Evaluation of rapid enzyme immunobinding assays for the detection of antibodies to HIV-1

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Abstract **Three rapid enzyme immunobinding assays, Abbott's TestPack HIV-1/HIV-2, Clonatec's Rapid HIV1/HIV2 AB and the DuPont HIVCHEK 1+2, were evaluated using a panel of 20 selected sera with Western blot-proven reactivity to at least one envelope glycoprotein of HIV-1. The Abbott assay had the highest sensitivity and detected 18 of the 20 sera with no indeterminates (i.e. specimens with high background coloration which interferes with interpretation of the assay). Both the Clonatec and DuPont rapid assays correctly identified 12 sera as HIV-1 antibody-positive; the former produced 5 false-negatives and 3 indeterminates and the latter 7 false-negatives and 1 indeterminate. The majority of the sera used in the evaluation showed evidence of early seroconversion as many had low absorbance ratios in a screening enzyme immunosorbent assay. Although they represent only a small portion of clinical specimens, they point to the need for careful evaluation of new methodologies, using appropriate, well-characterised sera, before such techniques are accepted for general use.**

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The rapid escalation of HIV-1 infection in South Africa[1,2] following the first locally reported AIDS cases in 1982,[3] has made many clinicians increasingly concerned about performing surgical procedures on patients without being aware of their HIV status. In an emergency situation the clinician may be unable to wait for routine HIV antibody testing, which is generally performed using highly sensitive enzyme immunosorbent assays (EIAs). This has led to the use of the recently introduced rapid enzyme immunobinding assays (REIBAs) that are simple to perform and do not require additional equipment. While hospital staff have been made well aware of the problem of false positivity in HIV antibody screening assays and the need for confirmatory testing, many are unaware of the sensitivities of such assays and of the possible implications of a false-negative result.

In this study we evaluated the ability of three REIBAs, Abbott's TestPack HIV-1/HIV-2, Clonatec's Rapid HIV1/HIV2 AB test and the DuPont HIVCHEK 1+2, to detect low levels of antibody to HIV-1, as would be seen during seroconversion.

Materials and methods

Twenty fresh, unfrozen sera with known HIV reactivity were selected to determine the sensitivities of the assays being evaluated. The sera had been received either for routine HIV screening and/or confirmatory testing or as part of an ongoing survey to determine the prevalence of HIV in individuals attending a sexually transmitted diseases clinic.

The sera had initially been screened using a second-generation EIA for detection of antibodies to HIV-1 and HIV-2 (Genelavia MIXT EIA, Diagnostics Pasteur, France). To correct for variations between EIA runs, the ratio between the absorbance value for each serum specimen and the cut-off value for positivity for that run was calculated. Specimens with ratios above 1 were then evaluated using commercial Western blots (WBs) (New Lav-Blot I or II, Diagnostics Pasteur) and the virus-specific bands were recorded. Sera giving at least 1 envelope glycoprotein band on the HIV-1 WB were then tested using all three REIBAs. None of the selected sera was HIV-2-positive.

The basic formats of the three REIBAs were similar; either synthetic or purified peptides or recombinant antigens for HIV-1 and HIV-2 were attached to the porous membranes. Minor differences in methodology existed, however (Table I). Both the DuPont HIVCHEK 1+2 (Ortho Diagnostic Systems, France) and the Rapid HIV1/HIV2 AB (Clonatec, France) required reconstitution of reagents; once reconstituted, the specimen diluent and conjugate in the Clonatec kit were stable at 2 - 8°C for 'one month at the most' (package insert). The kit contained 2 vials of lyophilised diluent and 2 vials of the lyophilised conjugate. In the DuPont kit the serum controls, wash buffer and conjugate were reconstituted before use. Both the controls (1 set/20 test kit) and wash buffer (2 vials/20 test kit) were stable for 6 months at 4°C while the conjugate (4 vials/20 test kit) was stable for 2 months at the same temperature. No prior dilution of the specimen was required for the DuPont HIVCHEK 1+2 assay. All the

TABLE I.
Comparison of the methodologies of the three rapid enzyme immunobinding assays evaluated

	REIBAs		
	Abbott	Clonatec	DuPont
No. tests/kit	40	30	20 or 100
Neg. control	Yes	Yes	Yes
Weak pos. control	No	Yes	Yes
Pos. control	Yes	Yes	Yes
Internal quality control indicator	Yes*	Yes*	No
HIV-1/HIV-2 detection	Yes	Yes†	Yes
Reconstitution of reagents required	No	Yes	Yes
Stability of reagents	Equal to shelf life of kit	1 mo. for diluent and conjugate 2 vials/kit	6 mo. for buffer 2 vials/kit of 20 2 mo. for conjugate 4 vials/kit of 20
Dilution of specimen required	Yes	Yes	No
Time required	5 min‡	3,5 min‡	

* The Abbott internal control assured both the stability and correct addition of reagents and specimen; Clonatec's internal control did not guarantee addition of the specimen.
† While all three REIBAs detected antibodies to both HIV-1 and HIV-2, only Clonatec differentiated between the two viruses.
‡ The Abbott and Clonatec REIBAs required the timing of several steps in addition to flow-through time of the reagents; for the DuPont REIBA flow-through time determined the amount of time required to perform the test.

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reagents in the TestPack HIV-1/HIV-2 (Abbott Laboratories, USA) were supplied ready for use; however, dilution of the specimen was necessary.

Both the Abbott and Clonatec membranes contained test validation markers indicating correct addition and stability of the reagents, although only the former REIBA included a control to ensure addition of the specimen. Both the Clonatec and Abbott REIBAs needed to be timed, while the DuPont assay merely required that the previous fluid had passed through the membrane before further reagents were added. All three REIBAs were designed to detect antibodies to both HIV-1 and HIV-2, although only the Clonatec kit differentiated between the two antibodies. All three of the package inserts warned that a negative test result did not exclude the possibility of infection with HIV.

The REIBAs were performed according to the manufacturers' instructions and were all done by the same technician. The results were recorded by two technicians who were unaware of the banding patterns on the WBs; specimens with high backgrounds were considered indeterminate as was recommended by the manufacturers.

Results

Of the 20 sera, 9 were correctly identified as HIV-positive by all three REIBAs (Table II). In general, these sera had the higher absorbance ratios although specimen 9 had a ratio of only 1,1 and gave positive results in all three REIBAs. In contrast, several sera with absorbance ratios in the 2,0 - 7,5 range were recorded as negative by one or more of the REIBAs. Specimen 13 was WB-positive with 3 envelope bands on the blot (i.e. glycoprotein 41, 120 and 160), which is indicative of full seroconversion. This specimen was positive using the Abbott TestPack but negative according to both the HIVCHEK and Rapid AB tests.

Only 2 specimens (16 and 19), both with low absorbance ratios, were missed by the Abbott TestPack and no specimen was recorded as indeterminate. Nine sera were missed by the DuPont HIVCHEK, while 1 specimen had a high background which interfered with the reading. In Clonatec's Rapid HIV1/HIV2 AB test 5 sera were falsely negative and 3 were recorded as indeterminate owing to high background coloration. In no instance did the same specimen cause high background coloration in more than one REIBA.

As HIV-1 WBs with reactivity to the group specific antigen (gag) p25 and the envelope glycoprotein 160 band are indicative either of early seroconversion or cross-reactions with HIV-2,[4] and none of the sera was HIV-2-positive by Western blotting, all the sera were considered HIV-1-antibody-positive for comparative purposes. The Abbott TestPack had the highest sensitivity and detected 18 of the 20 recombinant EIA-positive sera with at least one envelope glycoprotein band on the HIV-1 WB. The Clonatec assay detected 12 of the 20 sera, with 3 indeterminates, while the DuPont HIVCHEK also detected 12 of the sera but with only 1 indeterminate.

Discussion

We report here on an evaluation of three REIBAs designed for use in clinical and laboratory settings where standard EIAs for detection of antibodies to HIV-1 and HIV-2 are unavailable. Only the sensitivities of the assays for detection of antibodies to HIV-1 were evaluated as there were insufficient HIV-2 antibody-positive sera available for a meaningful evaluation. While only 20 sera were tested with all three REIBAs, the sera had been carefully selected and were recombinant EIA HIV antibody-positive with appropriate bands on an HIV-1 WB (and no significant bands on an HIV-2 WB) indicating either early or full seroconversion to HIV-1.

The Abbott TestPack was the only REIBA with a control mark that indicated the correct addition and stability of the reagents and the presence of the specimen. The Clonatec control ensured the correct addition of reagents but did not indicate whether the specimen had been applied to the membrane. While the REIBAs were all easy to perform and the instructions were clear and concise, the inclusion of the control line insured proper

TABLE II.
Evaluation of rapid enzyme immunobinding assays for detecting antibodies to HIV-1 in serum specimens with envelope glycoprotein reactivity on WBs

Specimen	REIBAs* D	C	A	EIA† ratio	HIV-1 WB bands
1	P‡	P	P	18,2	18, 25, 55, 68, 160
2	P	P	P	17,3	18, 25, 55, 68, 120, 160
3	P	P	P	16,0	18, 25, 34, 41, 55, 68, 120, 160
4	P	P	P	17,2	25, 55, 160
5	P	P	P	4,8	18, 25, 40, 55, 160
6	P	P	P	6,4	18, 25, 34, 41, 55, 68, 120, 160
7	P	P	P	21,1	25, 55, 160
8	P	P	P	21,2	18, 25, 52, 55, 68, 160
9	P	P	P	1,1	18, 25, 34, 41, 52, 55, 68, 120, 160
10	N‡	P	P	2,5	25, 55, 160
11	N	I‡	P	7,5	25, 34, 55, 160
12	N	I	P	1,3	25, 52, 160
13	N	N	P	1,7	18, 25, 34, 41, 52, 55, 68, 120, 160
14	N	I	P	2,2	25, 55, 160
15	I	P	P	6,3	18, 25, 34, 41, 52, 55, 68, 120, 160
16	N	N	N	1,6	25, 34, 55, 68, 160
17	N	N	P	1,5	25, 52, 55, 68, 160
18	N	P	P	2,0	25, 34, 55, 68, 160
19	P	N	N	1,2	18, 25, 55, 68, 160
20	N	N	P	4,8	34, 55, 160

*D = HIVCHEK 1+ 2 (DuPont); C = RAPID HIV1/HIV2 AB (Clonatec); A = TestPack HIV-1/HIV-2 (Abbott).
†Specimen/cut-off absorbance ratio.
‡P = positive result; N = negative result; I = indeterminate result.

performance of the methodology, an important feature in clinical situations where laboratory or hospital staff may be unfamiliar with such techniques.

Both DuPont and Clonatec included information on the handling of sera that have high background readings, a situation that arises when particulate matter is present in the specimen. However, all the specimens in this study were clear and did not contain visible particles. It is interesting to note that no specimen gave high background readings in more than one test system and most problems were experienced with the Clonatec REIBA, where 3 of 20 specimens were indeterminate.

The sensitivities observed with the REIBAs, especially with Clonatec's Rapid HIV1/HIV2 AB and the DuPont HIVCHEK, are considerably lower than those reported in other studies.[5-7] However, in this study, sera that showed seroconversion were deliberately selected and many had low specimen/cut-off absorbance ratios in the recombinant EIA used in the initial screening of the sera. With the spread of HIV in South Africa, increasing numbers of individuals who are in the process of seroconverting and who will have low levels of antibodies to HIV-1 will be seen in clinics and hospitals. It is worrying that clinicians and other hospital staff may rely on methods such as the REIBAs to screen patients before surgical procedures are performed without adequately considering the possibility of a false-negative result. While the sensitivity of the Abbott TestPack is acceptable for such a panel of 'difficult' sera with low levels of antibodies to HIV-1, the sensitivities of the other 2 REIBAs would be unacceptable, especially when screening blood from a high-risk population. Clinicians should also be aware that while a negative result in any HIV antibody assay does not rule out the possibility of the individual being in the 'window period' of infection before antibodies develop, the low sensitivities of two of the REIBAs evaluated increase the risk of a falsely negative assay result.

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REFERENCES

1. Martin DJ, Schoub BD, Padayachee GN, *et al.* One year surveillance of HIV-1 infection in Johannesburg, South Africa. *Trans R Soc Trop Med Hyg* 1990; **84:** 728-730.
2. Schoub BD, Smith AN, Johnson S, *et al.* Considerations on the further expansion of the AIDS epidemic in South Africa — 1990. *S Afr Med J* 1990; **77:** 613-618.
3. Ras GJ, Simson IW, Anderson R, Prozesky OW, Hamersma T. Acquired immunodeficiency syndrome. *S Afr Med J* 1983; **64:** 140-142.
4. Acquired Immunodeficiency Syndrome (AIDS). Proposed WHO criteria for interpreting results from Western blot assays for HIV-1, HIV-2 and HTLV-I/HTLV-II. *Wkly Epidemiol Rec* 1990; **65:** 281-288.
5. Mitchell SW, Mboup S, Mingle J, *et al.* Field evaluation of alternative HIV testing strategy with a rapid immunobinding assay and an agglutination assay. *Lancet* 1991; **337:** 1328-1331.
6. Spielberg F, Kabeya CM, Ryder RW, *et al.* Field testing and comparative evaluation of rapid, visually read screening assays for antibody to human immunodeficiency virus. *Lancet* 1989; **1:** 580-584.
7. De Cock KM, Porter A, Kouadio J, *et al.* Rapid and specific diagnosis of HIV-1 and HIV-2 infections: an evaluation of testing strategies. *AIDS* 1990; **4:** 875-878.