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Comparison of Enzyme Immunoassays and an Immunofluorescence Test for Detection of Antibody to Human Immunodeficiency Virus in African Sera

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A total of 152 sera from African subjects were tested for presence of antibody to human immunodeficiency virus using four enzyme immunoassays (EIA) marketed by Abbott Diagnostics, Organon Teknika, Wellcome and Diagnostics Pasteur respectively, an indirect immunofluorescence assay (IFA) and an immunoblot assay (IBA) as reference test. The sensitivity (95 % confidence limits, CL) of the EIAs and the IFA ranged between 80.9 % and 99.1 %. The specificity of the Abbott EIA was lower (95 % CL: 38.1–72 %) than that of the other assays (95 % CL: 83.5–100 %). The use of an IFA or the Wellcome competitive EIA as confirmatory test on initially EIA positive sera yielded a specificity of 85.5–100 % (95 % CL) compared with the IBA. The costs of screening by an EIA, followed by confirmatory testing of reactive sera with IFA or the Wellcome EIA and IBA on discrepant test results was similar for all combinations with the exception of initial screening with the Abbott EIA which was more expensive. Using a limited number of sera from African subjects no one test system yielded a significantly superior degree of specificity or sensitivity.

Enzyme immunoassays (EIA) are currently the most frequently used serologic tests for detection of antibody against the human immunodeficiency virus (HIV). The sensitivity and specificity of commercially available EIAs performed on European and American sera has been reported to be 98–100 % and 90.5–100 % respectively (1–4). However, conflicting results have been reported when EIAs for detection of HIV antibody were performed on stored or fresh sera collected in Africa. Thus, very high seroprevalence rates of up to 40 % were reported in sera from Central and East Africa using a prototype EIA (5, 6), whereas more recently introduced assays yielded much lower seroprevalence rates when applied to specimens from the same areas (7, 8, 9). In addition, cross-reactivity with *Plasmodium falciparum* antibody, non-specific reactions attributed to circulating immune complexes, high concentrations of immunoglobulins, and repeated freezing and thawing of sera have been reported to affect results (10, 11).

We report here on the ability of four EIAs and an indirect immunofluorescence assay to detect HIV antibody in African sera using results obtained with an immunoblot assay (IBA) for comparison.

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Materials and Methods

Sera. A total of 152 African sera were investigated. Sera from prostitutes and men with a sexually transmitted disease were collected in Africa between 1981 and 1985, and sent on dry ice to Antwerp where they were stored at – 70 °C until anti-HIV tests were performed. All persons were clinically healthy at the time of sampling.

Enzyme Immunoassays. Four commercial EIAs were used, including the HTLV III EIA (Abbott Diagnostics, USA), the anti-HTLV III Vironostika (Organon Teknika, The Netherlands), the Wellcozyme test (Wellcome, Great Britain) and the Elavia test (Diagnostics Pasteur Production, France). Tests were carried out according to the manufacturer's instructions.

Indirect Immunofluorescence Test. The indirect immunofluorescence assay (IFA) used is a modification of the method described by Sandström et al. (12). Slides were prepared from HTLV III infected and non-infected H9 cell suspensions stored in growth medium containing 10 % dimethylsulfoxide (v/v) in liquid nitrogen. After washing, these cells were resuspended in a 5 % foetal calf serum saline solution at a concentration of 5×10^5 cells/ml. Twenty µl were applied on a multispot slide (Wellcome, SM11), air dried and fixed in acetone. A 1/20 serum dilution was used for screening. Evans blue in a final concentration of 0.2 % was added to the conjugate (sheep anti-human IgG FITC, 1/80, Wellcome). Typical diffuse speckling cytoplasmic fluorescence and a "star" image were read as positive reaction. Reading was done blindly by at least two individuals.

Immunoblot Assay. Immunoassays (IBA) were performed on nitrocellulose strips containing HIV characteristic proteins (Organon Teknika). A 1/100 serum dilution in blotting buffer was applied. This buffer consisted of phosphate buf-

fered saline (PBS) containing 5 % non-fat milk and 4 % goat serum. Sera were incubated for 2 h at 37 °C while mixing on a horizontal shaker. Subsequently the strips were washed twice with PBS containing 0.05 % Tween 20 for 10 min at room temperature. The first conjugate, a biotinylated goat anti-huyman IgG (Vector Laboratories) diluted at 1/1000 in blotting buffer was added for 1 h at 37 °C on a rocker, and the strips were then washed twice for 15 min. This was followed by the second conjugate incubation with horseradish peroxidase labelled avidine (Vector Laboratories) diluted 1/1000 in PBS for 30 min at 37 °C while mixing. The strips were washed three times for 10 min. Antibodies to the viral proteins on the strip were made visible by addition of a substrate solution consisting of 0.06 % (w/v), 3,3-diaminobenzidine and 0.005 % (v/v) H_2O_2 in PBS. A western blot was considered positive if antibodies to p24 and gp41 were present.

Calculations. The sensitivity (true positive/true positives + false negatives) and the specificity (true negatives/true negatives + false positives) was calculated for each test. The predictive values of a reactive and non-reactive test result were calculated for varying HIV prevalences using Bayes' theorem (13).

Results

All 152 African sera were tested with the Organon EIA and the IFA. Some of the sera were also tested with the Pasteur EIA (146), the Abbott EIA (85) and the Wellcome test (74). All sera were tested in an IBA which was used as reference method to calculate the sensitivity and specificity of the different assays. The 104 (68 %) IBA positive sera demonstrated both p24 and gp41 reactive bands, mostly in addition to other characteristic HIV proteins. Weak antibody reactions to p24 were seen in five cases. Such sera were considered negative, and did not react in any EIA or the IFA. Of the IBA positive sera, 44 (44 %) showed a gp120 band. The number of reactive and non-reactive tests for each assay are shown in Table 1.

Sensitivity and specificity values are presented as 95 % confidence limits to account for variations resulting from unequal sample size. The sensitivity of the EIAs and IFA ranged from 80.9 % to 99.1 %, with no major differences between the five assays. The Abbott EIA yielded 2 % false negative results, whereas the other EIAs failed to detect 6.4–7.7 % of the IBA positive specimens. However, the specificity of the Abbott EIA was lower than that of the other tests (respective 95 % confidence limits of 38.1–72 % and 83.5–100 %) (Table 1). The Wellcome EIA did not score any false positive results, the IFA yielded one (1.2 %) false positive reaction. False positive tests were observed with the Organon, Pasteur and Abbott assays in 4.5 %, 4.7 % and 25 % of the sera respectively.

In order to increase the accuracy of screening with an EIA, initially reactive sera were tested with a different method, including an IFA or the Wellcome assay, a competitive solid-phase EIA. The results of such screening combinations are shown in Table 2. With the exception of the combination EIA Abbott followed by IFA (one false positive reaction, 2 %), no false positive test combinations were observed. The sensitivity and specificity were very similar for the different test combinations.

The predictive value of a positive test result was calculated by Baye's theorem. Figure 1 shows the variation of the positive predictive value of different assays and test combinations according to different seroprevalence rates. The predictive value of a reactive test result in a very low risk population (such as Belgian blood donors with a seroprevalence rate of 0.002 %) was 0.1 %. For a 2 % HIV prevalence rate (such as pregnant women in Nairobi), the predictive value of a positive test result ranged from 10 % to 51 % for a single test and from 22 % to 100 % for combined tests. In high-risk groups such as African prostitutes (40 % HIV prevalence) the predictive value of a confirmed positive test result was higher than 90 %.

The cost of each test combination is shown in Table 2. The cost per serum was calculated on the basis of the price charged for the test kits and of the labour costs. Confirmation with a commercially available

Table 1: Results of tests for HIV antibody in African sera, sensitivity, specificity and concordance as compared to an immunoblot assay (IBA).

Assay	Number of sera tested	Number of reactive sera (%)	Number of IBA positive sera (%)	Percentage of sensitivity[a]	Percentage of specificity[a]	Percentage of concordance with IBA[b]
EIA Abbott	85	64 (75)	49 (58)	89.1–100	38.1– 72.0	78.8
EIA Organon	152	89 (59)	91 (60)	86.2– 97.5	84.1– 98.2	93.4
EIA Pasteur	146	85 (58)	87 (60)	85.6– 97.4	83.5– 98.1	93.2
EIA Wellcome	74	40 (54)	43 (58)	80.9– 98.5	88.8–100	95.9
IFA	152	85 (56)	91 (60)	84.8– 96.9	94.2–100	94.7

[a] 95 % confidence limits.
[b] Concordance was calculated as identical results divided by the number of sera tested.

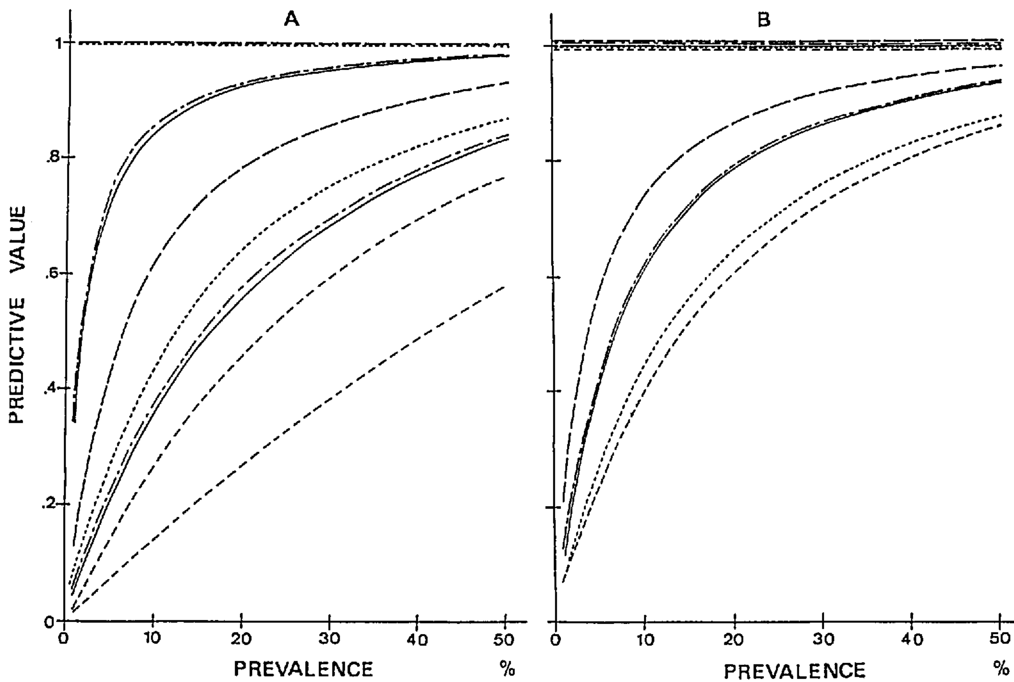

Figure 1: Predictive values of a reactive test result after single (A) and combined (B) serologic tests for HIV antibody in African sera. A: upper and lower limits for single tests: — — — — — EIA Abbott; ——— EIA Pasteur, —— . —— . EIA Organon, ... EIA Wellcome, —— —— IFA. B: upper and lower limits for combined tests: — — — — — EIA Abbott + IFA + WB, ——— EIA Pasteur + IFA + WB, —— . —— . EIA Organon + IFA + WB, ... EIA Wellcome + IFA + WB, —— —— IFA + EIA Wellcome + WB.

Table 2: Results of serologic test combinations for detection of HIV antibody in African sera, sensitivity, specificity and cost.

Assay[a]	Number of sera tested	Number of reactive sera (%)	Number of IBA positive sera	Percentage of sensitivity[b]	Percentage of specificity[b]	Cost[c]
EIA Abbott + IFA	85	48 (56)	49	89.1–100	85.5– 99.9	13.06
EIA Abbott + EIA Wellcome	74	42 (57)	43	87.7– 99.4	88.8–100	11.64
EIA Organon + IFA	152	85 (56)	91	86.2– 97.5	94.1–100	6.80
EIA Organon + EIA Wellcome	74	41 (55)	43	84.2– 99.4	88.8–100	6.70
EIA Pasteur + IFA	146	81 (55)	87	85.6– 97.4	93.9–100	6.60
EIA Pasteur + EIA Wellcome	69	38 (55)	40	83.1– 99.4	88.1–100	6.52
EIA Wellcome + IFA	74	40 (54)	43	80.9– 98.5	88.8–100	6.60
IFA + EIA Wellcome	74	41 (55)	43	84.2– 99.4	88.8–100	7.50

[a]EIA or IFA followed by IFA or Wellcome EIA on positive sera. IBA was performed on sera with discrepant results.
[b]95 % confidence limits.
[c]Cost per serum includes test price and labour costs (in ECU).

IFA or the Wellcome EIA yielded the same cost. Because of its lower specificity, resulting in more positive reactions, the cost of screening sera by the Abbott EIA was nearly double that of other EIAs.

Discussion

Except for a lower specificity of the Abbott EIA, major differences in performance of the different test

systems were not observed in this study using stored African sera with a high seropositivity rate. A disturbing finding in these sera from apparently healthy individuals was the fact that 2–7.7 % of reactions were false negative in the EIAs and IFA, compared with the IBA, the reference test. The precise specificity of the IBA is not known, and the assay can give both false positive and false negative results (14, 15). However, if a reaction with proteins representative of both the core and envelope proteins is sought, its specificity can be assumed to be high. As the prevalence of infection increases in a population, the observed rate of false negative reactions at initial testing has significant consequences for screening programmes aimed at reducing transmission of HIV through blood transfusion. Since seroprevalence rates as high as 18 % have been found among blood donors in Africa (16), an operational assessment of the rate of false negative reacting sera among blood donors is urgently needed.

According to the results obtained in the present study Abbott EIA followed by an IFA on the EIA-positive sera gave the highest sensitivity and specificity values in the African sera tested. However, the cost of this test combination is much higher than that of the other test combinations. Our results indicate that currently used EIAs marketed by Organon Teknika, Wellcome and Diagnostics Pasteur have an acceptable degree of specificity in the screening of African sera, and that an IFA or competitive EIA as confirmatory test represents an accurate and inexpensive alternative to the IBA. Earlier serum surveys in Africa yielding poor specificity values were not confirmed. The reasons for these false positive results are not clear – perhaps the prototype EIAs had still not been well adapted for African sera, or perhaps crossreactivity with other infectious agents or aspecific reactions due to circulating immune complexes occurred.

Using a limited number of sera in our study no one test system yielded a significantly superior degree of specificity or sensitivity in detection of HIV antibody in sera from African subjects.

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