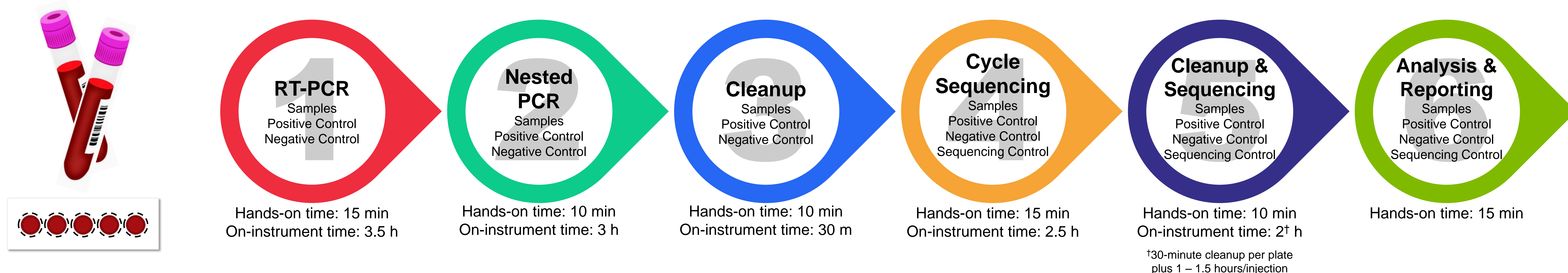


Cost-effective Sanger sequencing assay for detecting HIV-1 drug resistance in major group-M subtypes in resource-limited settings

Karen Clyde, Shiao-Min Chen, Amy Wong, Lauren Tracy, Stephen Williams
Thermo Fisher Scientific, 180 Oyster Point Boulevard, South San Francisco, California 94080, USA

WORKFLOW & TURNAROUND TIME



BACKGROUND

With the introduction of antiretroviral therapies and the UNAIDS 90-90-90 targets comes the need for detecting drug resistance. However, for an HIV-1 drug resistance assay to be successfully implemented worldwide, it must be both robust and cost effective. To this end, in collaboration with the U.S. Centers for Disease Control and Prevention (CDC), we have introduced a low-cost Sanger sequencing-based HIV drug resistance assay for reverse transcriptase (RT) and protease (PR) inhibitors that is specifically designed for resource-limited settings and a broad range of group-M subtypes.



MATERIALS & METHODS

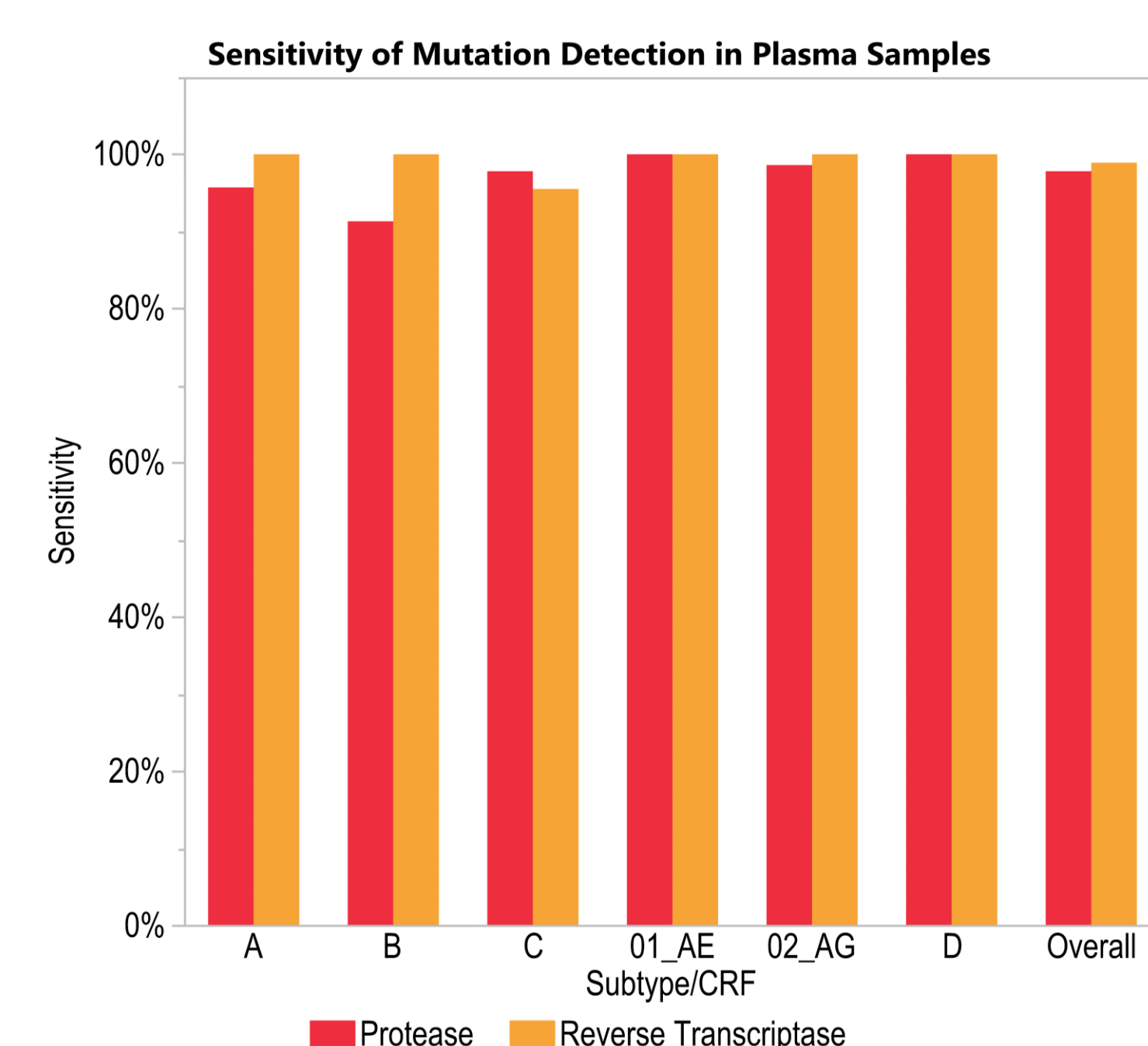
HIV-1 isolates comprising group-M subtypes A, B, C and D and CRF01_AE and CRF02_AG from plasma, dried blood spots (DBS) or culture were extracted with commercially available extraction kits and tested on the ProFlex, Veriti and GeneAmp 9700 thermal cyclers and 3730-series, 3500-series and 3130-series sequencers using the FastSeq50 sequencing protocol. Data were analyzed using the RECall sequence analysis tool (University of British Columbia) and Stanford University HIVDR Database.

RESULTS

The HIV-1 Genotyping Kit achieved a Limit of Detection of 1,000 copies/mL for plasma and 2,000 copies/mL for DBS across all subtypes and CRFs tested. The sensitivity of drug resistance mutation (DRM) detection was 98.1% for plasma and 98.4% for DBS, and specificity was 99.6% for both sample types at a viral load of 10,000 copies/mL. Turnaround time was approximately 14 hours from extracted RNA to results, with less than 3 hours of hands-on time. The assay performed well on all thermal cyclers and sequencers tested.

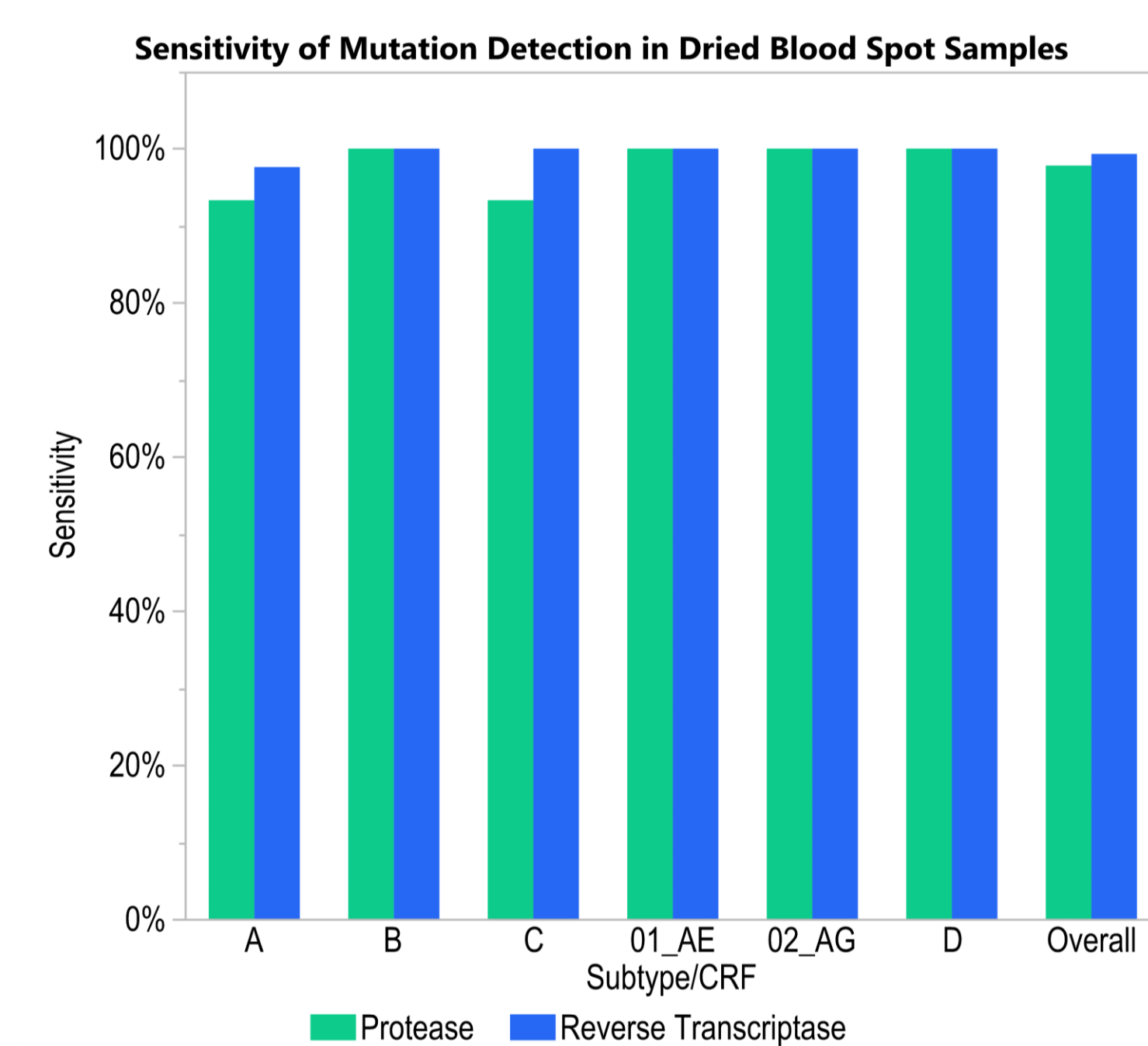
SENSITIVITY BY SUBTYPE AND SAMPLE TYPE

Figure 1. Sensitivity of Protease and Reverse Transcriptase Mutation Detection in Plasma Samples



Plasma samples with known drug resistance mutations from six different subtypes or CRFs were sequenced using the HIV-1 Genotyping Kit, and Sensitivity was calculated as $100\% \times TP/(TP+FN)$.

Figure 2. Sensitivity of Protease and Reverse Transcriptase Mutation Detection in Dried Blood Spot Samples



Dried Blood Spot samples with known drug resistance mutations from six different subtypes or CRFs were sequenced using the HIV-1 Genotyping Kit, and Sensitivity was calculated as $100\% \times TP/(TP+FN)$.

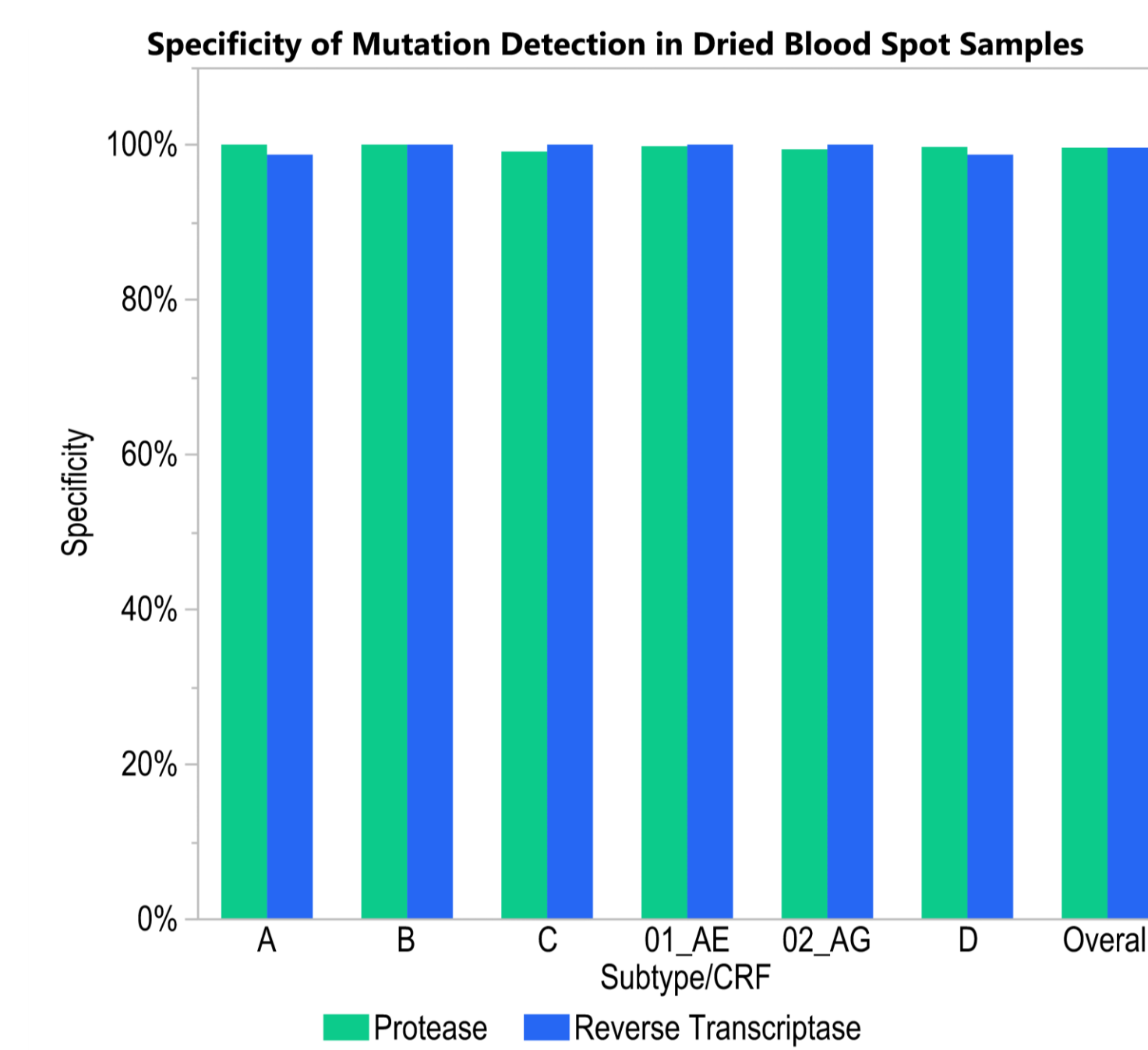
SPECIFICITY BY SUBTYPE AND SAMPLE TYPE

Figure 3. Specificity of Protease and Reverse Transcriptase Mutation Detection in Plasma Samples



Plasma samples with known drug resistance mutations from six different subtypes or CRFs were sequenced using the HIV-1 Genotyping Kit, and Specificity was calculated as $100\% \times TN/(TN+FP)$.

Figure 4. Specificity of Protease and Reverse Transcriptase Mutation Detection in Dried Blood Spot Samples



Dried Blood Spot samples with known drug resistance mutations from six different subtypes or CRFs were sequenced using the HIV-1 Genotyping Kit, and Specificity was calculated as $100\% \times TN/(TN+FP)$.

DATA ANALYSIS

Figure 5. Data Analysis Using RECall in Exatype™



Data are assembled and QC'ed in RECall or RECall implemented in Exatype™ (Hydrax Biosciences, Cape Town, South Africa).

CONCLUSIONS

The HIV-1 Genotyping Kit, which includes positive, negative and sequencing controls and pre-formulated master mixes for amplification and cycle sequencing, is a sensitive, specific and robust method of drug resistance genotyping designed for a broad range of settings and HIV-1 group-M genotypes. Moreover, the assay performs well on multiple thermal cyclers and on sequencers from 4 to 96 capillaries, making it highly flexible, scalable and practical. The low cost, range of subtypes detected and functionality on both new and legacy laboratory equipment make the HIV-1 Genotyping Kit ideal for use in resource-limited settings. Future efforts will focus on enabling DRM detection in Integrase, PR and RT in a single assay.

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