Use of DNA profiling to resolve discrepant HIV tests in the setting of injectable cabotegravir PrEP

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BACKGROUND

In clinical trials, discordant test results can complicate participant management and study endpoint determination.

In some cases, review of clinic and laboratory records and/or repeat testing may be sufficient to resolve these issues. However, in some cases, it may be difficult to identify the reason for unexpected laboratory results without further investigation.

HIV diagnosis can be challenging when long-acting cabotegravir (CAB-LA) is used for HIV pre-exposure prophylaxis (PrEP) since HIV antigen, antibody, and RNA levels are often low [1]. HIV assays can also revert from reactive/positive to non-reactive/negative in this setting.

We evaluated a case where discrepant HIV test results were obtained for a participant receiving CAB-LA in the HIV Prevention Trials Network (HPTN) 083 trial.

METHODS

Study Cohort

HPTN 083 was a randomized clinical trial that compared the efficacy of CAB-LA to daily oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC). The trial enrolled men and transgender women who have sex with men at 43 study sites [2, 3]. The trial was unblinded after demonstrating that CAB-LA PrEP was superior to TDF/FTC PrEP [2]. After completing the unblinded phase of the study, participants had the option to enroll in an open-label extension (OLE) phase.

HIV testing at the study site

The following assays were used at the study site for real-time testing: OraQuick Advance Rapid HIV-1/2 Antibody Test; Architect HIV Ag/Ab Combo assay; Geenius HIV 1/2 Supplemental Assay; RealTime HIV-1 Viral Load Assay. Whole blood was used to prepare plasma for storage.

Retrospective HIV testing

Testing was performed retrospectively at the HPTN Laboratory Center (LC) using the Architect HIV Ag/Ab Combo assay; Geenius HIV 1/2 Supplemental Assay; and the Aptima HIV-1 Qualitative test or Aptima HIV-1 Quant Dx assay.

Pharmacology testing

Plasma cabotegravir (CAB) was quantified using liquid chromatography-tandem mass spectrometry.

Blood Bank testing

Serologic ABO and Lewis testing was performed at the Johns Hopkins Hospital Transfusion Medicine Laboratory.

DNA profiling

Short tandem repeat (STR) DNA profiling was performed at the DNA Reference Laboratory. The Promega PowerPlex Fusion System was used for autosomal DNA STR testing and the Promega PowerPlex Y23 System was used for Y chromosome STR testing.

RESULTS

The participant received 19 CAB-LA injections according to the primary study protocol and then transitioned to oral TDF/FTC PrEP for ~16 months before enrolling in the OLE. In the OLE, the participant received one CAB-LA injection at the first OLE visit and then restarted TDF/FTC PrEP after an injection site reaction.

HIV test results

HIV test results from three study visits are shown in Table 1. All HIV test results, including an HIV RNA test, were negative/non-reactive at the first OLE visit (Sample 1); 26 days later, the HIV rapid test was non-reactive, the Ag/Ab test was reactive, the discriminatory test was positive, and the viral load was 2,588 copies/mL (Sample 2). All results at the next visit (Sample 3) and subsequent visits were non-reactive/negative. Retrospective test results from the HPTN LC were consistent with the results from the study site.

Pharmacology testing

CAB concentrations were evaluated at the visit where the reactive/positive HIV test results were obtained and the subsequent visit where HIV test results were non-reactive/negative. The CAB concentration was 2,882 ng/mL 26 days after CAB-LA injection (Sample 2) and was 2,643 ng/mL eight days later (Sample 3; Table 1). Both CAB concentrations are within expected ranges observed approximately one month after a single CAB-LA injection.

Table 1. Study events and laboratory results

Sample	Days in OLE	Site Testing (real-time)				HPTN LC Testing (retrospective)			
		Rapid Ab	Ag/Ab	RNA (c/mL)	HIV 1/2 discrim	Ag/Ab (s/co)	RNA (c/mL)	HIV 1/2 discrim	[CAB] ng/mL
1[a]	0	NR	NR	ND		NR (0.11)	ND		
2[b]	26	NR	**R**	**2,588**	**HIV-1 POS**	**R (460.4)**	**2,900**	**HIV-1 POS**	2,882
3[b,c]	34	NR	NR	ND		NR (0.09)	ND		2,643

Footnotes:
[a] The participant received a CAB-LA injection at this study visit.
[b] The participant was dispensed oral TDF/FTC at this study visit.
[c] Samples collected at study visits 38 days, 138 days, and 247 days after this visit had non-reactive (NR)/not detected (ND) HIV test results at the study site.

Blood Bank testing

We next analyzed the ABO blood group and Lewis phenotype for the three samples of interest (Table 2). All three samples contained anti-A and anti-B antibodies, indicating that they were all from a person with group O blood. The anti-A titer was identical for all three samples (128). The anti-B titer was 128 for Samples 1 and 2 and was 64 for Sample 3. Results of Lewis testing were identical for the three samples. All three samples had negative agglutination reactions after inhibition with anti-Le[b] antisera and positive agglutination (3+) after inhibition with anti-Le[a] antisera.

The combined results for three serologic tests (ABO grouping, ABO titer, Le phenotyping) suggest that the three samples were likely obtained from the same person; however, these results did not rule out the possibility of a sample mix-up.

Table 2. Results from Blood Bank testing

Sample	Blood Bank Testing		
	ABO group	Antibody titers	Lewis inhibition
1	O	anti-A 128 anti-B 128	Le(a-b+)
2	O	anti-A 128 anti-B 128	Le(a-b+)
3	O	anti-A 128 anti-B 64	Le(a-b+)

RESULTS

DNA profiling

As a final step, we performed autosomal and Y chromosome STR DNA profiling for Samples 1-3. Table 3 shows the results from autosomal STR testing at 24 loci for the three samples from the study participant. The autosomal DNA profiles for Samples 1 & 3 are consistent with results from one individual. Sample 2 had additional alleles detected at 5/24 loci tested (shown in red font). This is consistent with the presence of contaminating DNA from a second individual.

Table 3. Autosomal DNA profiles

Sample	Amelogenin	D3S1358	D1S1656	D2S441	D10S1248	D13S317	PENTA E	D16S539	D18S51	D2S1338	CSF1PO	PENTA D
1	X, Y	17, 18	15, 16.3	10,14	14, 15	12, 13	15	11, 12	14, 21	20, 24	11, 12	9, 10
2	X, Y	15, 17, 18	14, 15, 16.3	10,14	14, 15	12, 13	15, 21	11, 12, 13	14	20, 24	11, 12	9, 10
3	X, Y	17, 18	15, 16.3	10,14	14, 15	12, 13	15	11, 12	14, 21	20, 24	11, 12	9, 10
Sample	**TH01**	**vWA**	**D21S11**	**D7S820**	**D5S818**	**TPOX**	**DYS391**	**D8S1179**	**D12S391**	**D19S433**	**FGA**	**D22S1045**
1	9, 9.3	16,20	30,32.2	12,13	11, 12	8, 9	9	13	16, 20	12, 14	20, 24	15
2	9, 9.3	16,20	30	12,13	11	8, 9	9	13	16, 20	12, 14	20, 24	15, 16
3	9, 9.3	16,20	30,32.2	12,13	11, 12	8, 9	9	13	16, 20	12, 14	20, 24	-

Table 4 shows the results from Y chromosome STR testing at 22 loci for the three samples from the study participant. All three samples had the same Y-STR DNA profile. Since Sample 2 had the same single source male Y-STR DNA profile, the contaminating second contributor is likely a female.

Table 4 . Y-STR DNA profiles

Sample	DYS576	DYS3891-I	DYS448	DYS389-II	DYS19	DYS391	DYS481	DYS549	DYS533	DYS438	DYS437
1	19	13	19	29	13	9	24	14	13	12	14
2	19	13	19	29	13	9	24	14	13	12	14
3	19	13	19	29	13	9	24	14	13	12	14
Sample	**DYS570**	**DYS635**	**DYS390**	**DYS439**	**DYS392**	**DYS643**	**DYS393**	**DYS458**	**DYS385a/b**	**DYS456**	**Y-GATA-H4**
1	19	22	24	12	14	9	14	16	15, 21	15	10
2	19	22	24	12	-	9	14	16	15, 21	15	10
3	19	22	24	12	14	9	14	16	15, 21	15	10

Participant management

The study site was informed that the sample with the reactive/positive HIV test results was likely contaminated. Oral TDF/FTC PrEP was restarted ~3 months later. No further laboratory evidence of HIV infection was observed at subsequent study visits.

CONCLUSIONS

- In this case, DNA profiling revealed that plasma from a study visit with positive HIV test results was contaminated with DNA from an unrelated individual.
- DNA profiling helped guide clinical management of the study participant and excluded the case as a primary study endpoint.

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