Determining HIV status of African adults with discordant HIV rapid tests

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Background

In resource-limited settings, the WHO recommends using two HIV rapid tests for HIV diagnosis. If the test results are discordant, a third tie-breaker test is often used to determine HIV status. In this study, we evaluated the performance of different tie-breaker tests and characterized samples with discordant rapid tests. Samples were obtained from a population-based survey (NIMH Project Accept [HPTN 043]).

Methods

Study Cohort

HPTN 043 was a large, community-randomized clinical trial that assessed the impact of integrated behavioral interventions on HIV incidence [1]. HIV incidence was assessed at the end of the trial in a single cross-sectional survey [2].

We analyzed samples with discordant HIV rapid test results collected at three HPTN 043 sites (Kisarawe, Tanzania; Soweto and Vulindlela, South Africa, Table 1) [3].

Table 1. HIV rapid test discordant samples used for analysis

	HIV prevalence	HIV incidence	Participants tested	Discordant results[a]	Samples evaluated
Tanzania	5.9%	0.78%	9,041	187 (2.1%)	127 (67.9%)
South Africa	21.8%	2.3%	25,772	68 (0.3%)	46 (67.6%)
Soweto	14.1%	1.18%	13,929	22 (0.2%)	16 (72.7%)
Vulindlela	30.8%	3.90%	11,843	46 (0.4%)	30 (65.2%)
Total	17.7%	1.85%	34,813	255 (0.7%)	173 (67.8%)

[a] Samples were tested in-country using two HIV rapid tests. The number and percentage of samples that had discordant HIV rapid test results (one reactive and one non-reactive result) are shown.

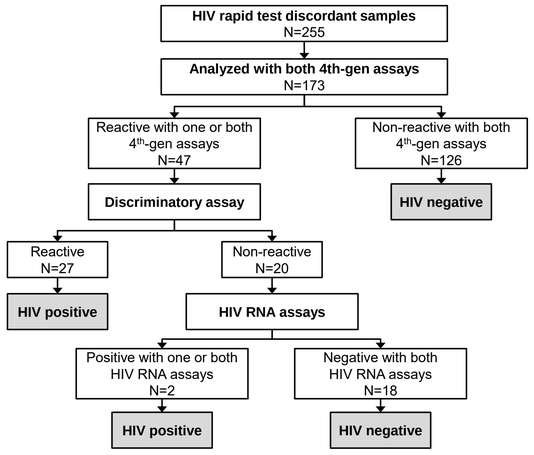
Laboratory Methods

Plasma samples (N=173) with discordant rapid test results were classified as HIV positive or negative using a testing algorithm adapted from the current US CDC testing algorithm for HIV diagnosis (Figure 1). The algorithm was modified to include two 4th-generation assays and two HIV RNA assays to increase sensitivity for detecting HIV infection.

Sensitivity, specificity, and accuracy were determined for four tie-breaker assays: a 3rd-generation HIV rapid test, a 3rd-generation enzyme immunoassay (EIA), and two 4th-generation assays (Figure 2). The rapid test was performed in-country (Tanzania only). The remaining tie-breaker assays and additional testing were performed at the HPTN Laboratory Center at Johns Hopkins University, Baltimore, MD.

Results

Figure 1. Testing algorithm used to classify samples with discordant HIV rapid tests as HIV positive or HIV negative*



* 4th-gen assays: GS HIV Combo Ag/Ab EIA , ARCHITECT HIV Ag/Ab Combo assay; Discriminatory assay: Multispot HIV-1/HIV-2 Rapid Test; HIV RNA assays: the APTIMA HIV-1 RNA Qualitative Assay and the Roche COBAS AMPLICOR HIV-1 MONITOR test, v1.5.

Classification of HIV discordant samples

Twenty-nine (16.8%) of the 173 rapid test discordant samples were classified as HIV positive using the testing algorithm shown in Figure 1. This classification was used as the gold standard for the evaluation of simpler, tie-breaker tests.

Comparison of the performance of tie-breaker assays

We next evaluated four assays as tie-breakers for resolving the HIV status of individuals with discordant HIV rapid test results (Figure 2). The HIV rapid test and 3rd-generation EIA had very low sensitivities (<25%) with specificities of 96.1% and 98.6%. The 4th-generation assays were more sensitive (93.1% and 96.6%), but had lower specificity (88.2% and 90.3%). The accuracy of the four tests as tie-breakers for rapid test discordant samples ranged from 79.5% to 91.3% (Figure 2).

Results

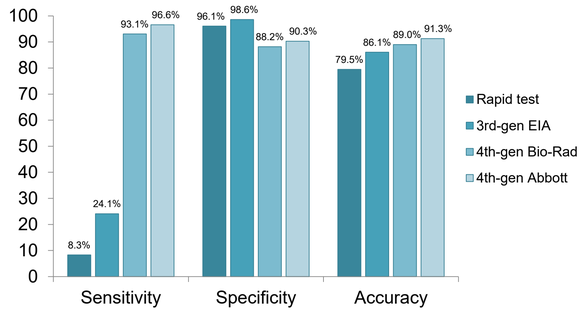
Further evaluation of HIV rapid test discordant samples

Additional testing was performed to characterize the 29 samples classified as HIV positive in this study. The laboratory tests included two FDA-approved HIV rapid tests, a Western blot, and a qualitative assay for antiretroviral (ARV) drug detection (Table 2). The sensitivity for the two HIV rapid tests were 25.0% and 42.3%. Sensitivity was lower when a Western blot was used to confirm HIV infection (range: 4.2% to 12.5%).

Only four samples had viral loads >400 c/mL; one of those samples had lamivudine (3TC) and nevirapine (NVP) detected (Case 4). ARV drugs were not detected in the other samples. One sample was identified as HIV-2 (Case 10). These results indicate that the majority of the HIV-infected samples with discordant HIV rapid test results were from individuals who were virally suppressed in the absence of ARV drugs (Table 2).

Among the 144 samples classified as HIV negative, 24 (16.7%) had at least one reactive serologic assay. Four had a reactive rapid test, two had a reactive 3rd-generation EIA, and 18 had a reactive 4th-generation assay. Additional testing was performed for this subset of HIV-negative samples; the samples were non-reactive using the discriminatory assay, had undetectable HIV RNA, and had no ARV drugs detected.

Figure 2. Evaluation of tie-breaker assays for samples with discordant HIV rapid test results*



* Rapid test: Uni-Gold HIV Test; 3rd-gen EIA: VITROS Anti-HIV 1+2 assay; 4th-gen Bio-Rad: GS HIV Combo Ag/Ab EIA; 4th-gen Abbott: ARCHITECT HIV Ag/Ab Combo assay.

Results

Table 2. Evaluation of samples classified as HIV positive

#	Rapid 1	Rapid 2	Rapid 3	3rd-gen EIA	WB	4th-gen Bio-Rad	4th-gen Abbott	Discrim assay	RNA qual[b]	Viral load	ARV drugs
1	R	R	R	R	Pos	R	R	R	n/a	9,052	No
2	R	NR	NR	NR	Ind	R	R	R	NR	<400	No
3[a]	n/a	NR	R	R	Pos	R	R	R	n/a	<400	No
4[a]	n/a	n/a	n/a	R	Pos	R	R	R	n/a	1,280	3TC/NVP
5	NR	NR	R	R	Pos	R	R	NR	n/a	2,651	No
6[a]	n/a	R	NR	R	Pos	R	R	R	NR	<400	No
7	NR	NR	NR	R	Neg	R	R	NR	n/a	459,246	No
8[a]	n/a	n/a	NR	R	Neg	R	NR	R	NR	<400	No
9	NR	NR	R	NR	Ind	NR	R	R	NR	<400	No
10[a]	n/a	NR	NR	NR	Neg	NR	R	R	NR	<400	No
11	NR	n/a	NR	NR	Ind	R	R	R	R	<400	No
12	NR	n/a	NR	NR	Ind	R	R	R	NR	<400	No
13	NR	NR	R	NR	Ind	R	R	R	NR	<400	No
14	NR	n/a	NR	NR	Ind	R	R	R	NR	<400	No
15	NR	n/a	R	NR	Ind	R	R	R	NR	<400	No
16	NR	n/a	n/a	NR	Ind	R	R	R	NR	<400	QNS
17	NR	n/a	NR	NR	Ind	R	R	R	NR	n/a	n/a
18	NR	n/a	NR	NR	Ind	R	R	R	NR	<400	No
19	NR	n/a	n/a	NR	Ind	R	R	R	NR	<400	No
20	NR	R	R	NR	Ind	R	R	R	NR	<400	n/a
21	NR	n/a	R	NR	Ind	R	R	R	NR	n/a	n/a
22	NR	R	NR	NR	Ind	R	R	R	NR	<400	No
23	NR	n/a	NR	NR	Ind	R	R	R	NR	<400	No
24	NR	NR	NR	NR	Ind	R	R	R	NR	<400	No
25	NR	NR	R	NR	Ind	R	R	R	NR	<400	No
26	NR	n/a	NR	NR	Ind	R	R	R	NR	<400	No
27	NR	NR	R	NR	Ind	R	R	R	NR	<400	No
28	NR	NR	R	NR	Ind	R	R	R	NR	<400	No
29	NR	NR	NR	NR	Neg	R	R	R	NR	<400	No

[a] Cases 3, 4, 6, 8, and 10 are from South Africa; the remaining cases are from Tanzania.
[b] Samples were not tested with the Aptima assay if the viral load was >400 c/mL.
Rapid test 1: Uni-Gold HIV test (performed in Tanzania only); Rapid test 2: Uni-Gold Recombigen HIV-1/2 Test; Rapid test 3: OraQuick ADVANCE Rapid HIV-1/2 Antibody Test; R: reactive; NR: non-reactive; n/a: sample not available or depleted in previous testing.

Conclusions

Rigorous testing approaches beyond a single tie-breaker assay should be considered when resolving HIV status in individuals with discordant rapid test results.

- Significant differences were observed in the performance of tie-breaker tests for determining HIV status in individuals with discordant HIV rapid test results.
- Twenty-five (86.2%) of the 29 HIV-infected adults with discordant HIV rapid tests had undetectable HIV RNA. Only one had ARV drugs detected. Therefore, most of these individuals are likely to have been elite controllers.
- False-positive test results were observed. The high frequency of other infectious diseases in Africa could contribute to false-positive tests due to immune stimulation and/or formation of antibodies that cross-react with target antigens in HIV assays.

ACKNOWLEDGMENTS: The authors thank the HPTN 043 study team and participants for providing samples and data for this study, and thank the laboratory staff at the study sites and at the HPTN Laboratory Center with assistance for sample management and testing.

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