in plasma specimens were also detected in the corresponding DBS specimens

# Nucleotide similarity

- The mean nucleotide similarity of the pol gene sequence of DBS and plasma was 99.64 ± 0.33% and ranged between 98.9 % and 100%.

# CONCLUSION

- DBS is an appropriate specimen type for surveillance of HIV-1 drug resistance among treatment naïve subjects in resource limited settings where logistic difficulties could prevent the use of plasma and serum specimens.
- The high efficiency of amplification and sequencing of a large *pol* fragment (1kb) in dried blood spots stored with desiccant at -200C suggests that -20°C may be an appropriate temperature for long term storage of DBS

Thank you