Guidance for the management of "discordant/discrepant" HIV testing results – HPTN 083 and 084

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Summary of key updates

This document has been update to reflect the following milestones/advances:

- Completion of screening and enrollment in both trials
- Unblinding of both trials
- Characterization of HIV infections in the blinded phases of both trials
- On-going transition to the open-label extension (OLE) phase of the studies that includes HIV RNA testing as a screening test for HIV infection; note that the OLE phase is included in HPTN 083 protocol v4 and HPTN 084 protocol v3.

Key changes to the guidelines document include:

- Revision of the text to reflect current information about infections that occur in the setting of CAB-LA PrEP
- Removal of information about HIV testing at the screening and enrollment visits
- Removal of the recommendation to perform testing with the Architect HIV-1 Ag/Ab Combo assay (as a specific brand of Ag/Ab assay), for sites that do not perform this assay as part of the screening algorithm)
- Removal of reference to the S/CO result for the Architect HIV-1 Ag/Ab Combo assay
- Revision of the list/type of clinical scenarios
- Use of different clinical management plans for participants who did vs. did not ever have a CAB-LA injection (regardless of randomized study arm or current PrEP regimen)
- Removal of a 4-week hold step for CAB cases
- Revision to the section on acute HIV infection so it only applies to sites that have not yet implemented the OLE phase of the trial (since the OLE phase includes HIV RNA testing at every study visit)
- The term “SOC ART” has been removed and replaced with “ART”.

Background

When providing PrEP, serial monitoring for HIV infection is important to ensure patient/participant safety, to avoid administration of PrEP agents after infection, and to reduce emergence of drug-resistant HIV. When using long-acting PrEP agents, prompt ART initiation likely will reduce the risk for emergence and/or accumulation of additional drug-resistance mutations.

Clinical trial data suggest that on-going TDF/FTC PrEP use after occult/undiagnosed HIV infection may delay or reduce the antibody response to HIV infection, making it difficult to detect and/or confirm HIV infection.[1-4] In one report, viral replication was suppressed by a median of 1.5 $\log_{10}$ copie/mL[5]. In in some cases, HIV RNA is undetectable in this setting[6], further complicating HIV diagnosis. Case reports have documented HIV seroconversion despite adherence to TDF/FTC PrEP; some of these infections occurred after exposure to highly-resistant virus[5] or after high-inoculum exposure.[6,7] In at least one case, nascent infection was only unmasked after discontinuation of TDF/FTC PrEP. However, discontinuation of PrEP in high-risk populations is not without risk, since HIV infection may occur in the absence of ongoing potent HIV prevention agents.

HPTN 083 and 084 demonstrated that daily oral TDF/FTC and CAB-LA are both highly effective for HIV prevention. While CAB-LA was superior to TDF/FTC for preventing infection, characterization of HIV infections in the setting of recent cabotegravir exposure demonstrated

significant and prolonged suppression of viral replication with substantial delays in anti-HIV antibody production that led to substantial delays in site detection of infection in many cases. In many cases, discordant/discrepant HIV test results were obtained when the sites first detected the possibility of HIV infection. These effects often persisted for several months or longer, even after discontinuing CAB-LA injections, reflecting the long-half life and potency of this drug. In some cases, HIV RNA fell below the level of detection even using a single copy RNA assay, and a positive discriminatory test was not obtained during >6 months of follow-up.

A nuanced and pragmatic approach for HIV diagnosis is needed in these on-going trials to minimize risk to participants who have evidence of possible HIV infection (e.g., an isolated reactive HIV screening test result). Standardized approaches to HIV testing and counseling are needed for consistent clinical management and messaging of results, and to maintain the integrity of the studies. Laboratory procedures must be used that maximize the ability to discriminate between false positive test results and true (atypical) infections. Distinguishing between these scenarios is not always straightforward. The problem with false positive test results has been extensively discussed.[8-14] Individuals who are considered to have false positive test results should be allowed to continue study products (oral cabotegravir, CAB-LA or TDF/FTC), depending on operational feasibility. Individuals who are confirmed to be infected or are likely to be infected based on available laboratory results should be offered fully-suppressive antiretroviral treatment (ART) selected based on HIV genotyping and/or prior PrEP agent exposure.

Considerations

All HIV tests/assays have some false positive test results. The positive predictive value of a reactive result is dependent on the pre-test probability of true infection. In most settings, this would be most impacted by the HIV prevalence/incidence in the population. However, in the presence of a highly effective PrEP agents, the positive predictive value of some HIV tests may be significantly lower than anticipated.

In settings where TDF/FTC PrEP is used and HIV test results may be atypical early in infection, temporary discontinuation of TDF/FTC should lead to an increase in viral replication with increased antibody levels, allowing detection/confirmation of HIV infection. In one case where diagnosis was complicated by viral suppression by TDF/FTC PrEP, HIV RNA was detected 3 weeks after TDF/FTC withdrawal.[6] Since the half-lives of TDF and FTC are short, short-term product holds can be used to “unmask” possible HIV infections with a limited risk for increasing drug-resistance.

HIV diagnosis is more challenging when long-acting agents, such as CAB-LA, are used for PrEP. When infections occur in the setting of CAB-LA, atypical test results, including very low or undetectable HIV viral load, may persist for long periods, even after injections are discontinued. Data from HPTN 077 shows that CAB concentrations remain quantifiable in 42% of HIV-uninfected women and 13% of uninfected men 76 weeks after the last CAB-LA injection. In the absence of suppressive ART, viral “escape” or increases in HIV viral load in breakthrough infections is often associated with INSTI resistance. For this reason, long-term product holds to “unmask” possible HIV infection are not advisable in persons using CAB-LA PrEP. In this setting, the risks of discontinuing CAB-LA to avoid unnecessary drug exposure and minimize the risk of drug resistance must be balanced against the increased risk of incident infection off PrEP in populations that are at high risk for HIV acquisition; these issues must be weighed against initiation of life-long ART without a definitive diagnosis. The complex decisional balance

requires shared decision making and a nuanced understanding of behaviors, virology, and pharmacology.

Objective

There are currently no guidelines for managing unconfirmed HIV infection in clinical or research settings where PrEP is used for HIV prevention. The purpose of this document is to provide updated guidelines for HIV diagnosis in HPTN 083 and 084 for participants who have discordant/discrepant HIV tests, based on available data and expert opinion. This document focuses on HIV diagnosis in participants receiving TDF/FTC and cabotegravir PrEP (the two drugs used for PrEP in HPTN 083 and 084).

Possible etiologies of discordant/discrepant HIV test results

Possible causes of discordant/discrepant HIV test results in the setting of PrEP include:

1. False positive test results due to non-biologic causes (e.g., sample contamination, technical error, data errors)
2. False positive test results due to biologic causes (e.g., cross-reactivity of antibodies to other pathogens)
3. HIV infection acquired prior to PrEP initiation, suppressed/immunologically altered by PrEP agents
4. HIV infection acquired while on PrEP, suppressed/immunologically altered by PrEP agents, including:
    a. Generalized infection
    b. Compartmentalized infection (e.g., HIV infection localized to GALT)
5. Antibody responses to repeated HIV exposure (immunological priming), without true infection
6. Aborted (cured) HIV infection with an immunological footprint without viremia or a latent HIV proviral pool.

Protocol-Specified HIV Testing Algorithms

In HPTN 083 and 084, HIV testing at study sites includes use of HIV rapid tests, instrumented HIV antigen/antibody (Ag/Ab) combination tests, HIV discriminatory tests (e.g., the Geenius HIV ½ Confirmatory Assay), and HIV RNA tests. An ultrasensitive HIV DNA test, performed in real-time at the HPTN LC, is also used to help determine HIV status in selected cases (see below).The locally-available assays and the algorithms used for HIV diagnosis vary among study sites, based on local HIV testing guidelines. For example, some African sites confirm HIV infection using two HIV rapid tests, while other sites use a discriminatory test to confirm HIV infection.

In the pre-OLE phases of the trials, study sites screened for HIV infection using one or two HIV rapid tests and an instrumented Ag/Ab test, using blood obtained by phlebotomy. In the OLE phase of the trials, the HIV testing algorithm was modified by the addition of an HIV RNA test with a limit of quantification of 50 copies/mL or less to screen for HIV infection at every study visit; this testing is in addition to the testing described in the HIV testing algorithm.

HIV DNA testing

HIV DNA testing is used for HIV diagnosis in infants[15] and offers another option for resolving HIV status in adults in some settings. Proviral HIV DNA can be detected and quantified using

cell pellets. The ability of HIV DNA tests to confirm or exclude HIV infection in the setting of PrEP has not been evaluated. Detection of proviral DNA in the setting of PrEP requires use of highly-sensitive assays, since the viral reservoir in these settings may be much smaller than the viral reservoir in newly-infected infants or in adults with no PrEP use/exposure. In HPTN 083 and 084, HIV DNA testing is performed in real-time at the HPTN LC to help resolve HIV status in selected cases using a highly sensitive assay developed for the HIV cure agenda. Data from the blinded phase of HPTN 083 and 084 demonstrates that DNA levels are very low in participants who were infected in the setting of CAB-LA PrEP. In these cases, the level of DNA was often below the limit of detection established for the highly-sensitive HIV DNA assay. The HPTN LC is evaluating results from HIV DNA testing in participants in the CAB arm with and without confirmed infection to evaluate the utility of this testing in this setting.

Overarching Diagnostic Principles

This document includes recommended clinical management plans for cases in which HIV testing yields discordant/discrepant results. The following principles were used for drafting this document:

(1) Any reactive/positive HIV test will trigger an immediate return visit for confirmatory testing and sample collection (the confirmatory visit must be conducted on a different day than the visit where the first reactive/positive test result was obtained). In this document, the first sample with a reactive or positive HIV test is referred to as the index sample; the sample from the subsequent visit is referred to as the confirmatory sample.
(2) In all cases where a reactive/positive HIV test result is obtained, all the HIV tests done at the index visit are also collected/ performed at the confirmatory visit (with the possible addition of HIV DNA testing in some cases)
(3) In some cases, additional HIV testing will be performed using a sample collected at a third visit (after a 4-week product hold) to help determine HIV status. A 4-week product hold will only be considered in participants who had no prior CAB-LA injections.
(4) The following test results at either the index or confirmatory visit are considered to be sufficient evidence of HIV infection to permanently discontinue study products and recommend immediate initiation of ART:
    - a positive discriminatory antibody test
    - a positive HIV RNA test above the limit of quantification/detection
    - a positive HIV DNA test above the assay's limit of detection

Other combinations of test results may also indicate infection (see below).

Clinical management plans

This document provides clinical management plans for the following scenarios where tests used to screen for HIV infection at the index visit are discrepant/discordant:

Scenario	One or both HIV rapid tests	Instrumented Ag/Ab test	HIV RNA test*
1	Reactive	Non-reactive	Not detected
2	Non-reactive	Reactive	Not detected
3	Reactive	Reactive	Not detected
4	Reactive or non-reactive	Reactive or non-reactive	Detected/positive

*Performed as a screening test in the OLE phase of the trials.

Different management plans are presented for two groups of participants. These groups are defined regardless of original study randomization arm or current PrEP regimen.

- No prior CAB-LA exposure (participants who received oral cabotegravir with no CAB-LA injections are included in this group)
- Participants with one or more prior CAB-LA injection (regardless of the time since the last injection)

NOTE: These guidelines are for the OLE phase of the studies, where HIV RNA testing is done at every study visit. For sites that have not yet transitioned to the OLE phase, HIV RNA testing should be performed at the at the index and confirmatory visits in all cases when a reactive Ag/Ab test is obtained.

(I) Clinical Management Plans for participants with no prior CAB-LA exposure

Notes: DNA testing is not indicated; includes a 4-week hold in some cases; a discriminatory test is not performed at all sites; testing criteria for ART initiation varies by site. "Ag/Ab test" refers to a laboratory instrumented antigen/antibody assay.

Scenario	Index visit test results*	Initial guidance	Confirmatory visit test results	Likely status	Follow-up guidance
1	Rapid test(s) reactive Ag/Ab test non-reactive RNA test not detected	Hold study product	All tests negative/NR except for rapid test(s)	HIV uninfected False reactive rapid test	Resume study product
			Ag/Ab test reactive; RNA and discriminatory tests negative	Infection likely (2 screening tests reactive, not confirmed)	Continue product hold; retest after a 4-week product hold; refer for ART based on local guidelines
			RNA and/or discriminatory test positive	Infection confirmed	Permanently discontinue study product, refer for ART
2	Rapid test(s) non-reactive, Ag/Ab test reactive RNA test not detected	Hold study product	All tests negative/NR except for Ag/Ab test	HIV uninfected False reactive Ag/Ab test	Continue product hold; retest after a 4-week product hold; refer for ART based on local guidelines
			Rapid test reactive; RNA and discriminatory tests negative	Infection likely (2 screening tests reactive)	Continue product hold; retest after a 4-week product hold; refer for ART based on local guidelines
			RNA and/or discriminatory test positive	Infection confirmed	Permanently discontinue study product, refer for ART
3	Rapid test(s) and Ag/Ab test reactive RNA test not detected	Hold study product	Rapid test(s) and Ag/Ab test reactive RNA and discriminatory tests not detected/negative	Infection likely (2 screening tests repeatedly reactive)	Continue product hold; retest after a 4-week product hold; refer for ART based on local guidelines
4	RNA test positive (regardless of other test results)	Hold study product	Repeat RNA test positive ** (regardless of other test results)	HIV infection confirmed	Permanently discontinue study product Refer for ART
			All tests non-reactive/negative	Consider false pos RNA result or other mixup	Continue product hold; retest after a 4-week product hold to see if infection can be confirmed
			Repeat RNA test not detected, any other test reactive/positive	Possible or confirmed infection	Continue product hold; retest after a 4-week product hold to see if infection can be confirmed

*HIV RNA testing is only included as an HIV screening assay at the index visit in the OLE phase of the studies. See the note above about the addition of HIV RNA testing at the confirmatory visit for participants with discordant/discrepant results.

**The following results are considered to be positive HIV RNA results: (1) viral load above the limit of quantification (for viral load assays); (2) detected above the limit of detection (for qualitative RNA assays). For results detected below the limit of quantification (for viral load assays), testing should be reviewed to be sure that the result was positive/detected; if this is the case, the assay should be repeated. In these cases, study product should be permanently discontinued and the participant should be referred for ART may be appropriate if the RNA result is "detected below the limit of quantification" on two separate dates.

(II) Clinical Management Plans for participants with at least one prior CAB-LA injection

Notes: Includes DNA testing; does not include a 4-week product hold

Scenario	Index visit test results	Initial guidance	Confirmatory visit test results	Likely status	Follow-up guidance
1	Rapid test(s) reactive Ag/Ab test non-reactive RNA test not detected	Hold study product Perform DNA testing	All tests negative/NR except for rapid test(s)	HIV uninfected False reactive rapid test	Resume study product
			Ag/Ab test reactive; RNA and discriminatory tests negative	Infection likely (2 screening tests reactive, not confirmed)	Permanently discontinue study product; refer for ART based on local guidelines
			RNA and/or discriminatory test positive	Infection confirmed	Permanently discontinue study product, refer for ART
2	Rapid test(s) non-reactive, Ag/Ab test reactive RNA test not detected	Hold study product Send DNA testing	All tests negative/NR except for Ag/Ab test	HIV uninfected Possible false reactive Ag/Ab test	Retest at subsequent visits; include testing with a second/different Ag/Ab test if available
			Rapid test reactive; Ag/Ab test still reactive; RNA, DNA and discriminatory tests negative	Infection likely (2 screening tests reactive)	Permanently discontinue study product; refer for ART based on local guidelines
			RNA, DNA and/or discriminatory test positive	Infection confirmed	Permanently discontinue study product; Refer for ART
3	Rapid test(s) and Ag/Ab test reactive RNA test not detected	Permanently discontinue study product Send DNA testing	Rapid test(s) and Ag/Ab test reactive RNA, DNA and discriminatory tests negative	Infection likely (2 screening tests repeatedly reactive)	Permanently discontinue study product; refer for ART based on local guidelines
4	RNA test detected (regardless of other test results)	Discontinue study product Send DNA testing	Repeat RNA test detected (regardless of other test results)	HIV infection confirmed	Permanently discontinue study product; Refer for ART
			All tests non-reactive/negative	Infection likely (vs. sample mixup)	Discontinue study product; repeat the testing algorithm; refer for ART*
			Repeat RNA test negative, any other test reactive/positive	Possible or confirmed infection	Permanently discontinue study product Refer for ART

*Available clinical information (e.g., low HIV risk) may be used to inform a decision about whether to stop ART and restart CAB-LA as PrEP. This decision should be made in discussion with the 083/4 HIV alias committee along with the site IoR and should be clearly documented in the participant's record.

ADDITIONAL GUIDANCE

Considerations for participants with signs/symptoms of acute HIV infection

Perform HIV testing if the participant has signs or symptoms of acute HIV infection. The clinical presentations of acute HIV infection may include fever, myalgia, pharyngitis, lymphadenopathy, rash, diarrhea, and headache in various combinations. HIV testing in this scenario should include a rapid test, instrumented Ag/Ab test, and HIV RNA test. If the site has not yet implemented protocol v4, include an RNA screening test in addition to protocol-required rapid testing and instrumented Ag/Ab testing. Note that RNA levels may be very low or undetectable for several months in persons who received at least one CAB-LA injection.

Persistently Positive/Reactive HIV Screening Tests

In scenarios above that allow resumption of study products, management of participants who have repeatedly reactive rapid tests or repeated reactive Ag/Ab tests is challenging. For participants who received at least one CAB-LA injection, the protocol-specified HIV testing algorithm used for follow-up visits will be modified to include cell pellet storage at all subsequent visits until HIV infection is confirmed, or until HIV infection is ruled out in consultation with the 083HIV or 084HIV@hptn.org alias teams. Stored cell pellets may be sent for HIV DNA testing at the HPTN LC at the direction of the HIV alias group. If the participant has new or different patterns of reactive/positive test results at subsequent study visits, clinical management should follow the algorithms presented above.

Long-term Clinical Management

For cases where study drug is permanently discontinued and participants are referred for ART, the decision whether participants should be continued on life-long ART or undergo a carefully-monitored treatment interruption at some future time point is a complex and nuanced clinical decision beyond the scope of clinical trial management. The HPTN 083 or 084 leadership teams will be happy to help local investigators with these decisions.

Discussion

Clinical management decisions in PrEP trials are complicated for participants who have discordant/discrepant HIV test results. Management in this setting requires clinical judgment, knowledge of the performance characteristics of each HIV test, knowledge of the potential impact of each PrEP agent on viral replication and antibody production, and an understanding of the current body of knowledge regarding HIV testing patterns in breakthrough infections that occur in clinical trials of long-acting PrEP agents. Decisions to observe participants off study PrEP agents are particularly nuanced, since it may be very difficult to discriminate between true HIV infection from false reactive/false positive test results if HIV RNA and HIV DNA are not detected or if HIV DNA is detected at a very low level, particularly if persons have been exposed to long-acting PrEP. Permanent discontinuation of PrEP in these cases could place study participants at increased risk for HIV acquisition. An argument that continuing CAB-LA in these cases would put participants at risk (due to unnecessary drug exposure and increased risk of drug resistance) is being further evaluated in the ongoing HPTN 083 and 084 trials; these risks are a potential consequence of ongoing CAB monotherapy exposure in the setting of occult/undetected HIV infection.

The possibility that participants could have compartmentalized infection or low-reservoir infection that could have potential for cure with ongoing administration of long-acting PrEP agents was considered in drafting this document; this requires further research.

The opposite extreme was also considered: permanent discontinuation of study products for all participants who have reactive HIV rapid test or instrumented Ag/Ab test only. However, this strategy would put uninfected study participants (those with false positive test results) at risk for HIV acquisition and/or commit them to lifelong ART in the absence of confirmed HIV infection. These approaches might also cause untold emotional distress, stigma, and other potential harms by implying that a participant is infected in the absence of confirmation of infection. These considerations must be carefully balanced against the profound ability of CAB and CAB-LA to suppress viral replication and delay/diminish antibody production below the level of detection available diagnostic tests.

The proposed management plan (subject to change as new information is obtained) provides the best pathway for evaluating discordant/discrepant HIV test results, minimizing risk to participants, and addressing critically-important questions about the efficacy and safety of CAB-LA as a PrEP agent, in anticipation of regulatory approval of CAB-LA for PrEP.

REFERENCES

Marzinke M., et al., Characterization of HIV infection in cisgender men and transgender women who have sex with men receiving injectable cabotegravir for HIV prevention: HPTN 083. J Infect Dis. 202; In Press.

Delany-Moretlwe S, et al. Long acting injectable cabotegravir is safe and effective in preventing HIV infection in cisgender women: results from HPTN 084. R4P meeting, January 2021. Abstract #HY01.02.

Eshleman, et al. Characterization of HIV infections in women who received injectable cabotegravir or tenofovir disoproxil fumarate/emtricitabine for HIV prevention: HPTN 084. Submitted.

Landovitz R, et al., Tail-phase safety, tolerability, and pharmacokinetics of long-acting injectable cabotegravir in HIV-uninfected adults: a secondary analysis of the HPTN 077 trial. Lancet HIV. 2020 Jul; 7(7):e472-e481.

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