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Evaluation of current rapid HIV test algorithms in Rakai, Uganda

Ronald M. Galiwango[1], **Richard Musoke**[1], **Lawrence Lubyayi**[1], **Robert Ssekubugu**[1], **Sarah Kalibbala**[1], **Viola Ssekweyama**[1], **Viola Mirembe**[1], **Gertrude Nakigozi**[1], **Steven J. Reynolds**[1,3,5], **David Serwadda**[1,4], **Ronald H. Gray**[2], and **Godfrey Kigozi**[1]

[1]Rakai Health Sciences Program, Kalisizo, Uganda [2]Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA [3]Laboratory of Immunoregulation, Division of Intramural Research, NIAID, National Institutes of Health, Bethesda, MD, USA [4]Makerere College of Health Sciences - School of Public Health, Kampala–Uganda [5]Johns Hopkins University School of Medicine

Abstract

Rapid HIV tests are a crucial component of HIV diagnosis in resource limited settings. In Uganda, the Ministry of Health allows for both serial and parallel HIV rapid testing using Determine, Stat-Pak and Uni-Gold. In serial testing, a non-reactive result on Determine ends testing. The performance of serial and parallel algorithms with Determine and Stat-Pak test kits was assessed. A cross-sectional diagnostic test accuracy evaluation using three rapid HIV test kits as per the recommended parallel test algorithm was followed by EIA-WB testing with estimates of the performance of serial testing algorithm. In 2520 participants tested by parallel rapid algorithms, 0.6% had weakly reactive result. Parallel testing had 99.7% sensitivity and 99.8% specificity. If Stat-Pak was used as the first screening test for a serial algorithm, the sensitivity was 99.6% and specificity 99.7%. However, if Determine was used as the screening test, sensitivity was 97.3% and specificity 99.9%. Serial testing with Stat-Pak as the initial screening test performed as well as parallel testing, but Determine was a less sensitive screen. Serial testing could be cost saving.

1. Introduction

Most HIV infection occurs in low resource settings particularly Sub- Saharan Africa(UNAIDS, 2010). HIV counseling and testing (HCT) is crucial for HIV prevention and access to care (Bunnell et al., 2008), and it is estimated that less than 40% of HIV-infected people in Sub-Saharan Africa are aware of their status (Anand et al., 2009; WHO, 2010). In Uganda, the 2011 AIDS Indicator Survey estimated HIV prevalence in Rakai at 10.6% among persons aged 15–49 years of age. (UAIS –MoH, 2011). Rapid HIV diagnostic tests as compared to standard ELISA/WB testing are simpler to perform, do not require laboratory facilities, are cost-effective, have a longer shelf life, and allow point of care provision of HCT with increased client linkage to care (Cabie et al., 2011; Delaney et al., 2011; Kannangai et al., 2000; Tung et al; 2010). Challenges to rapid test use include test sensitivity and the subjective interpretation of weak reactive results in field settings (Gray et

Corresponding Author: Ronald M. Galiwango, Rakai Health Sciences Program, Uganda. rmgaliwango@rhsp.org Tel: +256701333993.

al., 2007; Kagulire et al., 2011; Wolpaw et al., 2010). The World Health Organization (WHO) recommends that ideally rapid tests should have at least 99% sensitivity and 95% specificity. In Uganda, the Ministry of Health (MOH) recommends both serial and parallel algorithms for rapid HIV testing. In serial testing, Determine is recommended as a screen and testing is discontinued with a non-reactive result. A reactive result is subjected to Stat-Pak and results are issued if concordant. If Determine and Stat-Pak are discordant, Uni-Gold is used as a tie breaker test. In parallel testing, both Determine and Stat-Pak are run concurrently as a screen and concordant results issued, whereas discordant results proceed to Uni-Gold. The performance of the recommended MOH serial and parallel rapid testing algorithms was evaluated against standard EIA/WB.

2. Materials and methods

The Rakai Health Sciences Program in rural south western Uganda has conducted reproductive health research for over 20 years (Wawer et al., 1999). A cross-sectional evaluation of Rapid HIV Diagnostic test accuracy was conducted in adults aged 15–49, who provided written informed consent for HIV testing from November, 2011 to February, 2012. Participants provided whole blood obtained by finger prick for the rapid HIV tests which were done in the field. Venous blood was also collected in clot activator tubes for laboratory based serum enzymeimmuno assay and Western blot (EIA-WB) testing.

The three rapid tests used for field-based testing were Determine™ HIV-1 /2 (Alere Medical Company Limited, Chiba, Japan), HIV 1 /2 Stat-Pak® Dipstick (Chembio Diagnostic Systems, Medford, NY - USA) and Uni-Gold™ HIV (Trinity Biotech, Bray, Ireland). The test procedures followed manufacturer directions. All samples were subjected to parallel testing with Determine and Stat-Pak, and concordant results issued to participants with post-test counseling. Discordant results were tested with Uni-Gold as the tie breaker. Serum testing used two enzyme linked immunosorbent assays (Murex HIV-1, 2.O, Murex Biotech Limited, Dartford, UK, if reactive followed by Vironistika HIV Uni-Form II plus O Mircoelisa System, BioMerieux: Marcy I'Etoile, France) and discordant EIA results were confirmed by Western Blot (GS HIV-1 Western Blot, Bio-Rad Laboratories, Redmond, WA, USA. All testing was done by qualified and experienced laboratory technicians.

The sensitivity, specificity, false positive and false negative rates, negative and positive predictive values of the two rapid algorithms were compared against EIA-WB testing as the gold standard. Although all rapid tests were run in parallel, the performance of serial testing was inferred by assessing the Determine or Stat-Pak as the initial screening test. Proportions with exact binomial 95% confidence intervals and a two-sample test for proportions were used to compare differences between algorithms. Weakly reactive bands on rapid tests which were less intense than the positive control band on each test strip were assessed separately.

The study was approved by the Uganda Virus Research Institute Science and Ethics Committee, the Uganda National Council of Science and Technology and the Western Institutional Review Board (WIRB), Olympia, WA.

3. Results

A total of 2624 participants were tested using rapid tests. Using Determine, 43 (1.6%) were weakly reactive and 855 (32.6%) were reactive, while with Stat Pak, 61 (2.3%) were weakly reactive and 901 (34.3%) were reactive. There were 101 (3.8%) samples with discordant results on Determine and Stat-Pak kits. Of these, 94 were tested further using Uni-Gold kit. 2617 samples had complete rapid test results, of which 2520 were tested using EIA WB. The population was comprised of 1281 (50.8%) males and 1239 (49.2%) females. The age

distribution was 15–19 (10.5%), 20–29 (41.7%), 30–39 (37.4%) and 40+ (10.4%), and 24% were employed in fishing, which is a high risk occupation.

3.1 Sensitivity and Specificity of rapid test parallel algorithm

There were 888 (35.2%) reactive samples using the EIA-WB reference gold standard and 34.8% samples tested were reactive using the parallel rapid test algorithm, with an additional 0.6% (14) weakly reactive samples. Excluding weakly reactive samples, 874 samples were reactive on both the gold standard and parallel rapid test algorithm resulting in a sensitivity of 99.7% (95%CI 99.0–99.9) and a specificity of 99.8% (95% CI 99.2–99.9). The positive predictive value was 99.7%, with a false positive rate of 0.2% and false negative rate of 0.3% (Table 2).

When weak reactive samples were considered as reactive on the parallel rapid test algorithm, the sensitivity remained unchanged but the positive predictive value was reduced to 99.3% (95%CI 98.5–99.8%), the false positive rate increased to 0.4% and the false negative rate remained 0.3%. Specificity also decreased from 99.8% to 99.6%.

The 14 weakly reactive samples were tested further by EIA and 11(78.6%) tested reactive, while 3(21.4%) were non-reactive.

3.2 Sensitivity and Specificity of Determine for serial testing

As shown in Table 1, using Determine as a screening test, 834 (33.1 %) samples were reactive and 36(1.4%) were weakly reactive. Excluding the weakly reactive samples, 832 were reactive on both the EIA-WB reference and rapid test with a sensitivity of 97.3% (95%CI 96.0–98.3). The specificity was 99.9% (95%CI 99.6–99.99%). Positive predictive value was 99.8%, with a false positive rate of 0.1% and false negative rate of 2.7% (Table 2). The negative predictive value of the Determine test as an initial serial screen was 98.6%.

When weak reactive samples s were considered as reactive, the sensitivity was 97.4 (95%CI 96.1–98.4) and the positive predictive value was 99.4%. The false positive rate increased to 0.3% with false negative rate of 2.6%. Specificity also declined from 99.9% to 99.7%. The negative predictive value remained unchanged at 98.6%. Of the 36 weakly reactive Determine test results, 91.7% (33) were reactive on EIA.

3.3 Sensitivity and Specificity of Stat-Pak rapid test for serial testing

Using Stat-Pak, 874 (34.7 %) samples were reactive and 2.4% (61) were weakly reactive. Excluding weakly reactive results, 869 samples were reactive on both the EIA-WB reference and Stat-Pak tests with a sensitivity of 99.6% (95%CI 99.0–99.9) and specificity of 99.7% (95%CI 99.3–99.9%). The positive predictive value was 99.4% with both false positive and false negative rates of 0.3%. The negative predictive value of the Stat-Pak test was 99.8% (Table 2).

When weak reactive samples were considered as reactive, the sensitivity remained unchanged but the positive predictive value was reduced to 94.6%. The false positive rate increased to 3.1% with a false negative rate of 0.3%. Specificity also decreased from 99.7% to 96.9% ($p<0.0001$). The negative predictive value remained unchanged at 99.8%. Of the 61 weakly reactive on Stat-Pak, 26.2% (16) were reactive on EIA and 73.8% (45) were non-reactive.

The higher false negative rate with Determine as a screen (2.7%) was significantly higher than with Stat-Pak as screen (0.3%) and the parallel algorithm ($p<0.0001$).

4. Discussion

If weakly reactive samples are excluded, these results suggest that parallel testing has a sensitivity of 99.7% and specificity of 99.8%. Using Determine as a screen for serial testing yielded a lower sensitivity of 97.3% and a comparable specificity of 99.9%, whereas with Stat-Pak as the screening test for a serial algorithm, the sensitivity and specificity were both 99.7%. This suggests that if a serial testing algorithm is used, Stat-Pak is the preferable screening test. Serial testing is cheaper and simpler than parallel testing and these results suggest that Stat-Pak is provides the best initial screening test for serial testing. Thus, we recommend revision of the current Ugandan MOH recommendation that Determine be used as the initial screening test for a serial algorithm.

All three rapid test kits had weakly reactive results which cannot be interpreted; this suggests that samples with weak positive bands should be subjected to further testing before presenting results to clients. Weak positive bands were slightly more common with Stat-Pak (2.4%) than Determine (1.4%). In summary, serial rapid testing using Stat-Pak as the initial screening test performed as well as a parallel testing algorithm and could be used for routine point-of-care testing and counseling.

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References

Anand A, Shiraishi RW, Bunnell RE, Jacobs K, Solehdin N, Abdul-Quader AS, Marum LH, Muttunga JN, Kamoto K, Aberle-Grasse JM, Diaz T. Knowledge of HIV status, sexual risk behaviors and contraceptive need among people living with HIV in Kenya and Malawi. AIDS. 2009; 23:1565–73. [PubMed: 19542867]

Bunnell R, Opio A, Musinguzi J, Kirungi W, Ekwaru P, Mishra V, Hladik W, Kafuko J, Madraa E, Mermin J. HIV transmission risk behavior among HIV-infected adults in Uganda: results of a nationally representative survey. AIDS. 2008; 22:617–24. [PubMed: 18317003]

Cabie A, Bissuel F, Huc P, Paturel L, Abel S. Impact of rapid HIV testing on the return rate for routine test results in sexually transmitted infection testing centres. Int J STD AIDS. 2011; 22:757–8. [PubMed: 22174063]

Delaney KP, Branson BM, Uniyal A, Phillips S, Candal D, Owen SM, Kerndt PR. Evaluation of the performance characteristics of 6 rapid HIV antibody tests. Clin Infect Dis. 2011; 52:257–63. [PubMed: 21288853]

Global Report on HIV epidemic - UNAIDS. [accessed 7 March 2013] 2010. http://www.unaids.org/globalreport/documents/20101123_GlobalReport_full_en.pdf

Gray RH, Makumbi F, Serwadda D, Lutalo T, Nalugoda F, Opendi P, Kigozi G, Reynolds SJ, Sewankambo NK, Wawer MJ. Limitations of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study. BMJ. 2007; 335:188. [PubMed: 17545184]

Kagulire SC, Opendi P, Stamper PD, Nakavuma JL, Mills LA, Makumbi F, Gray RH, Shott JP, Serwadda D, Reynolds SJ. Field evaluation of five rapid diagnostic tests for screening of HIV-1 infections in rural Rakai, Uganda. Int J STD AIDS. 2011; 22:308–9. [PubMed: 21680664]

Kannangai R, Ramalingam S, Selvaraj PK, Damodharan K, Sridharan G. Hospital-based evaluation of two rapid human immunodeficiency virus antibody screening tests. J Clin Microbiol. 2000; 38:3445–7. [PubMed: 10970399]

Tung CS, Sangi-Haghpeykar H, Levison J. Rapid versus standard testing for prenatal HIV screening in a predominantly Hispanic population. J Perinatol. 2010; 30:30–2. [PubMed: 19741654]

Uganda AIDS Indicator Survey, Ministry of Health (UAIS - MoH). [Accessed 7 March 2013] Prevalence of HIV and Syphilis report. 2011. http://health.go.ug/docs/UAIS_2011_FACT_SHEET.pdf

Wawer MJ, Sewankambo NK, Serwadda D, Quinn TC, Paxton LA, Kiwanuka N, Wabwire-Mangen F, Li C, Lutalo T, Nalugoda F, Gaydos CA, Moulton LH, Meehan MO, Ahmed S, Gray RH. Control of sexually transmitted diseases for AIDS prevention in Uganda: a randomised community trial. Rakai Project Study Group. Lancet. 1999; 353:525–35. [PubMed: 10028980]

WHO. Towards Universal Access: Scaling up priority HIV/AIDS interventions in the health sector. 2010.

Wolpaw BJ, Mathews C, Chopra M, Hardie D, de Azevedo V, Jennings K, Lurie MN. The failure of routine rapid HIV testing: a case study of improving low sensitivity in the field. BMC Health Serv Res. 2010; 10:73. [PubMed: 20307310]

Table 1

Distribution of test results by Rapid Test Kits and the Parallel testing algorithm

Result	Test Kit			
	Determine	Stat-Pak	UniGold	Final Rapid Result (Parallel Testing)
Non reactive	1650 (65.5%)	1585 (62.9)	47 (47.5)	1629 (62.9)
Reactive	834 (33.1)	874 (34.7)	48 (48.5)	877 (34.8)
Weak reactive	36 (1.4)	61 (2.4)	4 (4.0)	14 (0.6)

Table 2

Comparison of Rapid test kits with the EIA-WB testing algorithm

	Sensitivity *(95% CI)*	**Specificity *(95% CI)***	**positive predictive value**	**negative predictive value**	**false positive rate**	**false negative rate**
Parallel Testing						
Parallel testing result (Weak reactive excluded)	99.7 *(99.0–99.9)*	99.8 *(99.5–100)*	99.7	99.8	0.2	0.3
Parallel testing result (Weak reactive included)	99.7 *(99.0–99.9)*	99.6 *(99.2–99.9)*	99.3	99.8	0.4	0.3
Serial testing						
Determine screen (Weak reactive excluded)	97.3 *(96.0–98.3)*	99.9 *(99.6–100)*	99.8	98.6	0.1	2.7
Determine screen (Weak reactive included)	97.4 *(96.1–98.4)*	99.7 *(99.3–99.9)*	99.4	98.6	0.3	2.6
Stat-Pak screen (Weak reactive excluded)	99.7 *(99.0–99.9)*	99.7 *(99.3–99.9)*	99.4	99.8	0.3	0.3
Stat-Pak screen (Weak reactive included)	99.7 *(99.0–99.9)*	96.9 *(96.0–97.7)*	94.6	99.8	3.1	0.3