

# The accuracy of an alternative confirmatory strategy for detection of antibodies to HIV-1: experience from a regional laboratory in Kagera, Tanzania

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## Abstract

**Background:** Constant improvement of HIV tests often results in withdrawal of poorer quality tests by the manufacturing companies. It is thus often necessary to evaluate new HIV testing kits and modify the existing testing strategies.

**Objectives:** To evaluate an alternative HIV antibody testing strategy which involves consecutive testing of sera by two enzyme-linked immunosorbent assays (ELISA), which both are recombinant antigen-based but utilise different test principles, followed by re-testing of sera giving discordant results.

**Study design:** Sera ( $n = 1558$ ) from a cross-sectional study of the HIV-1 seroprevalence in the Kagera region of Tanzania were tested using two ELISAs in parallel: Enzygnost anti-HIV-1/2 plus and Wellcozyme HIV-1 recombinant. Western blot analysis was done on all concordantly reactive and repeatedly discordant reactive samples as well as on 10% of concordantly ELISA negative sera.

**Results:** Two hundred and four sera (13.1%) were confirmed HIV-1-antibody positive. Both ELISAs had a sensitivity of 100%. The specificities of the ELISAs at initial and repeated testing were 99.8 and 99.9%, respectively, for Enzygnost and 97.7 and 99.5%, respectively, for Wellcozyme. None of the sera was concordantly false positive in both ELISAs. The mean ratio of the optical density of a sample to the cut off value of the test run (OD/CO ratio) was lower for samples giving false positive reactions than for confirmed HIV-1-antibody-positive samples. It is therefore important to interpret with caution HIV antibody ELISA test results on samples giving low OD/CO ratios. None of 10% of randomly selected concordantly ELISA negative sera gave a positive Western blot reaction.

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**Conclusions:** This field evaluation of an HIV antibody testing strategy involving the use of a recombinant antigen-based sandwich ELISA (Enzygnost) followed by a recombinant antigen-based competitive ELISA (Well cozyme) showed that it had a sensitivity and specificity of 100%. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** HIV-1; Antibody ELISA; Alternative confirmatory strategy; Africa; Human immunodeficiency virus; Alternative antibody testing

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## 1. Introduction

Enzyme linked immunosorbent assays (ELISAs) for serological detection of infection with human immunodeficiency virus (HIV) became commercially available in 1985, 2 years after the first isolation of HIV (Weiss et al., 1985). ELISAs are still the most commonly used HIV antibody screening assays. A variety of ELISAs based on different antigen preparations and/or different test principles have been developed (Constantine, 1993). Over time there have been improvements of the performance of ELISAs from the initial ones which utilised crude viral lysates to the more recent tests which utilise recombinant antigens, synthetic peptides or a combination of these types of antigens. Improvement in antigen preparation as well as in the ELISA detection systems has led to better sensitivity and specificity of the assays (Zaaijer et al., 1992; Constantine, 1993). Western blot (WB) analysis remains the gold standard in HIV antibody confirmation (Gurtler, 1996). However, the use of WB assays has been limited by the high cost; the need for well trained manpower; lack of consensus in interpretation criteria for WB and presence of indeterminate WB results (Tamashiro et al., 1993).

Affordable and simpler alternative strategies which utilise a combination of ELISAs and/or simple and rapid assays for the detection and confirmation of presence of HIV antibodies have been shown to be as sensitive and specific as the standard algorithm of an initial screening assay followed by WB analysis (Spielberg et al., 1990; Fonseca and Anand, 1991; Mitchell et al., 1991; van der Groen et al., 1991; Behets et al., 1992; Nkengasong et al., 1992; Mortimer, 1992; Urassa et al., 1992; WHO, 1992; Nunn et al., 1993; Urassa et al., 1994; Thorstensson et al., 1995; Carvalho et al., 1996; Ittiravivongs et al., 1996;

Andersson et al., 1997; Stetler et al., 1997; Wilkinson et al., 1997). There are some limitations of using alternative confirmatory testing strategies, the commonest being rapid withdrawal of various testing assays from the market. Ideally an alternative HIV testing strategy should include a combination of assays which utilise different antigen preparations as well as different test principles (WHO, 1992). We have previously used such a combination of ELISAs for HIV diagnostic testing in Tanzania (Urassa et al., 1994), but after withdrawal from the market of one of the assays which was based on synthetic peptide antigens we had to change the combination of assays. In the present study we have evaluated the accuracy of our currently used HIV-1 testing strategy which includes two ELISAs; Enzygnost anti-HIV-1/2 plus and Wellcozyme HIV-1 recombinant. Both are recombinant antigen-based assays but they utilise different test principles.

## 2. Methodology

The blood samples used in this study were collected in 1996 from a randomly selected population in Bukoba, the capital of the Kagera region in Tanzania, as part of a population-based cross-sectional study of the HIV-1 seroprevalence in the region. All samples were collected in empty sterile vacutainer tubes and allowed to clot after which sera were separated and stored at  $-20^{\circ}\text{C}$  until testing. None of the sera were thawed more than twice or were hemolyzed. All sera were tested in parallel using both Enzygnost anti-HIV-1/2 Plus ELISA (Behring, Marburg, Germany) which is a sandwich assay made up of recombinant proteins, and Wellcozyme HIV-1 recombinant ELISA (Murex, Dartford, UK) which is a competitive assay. The test procedures followed the manufacturers' instructions. Washing of ELISA plates was

done manually. A ratio of the optical density (OD) of a sample to the cut off (CO) value of the test run equal to or higher than 1.0 was considered a positive reaction. Samples giving discordant results on the two ELISAs were retested by both test assays. If the results were still discordant, WB analysis (HIV blot 2.2 Diagnostic Biotechnology (Pte) Science Park Singapore) was done. WB was also done on all sera which were reactive in both ELISAs and on 10% of randomly selected sera which were concordantly nonreactive in both ELISAs. WB results were interpreted according to WHO criteria requiring reactivity to at least two envelope bands for positivity (WHO, 1990). Sera which gave other WB bands but no envelope band were considered to be HIV antibody negative in this study.

### 3. Results

A total of 1558 sera were tested of which 204 (13.1%) were HIV-1-antibody-positive confirmed by WB analysis, 1353 were HIV-antibody-negative and one sample was considered to be indeterminate (see below). All 204 samples which were initially reactive on both ELISAs were positive on the WB assay. Thus both ELISAs had a sensitivity of 100%. Enzygnost anti-HIV1/2 ELISA gave three and one false positive reactions on initial and repeat testing, respectively. Thus the specificity was 99.8% (1350/1353) on initial testing and 99.9% (1352/1353) after repeat testing of initially reactive samples. Wellcozyme HIV-1 recombinant ELISA showed 31 and seven false positive reactions on initial and repeat testing respectively,

giving a specificity of 97.7% (1322/1353) on initial testing and 99.5% (1346/1353) after repeat testing. After repeat testing on both assays eight repeatedly discordant samples were run on WB assay; four showed a single p24 band, while the rest gave no band on the WB. One sample with p17, p55 and gp 160 bands on the WB was considered indeterminate and could not be processed further hence this was excluded from further analysis. None of the sera was concordantly false positive in both assays. None of the 120 randomly selected sera which were negative on both ELISAs showed a positive WB pattern. Thus the sensitivity and specificity were both 100% for a testing strategy involving initial screening of sera by the Enzygnost ELISA followed by testing of reactive sera by the Wellcozyme ELISA and retesting of discordant samples.

The mean OD/CO ratios of samples which were confirmed to be HIV-1-antibody-positive, and of those which were false positive are shown in Table 1. HIV-1-antibody-positive samples had similar mean value and 95% confidence interval of the ratio of the OD/CO in both ELISAs. Samples which gave false positive reactions had relatively lower mean value of the OD/CO ratio in both assays compared to the HIV-1-antibody-positive samples. Although there was an overlap in the ranges of the OD/CO ratios in sera from confirmed HIV-1 infected individuals and those which gave false positive reactions, none of the true antibody positive samples had a OD/CO ratio below 3.0 in both ELISAs.

There was no presence of HIV-2 antibodies as evidenced by the WB in the tested samples in the Kagera region.

Table 1

The mean ELISA OD/CO ratio (95% CI) and the range of the OD/CO ratio of confirmed HIV antibody-positive and false ELISA positive samples

| Assays                                    | True HIV-antibody positive |      |                      |          | False positive |      |           |         |
|---|----------------------------|------|----------------------|----------|----------------|------|-----------|---------|
|   | <i>n</i>                   | Mean | (95%CI) <sup>a</sup> | Range    | <i>n</i>       | Mean | (95% CI)  | Range   |
| Enzygnost anti-HIV-1/2 Plus               | 204                        | 7.5  | (7.3, 7.7)           | 2.3–18.5 | 3              | 2.4  | (0.6,4.1) | 1.6–2.9 |
| Wellcozyme HIV-1 recombinant <sup>b</sup> | 204                        | 7.3  | (7.1, 7.5)           | 1.8–17.3 | 31             | 1.6  | (1.1,2.1) | 1.0–7.9 |

<sup>a</sup> CI = confidence interval.

<sup>b</sup> The ratio for Wellcozyme was calculated by the formula CO/OD.

#### 4. Discussion

The importance of availability of simple, accurate and affordable assays for detection of HIV antibodies in blood before transfusion and for the diagnosis of HIV in individuals suspected of infection in order to inform and counsel them can not be overemphasised. Alternative HIV testing strategies which utilise a combination of antibody ELISAs and or simple and rapid antibody assays should ideally include assays based on different antigen sources and different test principles both of which are not always possible to fulfil (WHO, 1992; UNAIDS/WHO, 1997). Even if these conditions are fulfilled there is still a risk that the two assays can lead to false positive or false negative results. To explore such possibilities we did WB analysis on all test samples which were concordantly positive or discordantly positive on the two ELISAs and on randomly selected 10% of sera which were non reactive on both ELISAs in the present study. Our evaluation showed that a testing strategy involving the sequential use of two ELISAs (Enzygnost anti-HIV-1/2 Plus and Wellcozyme HIV-1 recombinant) based on similar antigen preparations (recombinant produced) but utilising different test principles had both a sensitivity and specificity of 100%.

UNAIDS/WHO has given recommendations for HIV testing strategies according to test objective and prevalence of infection in the sample population (WHO, 1992; UNAIDS/WHO, 1997). Our testing strategy based on two consecutive ELISAs corresponds to the UNAIDS/WHO strategy II which is recommended for diagnostic testing of individuals with symptoms of HIV infection or asymptomatic individuals in populations with a HIV seroprevalence above 10%.

An evaluation of a testing strategy utilising the two anti-HIV ELISAs included in the present work has not been published previously. However, we have previously reported a high diagnostic accuracy of a testing strategy utilising an earlier version of the Enzygnost ELISA based on synthetic peptide antigens, followed by the Wellcozyme recombinant HIV ELISA (Urassa et al., 1992, 1994). In the present study the Wellcozyme ELISA gave a somewhat lower specificity at initial

testing of samples (97.7%) than that found in a previous evaluation of this assay in Tanzania (99.5%) (Urassa et al., 1992). The reason for this difference could be that sera which had been stored frozen and manual washing of ELISA plates were used in the present study whereas the previous evaluation was done using fresh sera and a machine for washing of ELISA plates.

Objective interpretation of ELISA test results requires calculation of the OD/CO ratio. In the present study samples from confirmed HIV infected individuals had a higher mean OD/CO ratio (7.5 and 7.3 in Enzygnost and Wellcozyme ELISA, respectively) compared to samples giving false positive reactions (2.4 and 1.6 in Enzygnost and Wellcozyme ELISA, respectively). There was, however, an overlap in the ranges of the OD in those sera from confirmed HIV infected individuals with those which gave false positive reactions. Recent studies have shown that samples which are repeatedly reactive in sequential antibody screening assays but which are WB negative should be interpreted with caution regarding their HIV serostatus because some HIV-1 antibody assays have been found to be reactive earlier in the infection process than WB (Zaaijer et al., 1992; Tamashiro et al., 1993). It is therefore important to take special precautions in those samples showing low OD/CO values. The best option would be to take repeat samples from test subjects after 2–4 weeks. In our current diagnostic testing strategy on asymptomatic individuals repeat sequential testing by the two antibody ELISAs evaluated in the present work is done on two serum samples collected at an interval before an individual is confirmed to have HIV infection. In cases where repeatedly discordant ELISA results are obtained a WB test is done.

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