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Field evaluation of five rapid diagnostic tests for screening of HIV-1 infections in rural Rakai, Uganda

S C Kagulire, BSc[*], **P Opendi, MD**[*], **P D Stamper, BSc, MSc**[†,‡], **J L Nakavuma, BSc**[§], **L A Mills, MD, MSc**[*,†], **F Makumbi, PhD**[*,**], **R H Gray, MD**[‡], **J P Shott, BSc**[††,‡‡], **D Serwadda, MD, MPH**[**], and **S J Reynolds, MD, MPH**[‡‡]

[*]Rakai Health Sciences Program, Kalisizo, Uganda [†]School of Medicine [‡]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA [§]Faculty of Veterinary Medicine [**]School of Public Health, Makerere University, Kampala, Uganda [††]Clinical Monitoring Research Program, SAIC-Frederick, Inc, NCI-Frederick, Frederick, MD [‡‡]National Institute of Allergy and Infectious Diseases, NIH, Bethesda, MD, USA

Summary

The performance characteristics of HIV rapid diagnostic tests (RDTs) vary by test and by population. We assessed five commercial RDTs in Uganda where all but one RDT (Determine; Abbott Laboratories, Germany) performed close to manufacturer's expectations. Determine had low specificity (85.2%, positive predictive value 67.3%) due to false-positive results with weak-positive bands. Properly trained staff, good quality control programmes and validation of RDTs with laboratories having confirmatory testing capacity may be warranted to assure accuracy in each setting.

Keywords

HIV; rapid diagnostic testing; Uganda; point of care tests; resource-poor settings

There are several HIV rapid diagnostic tests (RDTs) that facilitate delivery of same-day results to improve voluntary counselling and testing in resource-poor settings. However, there is a need to evaluate RDTs to determine their performance characteristics in various settings. We and others have shown that interpretation of RDTs can be complicated by weak-positive bands with confirmatory testing by enzyme-linked immunosorbent assay (ELISA) and Western blot (WB) necessary before providing a diagnosis.[1–6]

We evaluated five commercially available RDTs for diagnosis of HIV-1 infection in 150 phlebotomized venous blood samples collected into EDTA tubes. All five RDTs are approved for use with venous blood samples (technical inserts). Study participants were enrolled following informed consent into the Rakai Community Cohort Study approved by the Western Institutional Review Board, the Uganda Virus Institute Science and Ethics Committee and the Uganda National Council for Science and Technology. One hundred samples were of unknown HIV status, 25 were known HIV-positives and 25 were known HIV-negatives. The sera were tested with five different RDTs in parallel to screen for HIV-1/2-specific antibodies. The RDTs evaluated were: Determine HIV 1/2 (Determine; Abbott Laboratories, Wiesbaden-Delkeheim, Germany); Uni-Gold HIV 1/2 (Uni-Gold; Trinity Biotech Plc, Bray, Co. Wicklow, Ireland); STAT-PAK HIV 1/2 (STAT-PAK;

Correspondence to: S J Reynolds, US Embassy Kampala, P.O. Box 7007, Kampala, Uganda, sjr@jhmi.edu.

Chembio Diagnostic Systems Inc, Medford, NY, USA); Advanced Quality Rapid Anti-HIV 1/2 (Advanced Quality; InTec Product Inc, Xiamen, China); and First Response 1–2.0 (First Response; PMC Medical, Nani Daman, India). All RDTs evaluated were immunochromatographic lateral flow platforms with a procedural positive control band incorporated in the test for quality control. The testing was performed according to the manufacturers' standard operation procedures for individual assays, in a well-lit laboratory.

The results of the RDTs were read by two qualified laboratory technicians and recorded as positive, negative or invalid; and positive band strength was noted. Weak-positive bands were defined as a sample with a positive band that was lighter than the positive control band on the test card as described elsewhere.[1] A positive result was defined as both the sample and the control line of the test developing; and a negative result was defined as only the control band developing. Invalid results were defined as no band developing and/or only the development of a band in the sample test area and none in the control zone. Two separate gold standard ELISAs (Abbott Murex, Murex Biotech Ltd, Dartford, UK and Vironostika HIV Uniform II Micro ELISA, bioMerieux, Geneva, Switzerland) and one WB assay (Calypte Biomedical Corporation, Portland, OR, USA) were performed by blinded laboratory technicians. WB was interpreted according to WHO criteria.[7] Double ELISA-negative results were considered as final results. ELISA discordant results as well as double ELISA-positive incident results were subjected to WB for confirmation. Participants received their HIV test results from trained programme counsellors based on the gold standard ELISA/WB result.

Data were double entered and statistical analyses were conducted using STATA (Version 8.2, Stata Corporation, College Station, TX, USA). The results of each RDT were compared with ELISA/WB for the calculation of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (Table 1). All tests demonstrated a sensitivity of 100%, no invalid results were encountered, there were no HIV-2-positive results and there were no technician-discordant interpretations of results. Determine had low specificity (85.2%, PPV 67.3% [Exact Clopper-Pearson 95% confidence interval (CI): 52.9, 79.7]). Uni-Gold and First Response had moderate specificity (97.4%, PPV 92.1% [Exact Clopper-Pearson 95% CI: 78.6, 98.3]). STAT-PAK performed well (sensitivity 100%, specificity 99.1%, PPV 97.2% [Exact Clopper-Pearson 95% CI: 85.5, 99.9]), and Advanced Quality had sensitivity, specificity and PPV of 100% (Exact Clopper-Pearson 95% CI: 90.0, 100.0).

Determine had unacceptably low specificity and Uni-Gold had somewhat low specificity, as has been reported previously in our setting,[1] elsewhere in Uganda,[8] in the Democratic Republic of Congo (DRC)[4] and Ethiopia.[6] In the DRC study,[4] weak-positive bands on Determine resulted in a high number (10.5% of total RDTs) of false-positive or indeterminate results as confirmed by WB. This false-positive rate fell to 3.3% when only strong-positive test results were considered. It is unclear why weak-positive bands occur; however, it is possible that a unique phenomenon involving serologic cross-reactivity or non-specific immune reactions are involved, particularly in east African individuals.[4] Another study indicated that batch-to-batch variations can affect the sensitivity and specificity, and ultimately could explain some variability in the performance of RDTs such as Determine and Uni-Gold in our setting.[5]

We previously reported on the potential difficulties of interpreting weak-positive bands, and that if these particular results are eliminated from the analysis, the test performance improves greatly (specificity 94.1% versus 99.6%; PPV 74.0% versus 97.7%; false-positive rate 26.0% versus 2.3%).[1] In this evaluation, weak-positive bands developed on 17 (11.3%) of 150 RDTs, yet all 17 were confirmed negative by gold standard ELISA/WB testing. Twelve (70.5%) of 17 weak-positive bands were seen with the Determine test. When these

tests with weak-positive bands were removed from the statistical analysis, the specificity of Determine increased to 95.1% from 85.2%. This suggests the need to consider band strength when interpreting Determine, and possibly other RDTs, before a result is assigned, and a diagnosis given to patients.

Numerous commercial HIV RDTs are now available; however, few have been evaluated in settings where they are employed. In our study, most of the RDTs in the evaluation performed with comparable sensitivity to current generation ELISAs. As RDTs are expanded in resource-poor settings, properly trained staff and good quality control programmes are imperative to ensure accurate RDT testing and validation of RDTs with laboratories having ELISA/WB capacity may be warranted to assure accuracy in each setting.

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Table 1

Performance characteristics of HIV RDTs

	Sensitivity% (*n* = 150)		Specificity% (*n* = 150)			
RDT	**Manufacturer's SEN***	**SEN†**	**Manufacturer's SPE***	**SPE (95% CI)†**	**NPV% (95% CI)†**	**PPV% (95% CI)†**
Determine	100	100	99.9	85.2 (77.4, 91.1)	100 (96.3, 100)	67.3 (52.9, 79.7)
STAT-PAK	100	100	100	99.1 (95.3, 99.9)	100 (96.8, 100)	97.2 (85.5, 99.9)
Uni-Gold	100	100	99.7	97.4 (92.6, 99.5)	100 (96.8, 100)	92.1 (78.6, 98.3)
First Response	100	100	99.2	97.4 (92.6, 99.5)	100 (96.8, 100)	92.1 (78.6, 98.3)
Advanced Quality	100	100	100	100 (96.8, 100)	100 (96.8, 100)	100 (90.0, 100)

PPV = positive predictive value; NPV = negative predictive value; CI = confidence interval; RDT = rapid diagnostic test; SEN = sensitivity; SPE = specificity

*Manufacturers' test characteristic performance (kit inserts) on serum; PPV and NPV dependent on the prevalence of population tested

†Evaluation findings

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