Evaluation of a quantitative double ELISA strategy for confirmation and differentiation of HIV infection

T.M. Rehle a, P. Mattke c, G.N. Liomba b, S. Krämer c, G.M. Gershy-Damet d, K. Konan d, A. Sangare d, L. Zekeng e, J.M. Tsague e, L. Kaptue e, J. Eberle f, L. Gürtler f,*

a Department of Infectious Diseases and Tropical Medicine, University of München, D-80802 Munich, Germany
b Queen Elizabeth Central Hospital, Blantyre, Malawi
c Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), D-65726 Eschborn, Germany
d Programme National de Lutte Contre le SIDA, Abidjan, Côte d'Ivoire
e Centre Hospitalier Universitaire, Laboratoire et Transfusion Sanguine, Université 1, Yaoundé, Cameroon
f Pettenkofer Institute, University of München, D-80338 München, Germany

Accepted 25 March 1997

Abstract

The current HIV pandemic is complicated by the spread of distinct types and subtypes of HIV. The currently used conventional diagnostic tests have shown limitations in the detection of antibodies against all HIV-I subtypes, as demonstrated by recent identification of HIV-1 subtype O. To evaluate quantitatively the diagnostic potential of a double ELISA strategy for the detection and partial differentiation of HIV-1, HIV-1 subtype O and HIV-2 infections blood samples were examined at five different test centers: Blantyre, Malawi; Abidjan and Daloa, Ivory Coast; Yaoundé, Cameroon; Munich, Germany. All test results, including ELISA extinction values and Western blot profiles, were forwarded to Munich for final interpretation. An indirect anti-HIV-1/2 ELISA and a competitive anti-HIV-1 ELISA were used in combination for the initial screening of blood specimens. All anti-HIV positive and anti-HIV negative samples were subjected to immunoblot analysis. Independent of the diversity of the extinction profiles, and of the test manufacturer, the quantitative evaluation of the ELISA extinction values could define two extinction areas with a 100% predictive value for HIV-1 seropositivity and HIV seronegativity: extinction values > 2 by the indirect ELISA and < 0.2 by the competitive ELISA for an anti-HIV-1 subtype A to I positive result; extinction values < 0.2 by the indirect ELISA and > 1.0 by the competitive ELISA for an anti-HIV negative result. Additionally, the quantitative evaluation of the extinction profile provides partial information on the HIV-1 subtype as far as the distinction in group M and group O is concerned. In conclusion, the quantitative evaluation of this double ELISA strategy can reduce the number of blood specimens that require additional confirmatory testing in developing countries and can be superior to the immunoblot method during early seroconversion. © 1997 Elsevier Science B.V.

Keywords: Quantitative ELISA; Indirect and competitive ELISA; HIV-1 group differentiation; HIV-1 confirmation

* Corresponding author. Tel: + 49 89 51605274; fax: + 49 89 5380584.
1. Introduction

The HIV/AIDS pandemic is composed of many separate epidemics, even within a single country, and strain variability becomes more complex over time. Of the two known HIV types, HIV-1 is the predominant virus worldwide. The current epidemiological interest is characterized by the analysis of the distinct subtypes of HIV-1 and HIV-2 (McCutchan et al., 1996).

In Thailand, differential transmission of HIV-1 subtypes has been observed: HIV-1 subtype B is acquired mainly through drug injection, while HIV-1 subtype E is spread as well by heterosexual contacts (Kunanusont et al., 1995; Mastro et al., 1997); in India and China HIV-1 subtype C is also found (Luo et al., 1995). In Brazil subtype B, a special clone of subtype B and to a lesser degree subtype F have been described (Potts et al., 1993). In sub-Saharan Africa all HIV-1 subtypes are prevalent. The pattern of subtypes in west-central Africa includes HIV-1 subtype O or group O which is present in Cameroon and neighboring countries since 1986 (Charneau et al., 1994; Zekeng et al., 1994; Kabeya et al., 1995). In contrast to the diversity found in sub-Saharan Africa, predominantly subtype B and also A, D and E, are prevalent in Europe (Arnold et al., 1995).

HIV-2 is divided in the subtypes A and B, and further subtypes C to E have been characterized by DNA sequencing (Sharp et al., 1994; McCutchan et al., 1996). All HIV-2 subtypes are found primarily in west Africa and also in Angola and Mozambique (WHO, 1994). Some European countries with close links to west Africa, such as Portugal and France, have reported substantial numbers of HIV-2 infections (Sharp et al., 1994).

The identification of HIV infected individuals is an essential element for prevention of spread. In developed countries, the enzyme linked immunosorbent assay (ELISA) is the most frequently used laboratory method for initial screening of anti-HIV and the Western blot for confirmation. However, cost, performance and interpretation of the Western blot pose difficulties for many laboratories in developing countries. Therefore, alternative confirmatory HIV testing procedures have been developed (Van der Groen et al., 1991; Sato et al., 1994). These studies have shown that combinations of ELISAs can provide test results with positive predictive values similar to that of Western blot confirmed results, especially when the optical density values obtained by the ELISAs were evaluated on a quantitative basis (Barin and Kabeya, 1994; Gürler, 1996).

For historical reasons most of the HIV-1 specific assays use HIV-1 subtype B antigens. The identification of HIV-1 subtype O has demonstrated that the currently used ELISAs are limited in their detection of antibodies against all HIV-1 subtypes. Because of crossreactivity with subtype B-antigen, these ELISAs can detect 80-90% of HIV-1 subtype O antibodies and 50-70% of HIV-2 antibodies (Schable et al., 1994; Simon et al., 1994; CDC, 1996).

The purpose of this study was to analyze the value of a quantitative evaluation of the ELISA extinctions, obtained by combining an indirect anti-HIV-1/2 ELISA and a competitive anti-HIV-1 ELISA, for detection and partial differentiation of HIV-1 (group M), HIV-1 subtype O (group O) and to a limited extent HIV-2 infections.

2. Materials and methods

2.1. Test centers and ELISA screening assays

This multicenter study was carried out in Blantyre, Malawi; Daloa and Abidjan, Ivory Coast; Yaoundé, Cameroon and at the Pettenkofer Institute in Munich, Germany. For initial screening, the following tests were used: the Enzygnost anti-HIV-1/HIV-2 (Behring, Marburg, Germany), an indirect anti-HIV-1/2 ELISA and a competitive anti-HIV-1 ELISA, for detection and partial differentiation of HIV-1 (group M), HIV-1 subtype O (group O) and to a limited extent HIV-2 infections.
nost anti-HIV-1/2 plus, Behring, Marburg, Germany). All tests were carried out according to the instructions of the manufacturer.

For final interpretation, all test results were forwarded to the Pettenkofer Institute in Munich where a quantitative evaluation of the ELISA absorbance values, hereafter referred to as extinctions, was carried out using a graphical program (GraphPad, San Diego, California).

2.2. Immunoblot performance

Any reactive and the anti-HIV negative specimens included in this evaluation, were subjected to immunoblot confirmation. The New LAV-Blot-I (Diagnostic Pasteur, Marnes-la-Coquette, France) was used in all African study centers, the Pasteur New LAV-Blot-II was used in addition in Ivory Coast. At the Pettenkofer Institute in Munich, which was the reference center for this study, in-house immunoblots prepared with the HIV-1 subtype B isolate MVP-899, the HIV-2 subtype A isolate MVP-11971 and the HIV-1 subtype O isolate MVP-5180 (Gürtl et al., 1994) were used. The differentiation between anti-HIV-1, anti-HIV-1 subtype O and anti-HIV-2 antibodies was usually done by a competitive immunoblot procedure with strips from the different viruses. Immunoblot interpretation was close to the proposed WHO criteria (WHO, 1990).

3. Results

3.1. Comparison of two different competitive ELISAs

As shown in Fig. 1, the two different competitive ELISAs produced quite similar extinction values with the 28 anti-HIV-1 positive specimens which, with a few exceptions, clustered in the lower left corner of the graph. In contrast, the 10 anti-HIV-2 positive specimens displayed a much broader range of extinctions due to their different competition; their extinctions ranged between borderline and negative. For logistical reasons only one competitive ELISA (Enzygnost) was used for further analysis.

![Fig. 1. Comparison of extinctions of the two competitive anti-HIV-1 ELISAs. The two dimensional presentation of the extinction values of 28 anti-HIV-1 positive and 10 anti-HIV-2 positive specimens shows narrow clustering for the HIV-1 samples but a wide distribution for the HIV-2 samples. (Beh) stands for the test manufactured by Behring and (Wel) by Wellcome/Murex. The coefficient of correlation r for anti-HIV-1 is 0.93 and for anti-HIV-2 is 0.78.](image1)

3.2. Combination of competitive and indirect ELISA in a HIV-1 only region

The value of this double ELISA strategy in a geographical region where only HIV-1 is prevalent was demonstrated in Blantyre, Malawi. As shown in Fig. 2, most of the ELISA extinctions of

![Fig. 2. Distribution of extinctions obtained by the combination of an indirect anti-HIV-1/2 ELISA and a competitive anti-HIV-1 ELISA with 105 anti-HIV-1 positive and 46 anti-HIV-1 negative specimens from Malawi. With a few exceptions, the extinctions for both groups cluster close together. The sample at the x-axis with an extinction value of 1.22 is from a patient during early seroconversion. Lines separate the areas of 100% predictive values for positivity (close to the x-axis with an extinction < 0.2 in the competitive ELISA and an extinction > 2.0 in the indirect ELISA) and for negativity (close to the y-axis with an extinction > 1.0 in the competitive ELISA and < 0.2 in the indirect ELISA). The coefficient of correlation r for anti-HIV-1 positive is 0.96 and for anti-HIV-1 -- 0.89.](image2)
the 105 anti-HIV-1 positive specimens were clustered in the area > 2 in the indirect ELISA and < 0.2 in the competitive ELISA. Only 4 (3.8%) of the extinction values were outside of this area (Fig. 2). In one of the four sera, the immunoblot profile was typical for a recent seroconversion, for the other three specimens the reason of the reactive ELISA was unknown. Extinctions of 46 anti-HIV-1 negative sera were close to the y-axis, as expected, and only 4 of these (8.7%) were reactive by the indirect but negative by the competitive ELISA using the manufacturer's cut off. The area with a 100% predictive value for a negative result was defined by extinction values < 0.2 by the indirect ELISA and > 0.8 by the competitive ELISA.

3.3. Combination of competitive and indirect ELISA in a region where HIV-1 and HIV-2 are prevalent

The extinction pattern produced by the two combined assays with 114 anti-HIV-1 positive, 29 anti-HIV-2 positive and 59 anti-HIV negative sera is shown in Fig. 3. Double reactive samples in which the differentiation of a HIV-1 and/or HIV-2 infection was not possible by the competitive immunoblot analysis were excluded from the quantitative evaluation because they behaved like HIV-1 specimens by both assays. Compared to the extinction profile obtained by the Malawian samples, the extinctions of positive and negative samples from the Ivory Coast were distributed over a much broader range. Eight (7.0%) of the 114 anti-HIV-1 positive specimens and six (20.7%) of the 29 anti-HIV-2 positive specimens showed extinction values < 2.0 in the indirect ELISA. In the competitive ELISA four (13.8%) of the 29 anti-HIV-2 positive samples were reactive, while the extinctions of the other specimens were negative. Two of them showing no competition at all. Despite the broader scatter of the extinctions of the anti-HIV positive samples, there was a clear difference in their distribution when compared to the extinctions of the anti-HIV negative specimens (Fig. 3). Only part of the extinctions of the 59 anti-HIV negative specimens was close to the y-axis of the graph, since 39 (66.1%) of those were reactive in the indirect anti-HIV-1/2 ELISA. As found with the Malawian samples, a predictive value of 100% for detecting anti-HIV-1 positive sera was only obtained when the samples had extinctions > 2 in the indirect ELISA and < 0.2 in the competitive ELISA.

In the analysis of the indirect ELISA extinctions, the cluster of anti-HIV-2 positive specimens was located in the area > 2, with the competitive ELISA however, they could not be distinguished from extinctions obtained with the anti-HIV negative samples (Fig. 3). Since all the anti-HIV negative samples had an extinction of < 1.0 by the indirect ELISA, a certain degree of differentiation between anti-HIV negative and positive samples was possible. There was no accurate prediction of the HIV antibody status within the range of extinction values 1.0 to 2.0. Therefore, in a geographical region where HIV-1 and HIV-2 are prevalent, a third assay is required for all reactive specimens with extinction values < 2 by the indirect ELISA and > 0.2 by the competitive ELISA.

3.4. Combination of competitive and indirect ELISA in a region where HIV-1 subtypes A to H and subtype 0 are prevalent

This study was carried out in Yaoundé, Cameroon, where the HIV-1 subtype O variant
was found recently. As observed in the two other African settings, the great majority of anti-HIV-1 positive specimens had extinctions of > 2 and < 0.2 by the indirect and competitive ELISA, respectively. All extinctions of the 20 anti-HIV-1 subtype O positive specimens were > 1.2 by the indirect ELISA, but were scattered between extinctions > 0.2 and < 1.0 by the competitive ELISA (Fig. 4). Compared to the distribution of the extinctions of anti-HIV-2 samples shown in Fig. 3, the extinctions of the anti-HIV-1 subtype O samples showed a more efficient competition by the competitive ELISA, which is in accordance with the amino acid sequence data of the proteins of both virus types. As observed in the previous graphs, also in the Cameroonian samples a clear distinction was possible between anti-HIV negative and positive specimens but 9 (23.7%) of the 38 anti-HIV negative specimens in this study had extinctions not close to the y-axis.

Fig. 4. Extinction pattern obtained by the combination of a double antigen anti-HIV-1/2 ELISA which included the HIV-1 subtype O antigen and a competitive anti-HIV-1 ELISA with 76 anti-HIV-1 subtype A to H (group M) positive, 20 anti-HIV-1 subtype O (group O) positive and 38 anti-HIV negative specimens from Cameroon. While the majority of the extinctions of anti-HIV-1 subtype O samples cluster closely together, the majority of the extinctions of the anti-HIV-1 subtype O samples is widespread, but still below the extinction of 1.0 of the y-axis, an area where the majority of the anti-HIV-2 positive specimens were found (see Fig. 3). The lines indicate the areas of 100% predictive values for positivity and negativity as described in Fig. 3. The coefficient of correlation r for anti-HIV-1 positive is 0.88, for anti-HIV-O + 0.56 and for anti-HIV - 0.77.

Fig. 5. Extinction pattern obtained by the combination of an indirect anti-HIV-1/2 ELISA and a competitive anti-HIV-1 ELISA with 35 samples, classified as indeterminate by the Western blot pattern, from Germany. The extinction values of the samples are distributed over the entire graph. Two of the three samples with extinction values of > 2.4 in the indirect anti-HIV-1/2 ELISA originated from patients during early seroconversion. Lines indicate the areas of 100% predictive values for negativity and positivity as described in Fig. 2.

3.5. Quantitative ELISA analysis of immunoblot indeterminate samples

Thirty five samples with indeterminate immunoblot profiles were reassessed by this double ELISA strategy at the Pettenkofer Institute. As shown in Fig. 5, the extinction values of 23 (65.7%) of the 35 samples clustered in the area < 1.0 by the indirect ELISA which is associated with a high probability of anti-HIV seronegativity. These 23 samples were from blood donors and hospitalized patients, none of whom seroconverted later. Three (8.6%) of the 35 samples showed extinctions > 2.0 by the indirect ELISA, two of them having extinctions < 1.0 by the competitive ELISA. These two specimens were obtained from patients during their time of early seroconversion. The extinction value of the third specimen was negative in the competitive ELISA and after reexamination of a second blood sample of this patient, the extinction was < 0.2 in the indirect ELISA, which is clearly negative. None of the samples with extinction values between 1.0 and 2.0 by the indirect ELISA were from patients who seroconverted later. When the distribution of the extinctions of the 35 immunoblot indeterminate samples was compared with the extinction pattern of specimens with a 100% predictive value for HIV seronegativity or HIV-1 subtype A to I
(group M) seropositivity, then this double ELISA strategy produced a more accurate result of the actual HIV antibody status than the immunoblot.

4. Discussion

The testing strategy used in this five center study was based on two ELISAs with a different antigen composition and different test principle. All samples have been reconfirmed by the Western blot method. Since the HIV prevalence in the tested samples was different in each study center (approximately 30% in Blantyre, 20% in Abidjan, 11% in Daloa and 7% in Yaoundé), no calculations were made for the predictive values of results obtained by a specific assay or assay combination.

One of the objectives of this study was to analyze whether this combination of two types of ELISA could be used with equal success in different African laboratories, using different assay lots and assays manufactured by different companies. As shown in Figs. 2–4, the proposed test algorithm was independent of the manufacturer of the indirect ELISA if the test interpretation is restricted to extinction areas representing results with a 100% predictive value.

The mean optical density values of the cut offs of the indirect ELISAs used in the study were approx. 0.25 and those of the competitive ELISAs approx. 0.75. We felt that adhering to these cut off values would not yield test results with the highest possible predictive values. Therefore, we divided the range of ELISA extinction values into three sections: 0 to 0.999, 1.0 to 1.999 and > 2.0 for the indirect ELISA, and 0 to 0.2, 0.201 to 0.999 and > 1.0 by the competitive ELISA. In the quantitative analysis of the extinction values produced by this double ELISA strategy we defined two extinction areas with a 100% predictive value for an anti-HIV-1 subtype A to I (group M) positivity (extinctions values by the indirect ELISA > 2 and < 0.2 by the competitive ELISA) and for an anti-HIV negativity (extinction values in the indirect ELISA < 0.2 and > 1.0 in the competitive ELISA). The application of these areas will enable a further reduction in the number of samples that require confirmation, for example by immunoblot. Most of the samples tested in our study had extinction values which were associated with a 100% predictive value for HIV-1 seropositivity or HIV seronegativity. Not more than 3% of all anti-HIV-1 positive specimens had extinction values outside of the defined areas with a 100% predictive value and would have required reassessment with an additional confirmatory test.

As shown in Fig. 3, the quantitative evaluation of this test strategy is also quite effective in countries where HIV-1 and HIV-2 are prevalent. However, the double ELISA strategy would be more effective for identifying HIV-2 positive specimens when the anti-IIV-1 competitive ELISA is replaced by another HIV-2 specific ELISA (Barin and Kabeya, 1994). An additional advantage of combining an indirect and a competitive ELISA is that by the quantitative evaluation the extinction profile may also give a clue as to the HIV-1 group, i.e., differentiating between HIV-1 group M and HIV-1 group/subtype O.

In conclusion, the quantitative strategy of two different ELISAs in combination and adaption to the different HIV types and subtypes prevalent in a given geographical region, will yield test results that are in part even more reliable than those obtained with conventional immunoblot (CDC, 1996).

Acknowledgements

We are grateful to Dr B. Lorbeer for his valuable assistance in the data analysis and for all African laboratory members that are not cited as authors. The study was supported by the Ministry for Economic Cooperation and Development, Germany.

References


