Does antiretroviral therapy use affect the accuracy of HIV rapid diagnostic assays? Experience from a demographic health and surveillance site in rural South Africa☆☆☆

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A B S T R A C T

Rapid diagnostic tests (RDTs) are the mainstay of HIV diagnosis in the developing world but might have poor sensitivity among individuals taking antiretroviral therapy (ART). We leveraged a home-based HIV testing program linked to clinical data to compare the sensitivity of RDTs between individuals using versus not using ART. Field workers tested 6802 individuals using 2 HIV RDTs, which were compared to a single HIV immunoassay tested on dried blood spots. Approximately 5% (371/6802) tested positive by immunoassay, of whom 157 (42%) were currently on ART. The sensitivity of the Abon RDT among those never versus currently on ART was 91.6% (95% CI 88.3–94.3) and 96.6% (95% CI 88.3–94.3), respectively, and 95.4% (95% CI 92.8–97.3) versus 99.3% (95% CI 95.2–99.7) for the Advanced Quality assay. We report similar sensitivity of RDTs in ART-naïve and ART-experienced individuals, which mitigates concerns about their use among treated individuals in population-based epidemiologic surveys and those transferring care.

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1. Background

The World Health Organization supports use of rapid diagnostic tests (RDTs) for diagnosis of HIV in settings where laboratory-based confirmatory assays are not available (Consolidated guidelines on HIV testing services, 2015). RDTs allow rapid, low-cost, point-of-care diagnostic evaluation for HIV without need for complex laboratory infrastructure or extensive human resource expertise. These characteristics make them a cornerstone of HIV diagnostic in much of the developing world. Recently updated WHO guidelines in 2019 now suggest use of 3 sequential positive tests that rely on excellent sensitivity and moderate specificity of RDTs (World Health Organization, 1997; 2019).

RDTs have been primarily developed and evaluated to make new diagnoses of HIV. More recently, such assays have been applied at a population scale within generalized epidemics, for the purpose of HIV surveillance (Kim et al., 2016) among individuals transferring care who sometimes do not disclose their ART status (Grabowski et al., 2018; Manne-Goehler et al., 2019; Sykes et al., 2019), to determine inclusion criteria for research studies (Coleman et al., 2018) and in the context of community-based test-and-treat ART initiatives (Hayes et al., 2019). These scenarios will increasingly include individuals on ART. However, the high sensitivity of RDTs for the detection of HIV among people on ART has been challenged. Although a relatively rare phenomenon in practice, early initiation of ART during acute HIV infection prevents development of an antibody response to HIV (de Souza et al., 2016). Some studies have also suggested antiretroviral therapy use might decrease the sensitivity of these assays (O’Connell et al., 2003; Merchant et al., 2014; Fogel et al., 2017). The biological mechanism for declining sensitivity of RDTs is that the titers of anti-HIV envelope and other antigens decline after years of ART use ( Merchant et al., 2014; Fogel et al., 2017). If true, high rates of false-negative RDT results would have important implications both for...
population-based epidemiologic studies and for testing patients currently or previously in care, which is commonly done at the time of clinic transfer. Repeat testing of individuals who have surreptitiously transferred care or are seeking to enroll in studies as ART naïve is widely reported in both clinical and programmatic settings (Fogel et al., 2013; Sullivan et al., 2013; Coleman et al., 2018).

We sought to answer 2 questions: 1) What proportion of individuals actively taking ART test positive by RDT and HIV 1/2 antigen/antibody enzyme immunoassay? 2) Is the sensitivity of RDTs, compared to HIV 1/2 antigen/antibody enzyme immunoassays, decreased among individuals taking ART versus those ART naïve? To do so, we leveraged a demographic health and surveillance (DHS) program that routinely performs home-based HIV testing using RDTs with paired HIV 1/2 antigen/antibody immunoassays on dried blood spot (DBS) specimens. We hypothesized that, in a programmatic setting in rural South Africa, home-based RDTs would perform equally well among those currently taking or naïve to ART.

2. Methods

2.1. Study design

The African Health Research Institute (AHRI) (formerly the Africa Centre for Health and Population Studies) is a Wellcome Trust–funded research institute in South Africa. In 2000, AHRI established a DHS in rural uMkhanyakude District, northern KwaZulu-Natal, which now covers an area of 845 km² with a population of approximately 150,000 (Tanser et al., 2008). Annual household-based surveys are used to collect information on births, deaths, and migration patterns for all household members, including nonresidents. Resident members who are aged ≥15 years are also invited to participate in an individual survey, which includes an interview on general health and sexual behavior, and collection of a DBS for anonymized HIV testing. In addition, HIV counseling and testing (HCT) using RDTs is offered to all residents aged ≥15 years. Although we encourage testing in individuals who do not know their status, we do not exclude testing among individuals who have a recent negative test or have had prior positive test result. Participants who newly test HIV positive are referred for care at 1 of the 11 government primary health care clinics in the surveillance area.

AHRI has a memorandum of agreement with the KwaZulu-Natal Provincial and District Department of Health to receive data from an HIV care electronic patient record system that is used in the government clinics (TIER.net). The TIER.net database contains information on clinic visit attendance, laboratory results, and ART dispensing records for all patients on ART. Clinical records of patients in the TIER system are linked with their household and individual-level data gathered through the AHRI demographic surveillance system. Individuals are linked using their unique South African identification number or by first name, surname, age, and sex using algorithms developed by AHRI. On a monthly basis, TIER.net data are transferred to AHRI from the central Department of Health. Individuals who had any record of receiving ART in TIER were considered ever on ART. Those who had initiated ART at least 1 year before the RDT and who had attended a clinic for ART care in the last 6 months were considered to be currently on ART. We categorized the cohort into those never, currently, or previously on ART and summarized demographic and clinical indicators. For this analysis, we calculated the sensitivity and specificity of both RDTs compared to the EIA immunoassay performed on DBS samples as a reference standard for the total cohort, and stratified by ART use categories. We compared the sensitivity of each assay in individuals currently on ART to those individuals never on ART using a Fisher’s exact test.

In a secondary analysis, true positive (reference) was defined by either a positive test on our EIA or a record of ART use in the TIER electronic medical record, whereas a true negative was defined as negative immunoassay and no record of ART care. Sensitivity and specificity of each RDT, and of the parallel strategy, were calculated among all individuals and stratified by ART status, as described above.

2.3. Statistical analysis

Ethical approval for the demographic surveillance study, linkage to the government ART records (TIER.Net), and analyses of these data was granted by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal, South Africa. Separate informed consent was obtained for the main household survey, the individual-level questionnaires, HCT, and provision of the DBS.

3. Results

The study population comprised 6802 individuals who accepted HCT and provided a DBS specimen between 1 June and 20 December 2017; median age was 35 years (Table 1). The majority (4778, 70%) were female, and most (6613, 97%) had never been on ART, as determined by the TIER.Net electronic record. Among the 189 who had ever been on ART, 157 (83%) were currently on ART for a median duration of 4.9 years. There was no evidence of a difference in age between individuals on ART and those who had never been on ART (P = 0.19, by Wilcoxon rank sum test), but a higher proportion of those on ART were female (90% versus 70%, respectively, P = 0.001 by χ² test).

All 6802 individuals completed EIA testing, of whom 6252 (91.9%) tested negative and the remaining 550 (8.1%) tested positive (Supplementary Table 1). Of these, 6796 (99.9%), 6800 (99.9%), and 6794 (99.9%) also completed testing by Abon, Advanced Quality, and both tests in parallel, respectively. The sensitivity of the Abon assay among individuals currently on ART was 99.1% (95% CI = 98.3–99.9%) and 96.6% (95% CI = 92.3–98.3%) and 91.6% (95% CI = 88.3–94.3%) among those never on ART (P = 0.05) (Table 2 and Fig. 1). Similarly, sensitivity of the Advanced Quality assay was higher among those on ART versus those never on ART (99.3%; 95% CI = 96.3–100.0% and 95.4% 95% CI = 92.8–97.3%, respectively) (P = 0.03). When both immunoassay and ART records were used as the reference standard, the sensitivity of the individual assays

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and parallel RDT strategy decreased slightly (Table 3), but there was no evidence of a difference in the sensitivity of any of the assays in individuals on ART compared with those never on ART.

4. Discussion

Home-based HIV RDT assays conducted by field workers in rural South Africa had a sensitivity among people taking ART for a median of 5 years that is equal to or better to that when used among ART-naive individuals. We found that approximately 95% of HIV enzyme immunoassay-positive individuals actively taking ART in the public sector in South Africa tested positive by RDT compared to approximately 92% in those without a history of ART use. While we identified an overall imperfect sensitivity of the RDT assays, which requires additional attention, our results do not suggest that long-term ART has a major impact on the sensitivity of RDTs in this setting.

The first study to suggest a lower sensitivity of RDTs for detection of HIV infection among those on ART found a sensitivity of only 89% among...
Importantly, neither of the 2 latter studies included a control. A substudy of the HPTN 051 clinical trial assessed the validity of third-generation RDTs with multiple antigens among 207 specimens (5%) were either nonreactive (median 234), including those who had false-negative RDT results (median 95.1%, respectively. In our study which observed individuals (n=27) who underwent repeated testing over calendar time, and found a negative-testing rate ranging from 2% to 20%, depending on the assay (Merchant et al., 2014). This study also reported decreasing titers by enzyme immunoassay with increased duration of ART use, which was up to 15 years in duration, in many participants. Finally, a sub-study of the HPTN 051 clinical trial assessed the validity of third-generation RDTs with multiple antigens among 207 adults with prospectively collected specimens (Fogel et al., 2017). Ten of 207 specimens (5%) were either nonreactive (n=1) or weakly positive by RDT (n=9) at follow-up testing, for a sensitivity of 99.5 and 91.5%, respectively, depending on whether or not weakly positive bands were considered positive. Four of the 10 individuals also had indeterminate or negative Western blot results, suggesting a more extensive effect of ART on production of anti-HIV antibodies. Counterintuitively, the frequency of weak or nonreactive bands was lower among those in the early ART arm (350–550 cells/μL) versus the delayed ART arm (CD4 <250 cells/μL) with 7/180 (3.9%) and 3/27 (11.1%, P=0.13), respectively. In our study which observed individuals in routine care, the most individuals started ART with a low CD4 count (median 234), including those who had false-negative RDT results (median 109). Importantly, neither of the 2 latter studies included a control group of individuals who were also tested without exposure to ART, whether the decrease in accuracy was due to ART or variations in test performance with repeat testing cannot be determined.

Our data, along with prior studies that directly compared RDT sensitivity by ART use, are less suggestive of a large decrease in sensitivity due to ART use (Table 4). For example, a prior study from the US comparing 6 RDTs to an enzyme immunoassay/Western blot sequential reference standard in 386 individuals found ~98% sensitivity in all assays among those on ART (Delaney et al., 2011). Although none of the assays showed statistically significant differences in test sensitivity by ART status, second-generation tests had larger nominal reductions in sensitivity for those on ART than third-generation tests, for which sensitivity was 100% for all assays. Note, that study did demonstrate poorer sensitivity among people on ART for RDTs using oral fluid as an analyte (97.7% versus 100%), which is known to be less sensitive than blood specimens (Pant Pai et al., 2012). Although the assays were routinely 100% sensitive among those not on ART (n=105), there was no statistically significant difference in assay sensitivity by use or nonuse of ART. In our study, we found that 9 (5%) of 157 individuals currently in HIV care and on ART based on clinic records tested negative on all 3 assays studied (immunoassay and 2 RDTs). We also found a moderately increased sensitivity of RDTs compared to an immunoassay in people currently on ART versus those never exposed to ART.

The overall sensitivity of our RDTs reported (93–96%) was lower than that reported in many community-based studies (Molesworth et al., 2010; Jackson et al., 2013) and below WHO recommendations (Consolidated guidelines on HIV testing services, 2015). Nonetheless, our results are similar to other field-based studies comparing RDTs to immunoassays in the region (Wolpaw et al., 2010; Kufa et al., 2017). In 1 field-based study, low test sensitivity was drastically improved after implementation of augmented quality control procedures and a repeat testing algorithm (Bock et al., 2017). Testing was done in our study by lay health workers.

### Table 2

Sensitivity and specificity of rapid diagnostic tests.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Reference = ELISA</th>
<th>Reference = ELISA + TIER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test + / True +</td>
<td>Sensitivity</td>
</tr>
<tr>
<td><strong>All participants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abon RDT</td>
<td>513 / 550</td>
<td>93.3 (90.8–95.2)</td>
</tr>
<tr>
<td>Advanced Quality RDT</td>
<td>530 / 550</td>
<td>96.4 (94.4–97.8)</td>
</tr>
<tr>
<td>Parallel RDT</td>
<td>509 / 550</td>
<td>92.5 (90.0–94.6)</td>
</tr>
</tbody>
</table>

a ELISA testing of DBS sample used as reference (true status).
b Reference is based on ELISA results and presence of record in TIER. Individuals with positive ELISA or record in TIER are classified as HIV positive; individuals with negative ELISA and no record in TIER are classified as HIV negative.
c Test positive defined as being positive on both rapid tests (Abon and Advanced Quality); test negative defined as being negative on at least one of the rapid tests.

### Table 3

Sensitivity of rapid diagnostic tests by ART status.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Reference = ELISA</th>
<th>Reference = ELISA + TIER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test + / True +</td>
<td>Sensitivity</td>
</tr>
<tr>
<td><strong>Never on ART</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abon RDT</td>
<td>340 / 371</td>
<td>91.6 (88.3–94.1)</td>
</tr>
<tr>
<td>Advanced Quality RDT</td>
<td>354 / 371</td>
<td>95.4 (92.8–97.3)</td>
</tr>
<tr>
<td>Parallel RDT</td>
<td>337 / 371</td>
<td>90.8 (87.4–93.6)</td>
</tr>
<tr>
<td><strong>Any current or prior ART</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abon RDT</td>
<td>173 / 179</td>
<td>96.6 (92.8–98.8)</td>
</tr>
<tr>
<td>Advanced Quality RDT</td>
<td>176 / 179</td>
<td>98.3 (95.2–99.7)</td>
</tr>
<tr>
<td>Parallel RDT</td>
<td>172 / 179</td>
<td>96.1 (92.1–98.4)</td>
</tr>
</tbody>
</table>

- ELISA testing of DBS sample used as reference (true status).
- Reference is based on ELISA results and presence of record in TIER. Individuals with positive ELISA or record in TIER are classified as HIV positive; individuals with negative ELISA and no record in TIER are classified as HIV negative.
- Test positive defined as being positive on both rapid tests (Abon and Advanced Quality); test negative defined as being negative on at least 1 of the rapid tests.
- Test positive defined as being positive on both rapid tests (Abon and Advanced Quality); test negative defined as being negative on at least 1 of the rapid tests.
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at patient homes. Decreased test accuracy has also been reported previously in field settings owing to difficult interpretation of weak test bands, high rates of indeterminate results (i.e., absent control band), and lower test accuracy among lay workers and nurses compared to laboratory staff (Gray et al., 2007; Klarkowski et al., 2009; Kagulire et al., 2011; Mwangala et al., 2016). Divergent test results in field sites compared to clinical and laboratory settings deserve further attention and likely have implications for the emergence of home and self-based testing programs (Sabapathy et al., 2012; Pant Pai et al., 2013).

Our study has a number of limitations. We conducted HIV immunoassay testing on dried blood spots, which might decrease the sensitivity of our reference standard assay. If the sensitivity of the immunoassay was lower than would have been expected with samples from serum or plasma specimens, this might have affected our estimate of RDT specificity. Moreover, we conducted confirmatory testing with only a single immunoassay in contrast to WHO guidelines, which suggest use of 2 confirmatory assays (World Health Organization, 2019). The fourth-generation EIA we used detects both antibody and antigen, as well as immunoassay in contrast to WHO guidelines, which suggest use of 2 confirmatory assays. If the sensitivity of the immunoassay in contrast to WHO guidelines, which suggest use of 2 confirmatory assays. The fourth-generation EIA we used detects both antibody and antigen, as well as weak bands considered positive was 99.5% (95% CI 97.3–100%).

Table 4
Summary of studies that assessed the sensitivity of HIV rapid diagnostic tests (RDTs) among individuals on antiretroviral therapy (ART).

<table>
<thead>
<tr>
<th>Author, year (REF)</th>
<th>Assay(s) tested</th>
<th>Analyte tested</th>
<th>Participants on ART</th>
<th>Sensitivity of RDTs among those on ART (95% CI)</th>
<th>Participants not on ART</th>
<th>Sensitivity of RDTs among those not on ART (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Connell et al., 2003</td>
<td>OraQuick</td>
<td>Serum</td>
<td>91</td>
<td>95.6% (89.1–98.8%)</td>
<td>10</td>
<td>100% (69.1–100%)</td>
</tr>
<tr>
<td>O’Connell et al., 2006</td>
<td>Multisopt HIV-1/HIV-2</td>
<td>Serum</td>
<td>248</td>
<td>100% (98.5–100%)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Piwowar-Manning et al., 2014</td>
<td>OraQuick</td>
<td>Not specified</td>
<td>101</td>
<td>98.0% (93.0–99.8%)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Delaney et al., 2011</td>
<td>Clearview Complete</td>
<td>Whole blood</td>
<td>384</td>
<td>98.7% (97.0–99.6%)</td>
<td>103</td>
<td>100% (97.1–100%)</td>
</tr>
<tr>
<td>Merchant et al., 2014</td>
<td>OraQuick Advance</td>
<td>Whole blood</td>
<td>386</td>
<td>99.2% (97.8–99.8%)</td>
<td>106</td>
<td>100% (97.1–100%)</td>
</tr>
<tr>
<td>Fogel et al., 2017</td>
<td>OraQuick Advance</td>
<td>Not specified</td>
<td>98</td>
<td>90.8% (83.2–95.7%)</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>Siedner, 2020 (current)</td>
<td>Abon Advanced Quality</td>
<td>Whole blood</td>
<td>157</td>
<td>96.6% (92.8–98.8%)</td>
<td>214</td>
<td>91.6% (88.3–94.3%)</td>
</tr>
</tbody>
</table>

References


Please cite this article as: M.J. Siedner, K. Baisley, O. Koole, et al., Does antiretroviral therapy use affect the accuracy of HIV rapid diagnostic assays? Experience from a demographic HIV surveillance study setting in rural South Africa. Our results support continued use of RDTs for population-based studies of HIV epidemiology, as well as for individuals who are transferring care or reinitiating therapy. Further attention should be paid to the test performance of RDTs in field-based settings to ensure adequate performance of the most common modality of HIV testing in such settings.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.diagmicrobio.2020.115031.

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