Supplement

Sites

Most sites had more than one enrollment location, and the specific address for study visits may have changed over time to accommodate local circumstances.

"Los Angeles" included enrollment sites in Los Angeles, Pasadena, and Culver City, California.
"San Francisco" included enrollment sites in San Francisco, Oakland, and Palo Alto, California.
"Miami" included enrollment sites in Miami and Fort Lauderdale, Florida.

Sample preparation

Whole blood samples were shipped to Vitalant Research Institute (VRI) for processing into whole blood and plasma aliquots; repository aliquots were stored. Samples were distributed to the two testing labs used for this study.

Laboratory Testing

HIV-1 NAT

HIV-1 NAT used in this study is the transcription mediated amplification assay. The detection probabilities (international units/mL) for HIV-1 NAT are a 50% limit of detection of 4.7 IU/mL (95% fiducial limits 4.0 – 5.3), and a 95% limit of detection of 21.2 IU/mL (95% fiducial limits of 18.2 – 25.7). The limit of detection for this assay is 12 copies/mL. This equates to a window period of approximately 10-13 days since infection acquisition.[35,36]

HIV Serology

Antibody testing is conducted on the Bio-Rad GS HIV-1/HIV-2 PLUS enzyme immunoassay (EIA). This test uses recombinant proteins and synthetic peptides for the detection of antibodies to HIV-1 (Groups M and O) and/or HIV-2 in human serum or plasma. The GS HIV-1/HIV-2 PLUS O EIA testing was conducted on the automated ORTHO® Summit System (OSS).

HIV Limiting Antigen

Limiting Antigen (LAg)-Avidity enzyme immunoassay testing (Sedia Biosciences®, Portland, OR) was conducted at VRI. Among those who tested HIV NAT and serology reactive, the LAg Avidity assay is used to define the infection as recently acquired or long-standing.[28] The estimate of time of infection is based on antibody maturation kinetics and the assay has been shown to classify recent infection as an infection acquired four to six months before testing.[37,38]

Tenofovir

Testing was conducted at VRI. Tenofovir is one of two antiretroviral drugs in oral PrEP medications. Tenofovir is a nucleotide analog reverse transcriptase inhibitor with two active forms, tenofovir alafenamide and tenofovir disoproxil fumarate. Both are detected by the whole blood enzyme linked immunosorbent assay (ELISA). We used the research use only (OraSure®, Bethlehem, PA) plate-based ELISA with whole blood as the specimen input type. In brief, if the specimen has no detectable tenofovir

it will show maximum intensity whereas if tenofovir is present it will show a reduced signal inversely related to the time since last ingestion of oral PrEP. The assay has a 50% reduction in signal at a lower limit of detection of 5 ng/ml (unpublished data per OraSure®). As part of the ADVANCE study protocol, results above 55% are classified as tenofovir detected (PrEP reactive) and below 45% as tenofovir not detected (PrEP non-reactive). Those specimens with between 45 – 55% inhibition were retested and if they remained in the range of 45 – 55% inhibition were considered tenofovir inconclusive.

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Supplement Table 1. Blood Test Results for Enrolled Study Participants Who Tested HIV-positive.

Participant	HIV Nucleic Acid Test S/CO*	HIV-1/2 Antibody Test S/CO*	LAg Avidity Result	Tenofovir Testing†		
				1st	2nd	Final Interpretation
1	21.6	>10.0	Long Term	20.2%	–	Not Detected
2	13.05	>10.0	Long Term	50.6%	66.9%	Detected
3	15.36	>10.0	Long Term	32.9%	16.2%	Not Detected
4	18.41	>10.0	Long Term	50.1%	41.9%	Not Detected

*****Signal to cutoff (S/CO)

†Tenofovir is used in both HIV treatment and prevention formulations.