

Limitations of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study

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ABSTRACT

Objective To evaluate the limitations of rapid tests for HIV-1.

Design Diagnostic test accuracy study.

Setting Rural Rakai, Uganda.

Participants 1517 males aged 15-49 screened for trials of circumcision for HIV prevention.

Main outcome measures Sensitivity, specificity, negative predictive values, and positive predictive values of an algorithm using three rapid tests for HIV, compared with the results of enzyme immunoassay and western blotting as the optimal methods.

Results Rapid test results were evaluated by enzyme immunoassay and western blotting. Sensitivity was 97.7%. Among 639 samples where the strength of positive bands was coded if the sample showed positivity for HIV, the algorithm had low specificity (94.1%) and a low positive predictive value (74.0%). Exclusion of 37 samples (5.8%) with a weak positive band improved the specificity (99.6%) and positive predictive value (97.7%).
Conclusion Weak positive bands on rapid tests for HIV should be confirmed by enzyme immunoassay and western blotting before disclosing the diagnosis. Programmes using rapid tests routinely should use standard serological assays for quality control.

Trial registration Clinical Trials NCT00425984.

INTRODUCTION

Rapid tests for the detection of antibodies to HIV-1 allow point of care provision of results and do not require the laboratory facilities needed for conventional testing.¹ An FDA application is now pending for an over the counter home testing kit,² and the expansion of requirements for HIV testing in the developing world will require use of these tests.³ We have encountered problems with the interpretation of positive results of rapid tests during the screening of populations in rural Uganda for two randomised trials. We evaluated limitations of these tests in such settings.

METHODS

The Rakai health sciences programme used rapid HIV tests to screen uncircumcised males aged 14-49 for two randomised trials of circumcision for the prevention of HIV in a rural population in Rakai, Uganda. One trial enrolled males who were HIV negative and the other

males who were HIV positive. The rapid tests were used to initially screen the males for enrolment into these trials.

During 2003-4 we used an algorithm incorporating three rapid HIV tests for the screening of potential participants for a randomised trial. The algorithm (see bmj.com) consisted of an initial screening using Determine HIV-1/2/O (Abbott Laboratories, Abbott Park, IL). If the test result was negative the participant was given a diagnosis of HIV negative with no further testing. If the result was positive the sample was retested using HIV 1/2 Stat-Pak Ultra Fast (Chembio Diagnostic Systems, Medford, NY). If both test results were positive the participant was given a diagnosis of HIV positive with no further testing. If the results were discordant, the sample was evaluated using Uni-Gold Recombinant HIV-1/2 (Trinity Biotech, Bray, Ireland). For those samples assessed by all three tests, two positive results were interpreted as a positive diagnosis. If two of the three test results were negative then the participant was diagnosed as HIV negative.

Tests were run on serum from blood centrifuged immediately before testing. The reading of each result was timed according to the manufacturers' specification. Each test was read by two laboratory technicians, with the test card flat on the table. The tests were interpreted according to manufacturers' instructions, which recommend that bands in the positive region be considered a positive result for HIV, irrespective of strength. All samples were subsequently batch retested for quality control, using two enzyme immunoassays (Vironostika HIV-1, Organon Teknika, Charlotte, NC and Cambridge Biotech, Worcester, MA). Discordant results were confirmed by western blotting (HIV-1 Western Blot; BioMerieux-Vitek, St Louis, MO).

We tested one sample from each of 1517 participants. An initial assessment of 878 samples suggested problems with false positive results, so after March 2004 the technicians recorded the intensity of positive bands in 639 samples. In this subgroup 125 samples tested positive for HIV, of which 37 were classified as weak positive bands (5.8%)—a sample with an apparent positive band that was lighter than the control positive band on the test card. We estimated the sensitivity, specificity, negative predictive values, and positive

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predictive values of this algorithm, compared with the results of the enzyme immunoassay and western blotting as the ideal methods. Analyses were carried out for the 1517 samples, for the 639 samples for which band intensity had been coded, and for the subgroup of tests with exclusion of positive results coded as weak positive bands (n=602).

RESULTS

The study population comprised 1517 males aged 15-49. Fifty one per cent were married and most (65.2%) had achieved primary or secondary education (22.1%) or higher (7.3%). Most were sexually active (84.3%).

The table shows the results of the rapid tests for HIV. In the total sample of 1517 tests the algorithm had reasonable sensitivity (97.7%, 95% confidence interval 94.1% to 99.4%) and negative predictive value (99.7%), but the specificity was low (90.4%, 95% confidence interval 88.7% to 91.9%) and the positive predictive value was unacceptably low (56.3%). Overall, 129 of 295 positive test results were false positives (43.7%) and four of 1222 negative results were false negatives (0.3%). Of the 129 false positives, 123 (95%) resulted from the Determine and Uni-Gold tests.

In the subsample of 639 tests with weak bands coded as HIV positive, the specificity (94.1%, 95% confidence interval 91.8% to 96.0%) and the positive predictive value (74.0%) were unacceptably low and the false positive rate (26.0%) was high. Exclusion of the 37 samples with weak positive bands noticeably improved the specificity (99.6%, 98.6% to 100.0%) and positive predictive value (97.7%) and reduced the rate of false positives to 2.3% (2/86). Among the 37 samples coded as having weak positive bands 86.0% were HIV negative on enzyme linked immunoassay and western blotting and 8.1% had indeterminate results on western blotting. Overall, 94.1% of weak positive bands were not confirmed as positive by enzyme linked immunoassay and western blotting. Among the 37 samples with weak positive bands 70.3% had weak bands on the Determine test and 29.7% on the Uni-Gold test. This problem was not observed with Stat-Pak.

DISCUSSION

An HIV testing algorithm consisting of three rapid tests in a Ugandan setting showed low specificity and low positive predictive values if weak bands were interpreted as positive, according to the manufacturers' recommendations. Exclusion of weak positive results noticeably improved the performance of the algorithm and reduced the proportion of false positives to acceptably low levels.

The interpretation of positive bands is subjective—the instructions for the Determine test state that any visible red should be interpreted as positive. It is possible that the technicians reading over-interpreted this instruction; it is noteworthy that the specificity was higher when the technicians were asked to code the band strength (table), suggesting that they became more cautious.

It is possible that the weak bands reflect cross reactions with other infections, but we have no data in this regard. One other rural programme reported similar problems with false positives from weak positive bands (H Grosskurth, personal communication, 2005), but two other evaluations of rapid tests in urban Uganda did not report these problems.^{4,5} We do not know whether the interpretation of weak positive results in rural Uganda affects other testing programmes in Africa. It is possible that this problem is caused by the dominant HIV-1 subtypes D, A, and AD recombinants found in Uganda, but high rates of false positive rapid test results have been reported in the United States, suggesting that this problem is not restricted to specific viral subtypes.³ Our findings may only pertain to the Determine and Uni-Gold tests. Also, because these tests were only assessed in males we cannot determine whether the findings apply to females, although a gender specific difference in test performance seems unlikely. About 50% of males enrolled in the trials were identified from a parallel population cohort, and participants were fairly representative of uncircumcised males in the general population of Rakai. Thus although the external validity of our findings to other populations cannot be fully defined, it is likely that the problems we encountered may occur in other settings. Our observations suggest a need to assess the performance of rapid tests in a variety of settings, and it would be prudent to routinely retest a batch of samples

Sensitivity, specificity, positive predictive value, and negative predictive value of rapid tests for HIV before and after exclusion of results with weak positive bands

Study sample	No of samples tested	Positive on EIA and western blotting	Negative on EIA and western blotting	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	False positives (%)	HIV prevalence (%)
All rapid tests:									
Positive result	295	166	129	97.6	90.4	56.3	99.7	43.7	11.2
Negative result	1222	4	1218						
Subsample with weak positive bands coded:									
Positive result	123	91	32	97.8	94.1	74.0	99.6	26.0	14.6
Negative result	516	2	514						
Subsample excluding weak positive bands:									
Positive result	86	84	2	97.7	99.6	97.7	99.6	2.3	14.3
Negative result	516	2	514						

EIA=enzyme immunoassay; PPV=positive predictive value; NPV=negative predictive value.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Rapid HIV tests provide timely, point of care methods for screening and diagnosis, but interpretation of positive bands is subjective

WHAT THIS STUDY ADDS

Weak positive bands on rapid HIV tests are mainly false positives and should be confirmed by enzyme immunoassay and western blotting before providing a diagnosis

by enzyme immunoassay and western blotting to maintain quality control in programmes using rapid tests.³ This is of particular importance given the proliferation of rapid tests and given the potential social and psychological consequences of a false positive HIV diagnosis. The proportion of samples yielding weak positive bands was relatively low in our study (5.8%), so retesting of weak positive results would not impose a heavy burden on most programmes. It would, however, require laboratory backup which could lead to delay in disclosure of some results.

We conclude that weak positive bands on rapid tests cannot be interpreted as positive in serum from Ugandan populations.

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Competing interests: None declared.

Ethical approval: This trial was approved by the scientific and ethics committee of the Uganda Virus Research Institute, Entebbe and the National Committee of Science and Technology, Kampala and the Committee on Human Research, Johns Hopkins University, Bloomberg School of Public Health, Baltimore.

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Diagnostic accuracy and clinical utility of a simplified low cost method of counting CD4 cells with flow cytometry in Malawi: diagnostic accuracy study

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ABSTRACT

Objectives To assess the diagnostic accuracy and clinical utility of a simplified low cost method for measuring absolute and percentage CD4 counts with flow cytometry.

Design A CD4 counting method (Blantyre count) using a CD4 and CD45 antibody combination with reduced blood and reagent volumes. Diagnostic accuracy was assessed by measuring agreement of the index test with two other assays (TruCount and FACSCount). Clinical utility was investigated by comparing CD4 counts with the new assay with WHO clinical staging in patients with HIV.

Setting Research laboratories and antiretroviral therapy clinic at a medical school and large government hospital in southern Malawi.

Participants Assay comparisons were performed on consecutive blood samples sent for CD4 counting from 129 patients with HIV. Comparison of CD4 count with staging was conducted on 253 consecutive new patients attending the antiretroviral therapy clinic.

Main outcome measures Limits of agreement with 95% confidence intervals between index test and reference standards.

Results The limits of agreement for Blantyre count and TruCount were excellent (cell count -48.9 to $27.0 \times 10^9/l$ for absolute counts in the CD4 range $<400 \times 10^9/l$ and

-2.42% to 2.37% for CD4 percentage). The assay was affordable with reagent costs per test of \$0.44 (£0.22, €0.33) for both absolute count and CD4 percentage, and \$0.11 for CD4 percentage alone. Of 193 patients with clinical stage I or II disease, who were ineligible for antiretroviral therapy by clinical staging criteria, 73 (38%) had CD4 counts $<200 \times 10^9/l$. By contrast, 12 (20%) of 60 patients with stage III or IV disease had CD4 counts $>350 \times 10^9/l$.

Conclusions This simplified method of counting CD4 cells with flow cytometry has good agreement with established commercial assays, is affordable for routine clinical use in Africa, and could improve clinical decision making in patients with HIV.

INTRODUCTION

CD4 counting could improve appropriate allocation of antiretroviral therapy for people infected with HIV.¹ Despite initiatives to reduce the price of the necessary reagents for developing nations to \$3-6 (£1.5-3.0; €2.2-4.4) per test,² this cost is still high for Africa.³ CD4 counting with flow cytometry is perceived by many to be too complex for use in Africa. WHO guidelines state that where CD4 counting is available, adults and children over 5 years with HIV should start