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Testing and confirmation strategy for HIV using quantitative ELISA results

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Summary

Objective: This study is an evaluation of the utility of quantitative ELISA reactivity in a survey of sera from Brazilian blood donors and AIDS patients.

Study Design: The evaluation was performed on 298 sera of the Brazilian reference panel. In a previous investigation with these sera the operational characteristics of four different anti-HIV tests were compared using the cut-off value given by the manufacturer and with an alternative cut-off value calculated by receiver operating curves (ROC). Based on these new cut-off values, quantitative ELISA results were used to develop an alternative strategy for the confirmation of HIV EIA results, thus diminishing the number of costly HIV Western blots normally necessary for confirmation. Normalized EIA O.D. ratios were derived by dividing the test O.D. by the cut-off O.D., using the cut-off values calculated in the ROC analysis. For each serum, the signal/cut-off ratios of the six possible HIV assay combinations were compared and the operational characteristics of the test combinations calculated.

Conclusion: It was possible to demonstrate that HIV testing employing combinations of different ELISAs, followed by quantitative interpretation of the ELISA reactivities, is a useful tool for HIV diagnosis. Since the method of HIV testing proposed in this study was shown to be as accurate as the standard procedure performed in most laboratories (ELISA followed by confirmation of ELISA reactive sera with HIV Western blot), while avoiding up to 90% of the costs of Western blots, it is recommended for use in routine testing, especially in settings with limited resources (e.g. developing countries).

Key Words

Accuracy, Brazil, cut-off value, HIV, quantitiative EIA results, ROC, Youden's Index

Introduction

In recent years, tests for detection of antibodies against human immunodeficiency virus type 1 and 2 (HIV-1, HIV-2) have became widely available [3]. Testing strategies for the diagnosis of HIV infection have therefor been developed. Generally, sera are screened with an anti-HIV ELISA and the reactivity of ELISA-positive sera is confirmed in a HIV Western blot. Since this procedure is time consuming and expensive, alternative strategies have been developed using a sequence of different ELISAs and/or agglutination assays for anti-HIV detection [4, 6, 7, 8, 10, 11, 12]. All but one [5] study are using only qualitative ELISA results, characterizing a serum either as reactive or nonreactive. They evaluated the utility of quantitative interpretation of the ELISA results using low- and high-prevalence sera from laboratory testing in USA. Although sera from tropical areas sometimes presenting higher backgrounds, it was possible to corroborate their results, and to demonstrate that quantitative ELISA is useful utility for the confirmation of HIV infection after adjusting the cut-off values proposed by the manufacturers with receiver operating analysis [2].

Materials and Methods

Patients' sera

The evaluation was performed on 298 sera from a Brazilian reference panel. The sera were collected from Brazilian blood donors, healthy individuals and AIDS patients between 1988-1991 at blood banks and hospitals in the cities of Rio de Janeiro and Salvador (Bahia) [1].

Serologic assays

All 298 sera were tested with four HIV screening assays (Abbott HIV-1 EIA, Abbott Laboratories Ltd., BR; Biochrom HIV-1/-2 ELISA, Biochrom KG, D; Vironostika anti-HTLV III, Organon Teknika Inc., BR; Wellcozyme HIV-1+2, Murex Diagnostika GmbH, D) and were confirmed by Western blot analysis (DuPont HIV-1 Western Blot Kit, DuPont Nemours Deutschland GmbH, D; HIV Blot 2.2, Diagnostic Biotechnology Ltd., Singapore; Western Blot HIV-1 IgG Assay, Diagnostic Biotechnology Ltd.).

All sera were aliquoted and stored at -20°C. Initially reactive sera were retested. Sera with discrepant ELISA results were tested a third time. The outcome of the two results which were in agreement was taken as the final result and used for further analysis. HIV positivity was defined as a positive Western blot result (WB) with at least one band from each gene product Env, Gag and Pol (following the guidelines of the American Red Cross). Sera with no bands in Western blot were termed HIV negative. All other reaction patterns were designated as HIV "indeterminate". All tests were performed according to the manufacturers' instructions.

Statistical analysis

Recently we compared in our lab the operational characteristics of the single HIV tests such as accuracies, negative and positive predictive values [9] using the cut-off value given by the manufacturer, and with an alternative cut-off value calculated with receiver operating curves (ROC) [2]. Using the cut-off values obtained by ROC analysis we were able to improve the assay specificities of without affecting sensitivities.

Based on the ROC-derived cut-off values (Table 1), 255 out of 298 sera classified either as HIV positive or negative were used to develop an alternative strategy for HIV EIA result confirmation, diminishing the number of HIV Western blots normally necessary for confirmation.

Table 1: Calculation methods for estimation of the cut-off values.

	Abbott	Biochrom	Murex	Organon
Cut-off formula [a]	NC + (K × PC)	NC + K	NC + K	K × (NC + PC)
K manufacturer	0.15	0.2	0.2	0.5
K ROC-analysis	0.3	0.3	0.5	0.9

[a] NC: Mean of negative controls, PC: Mean of positive controls.

Accuracy : The ability of a test to classify samples correctly = (TP + TN) / (TP + FN+ TN + FP)
NPV : Negative predictive value = TN (TN + FN) × 100
PPV : Positive predictive value = TP (TP + FP) × 100
ROC : Receiver-operating characteristics
Sensiticity : The ability of a test to classify positive samples as positive (diagnostic or clinical sensitivity) = TP / (TP + FN) × 100
Specificity : The ability of a test to classify negative samples as negative (diagnostic or clinical specificity) = TN / (TN + FP) × 100
Youden's Index: J = ((TP × TN) - (FN × FP)) / ((TP + FN) × (FP + TN))

Table II: Operational characteristics of the different HIV ELISA combinations (n=255).

	Abbott ∞ Biochrom	Abbott ∞ Murex	Abbott ∞ Organon	Biochrom ∞ Murex	Biochrom ∞ Organon	Murex ∞ Orgnon
Sensitivity	98.8%	98.9%	97.8%	97.8%	97.3%	97.3%
Specificity	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Accuracy	98.8%	99.2%	98.4%	98.4%	98.0%	98.0%
NPV	95.9%	97.3%	94.7%	94.7%	93.4%	93.4%
PPV	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Youden's Index J	0,984	0,989	0,978	0,978	0,973	0,973
Reduction of WB	90.9%	90.5%	82.3%	91.5%	84.3%	83.5%

Normalized EIA O.D. ratios were derived by dividing the test O.D. by the ROC-derived cut-off O.D. For each of the six possible HIV assay combinations, the signal/cut-off ratios of each serum were compared (Figure 1) and the operational characteristics, including Youden's Index J [13], of the test combinations were calculated (Table 2).

The analysis shown here is easy to perform since it can be done on an electronic spread sheet without means of specialized statistical software packages.

Results

The signal/cut-off ratio in any assay has no predictive value for the signal/cut-off ratio in any other assay, since the signal/cut-off ratios do not correlate for any of the investigated ELISA pairs (Figure 1). On the other hand, all sera with a signal/cut-off ratio greater than 2.0 for all ELISA pairs were also positive in Western blot. This fact was used to develop a simplified confirmation strategy for HIV testing (Figure 2).

In daily routine testing, sera which are non-reactive in a given HIV screening test are classified as HIV negative (neglecting the possibility of false negative reactions), and all sera which are reactive in the screening assay are confirmed with HIV Western blot. Since the Western blot is expensive and time consuming the usefulness of paired ELISA testing for confirmation of HIV infection was investigated. The results show that sera with a signal/cut-off ratio greater than 2.0 in two different ELISAs can be classified as truly HIV (Figure 1). Sera which are either reactive in only one assay, or show ratios between 1.0 and 2.0 for both assays, should be confirmed by Western blot (Figure 2). With this strategy it is possible to diminish the use of Western blots by about 80-90%, without losing diagnostic accuracy (Table 2).

Furthermore, the 43 sera with indeterminate Western blot patterns were analysed (Table 3). More than 50% (23/43) of these sera would have never been identified as WB indeterminate either using the standard procedure for HIV testing or our alternative strategy, since they gave negative results in all four ELISAs. Forty percent of the WB indeterminate sera would be characterized either as negative or indeterminate in the standard procedure as well as in the proposed strategy (depending on the combination of ELISAs). Three sera were strongly reactive in all four ELISAs, and showed WB activity against either ENV/POL or ENV/GAG.

Discussion

Since the method of HIV testing proposed in this study is as accurate as the standard procedure, but avoids up to 90% of the costs of Western blots, it is recommended for use in routine testing, especially in settings with limited resources (e.g. developing countries). The advantage of the method described here over the other methods, which use solely different ELISAs and/or agglutination tests, is that in any doubtful case a confirmation by Western blot would be performed. The

Table III: Reaction patterns of the Western blot indeterminate Sera (n=43).

	n	WB patterns of WB indeterminate sera																	
M_r of virus specific bands in HIV Western blots		160	120	66	55	32	24	17	160 120	160 120 66	160 55	66	66	66	51	32	24 17	160 120 17	160 120 66 51 41 32
All ELISA negative	23	3	1		5		6	5			1		1	1					
1-3 ELISA positive	17		1	1		1	4	4	4	1		1							
All ELISA positive	3																	1	2

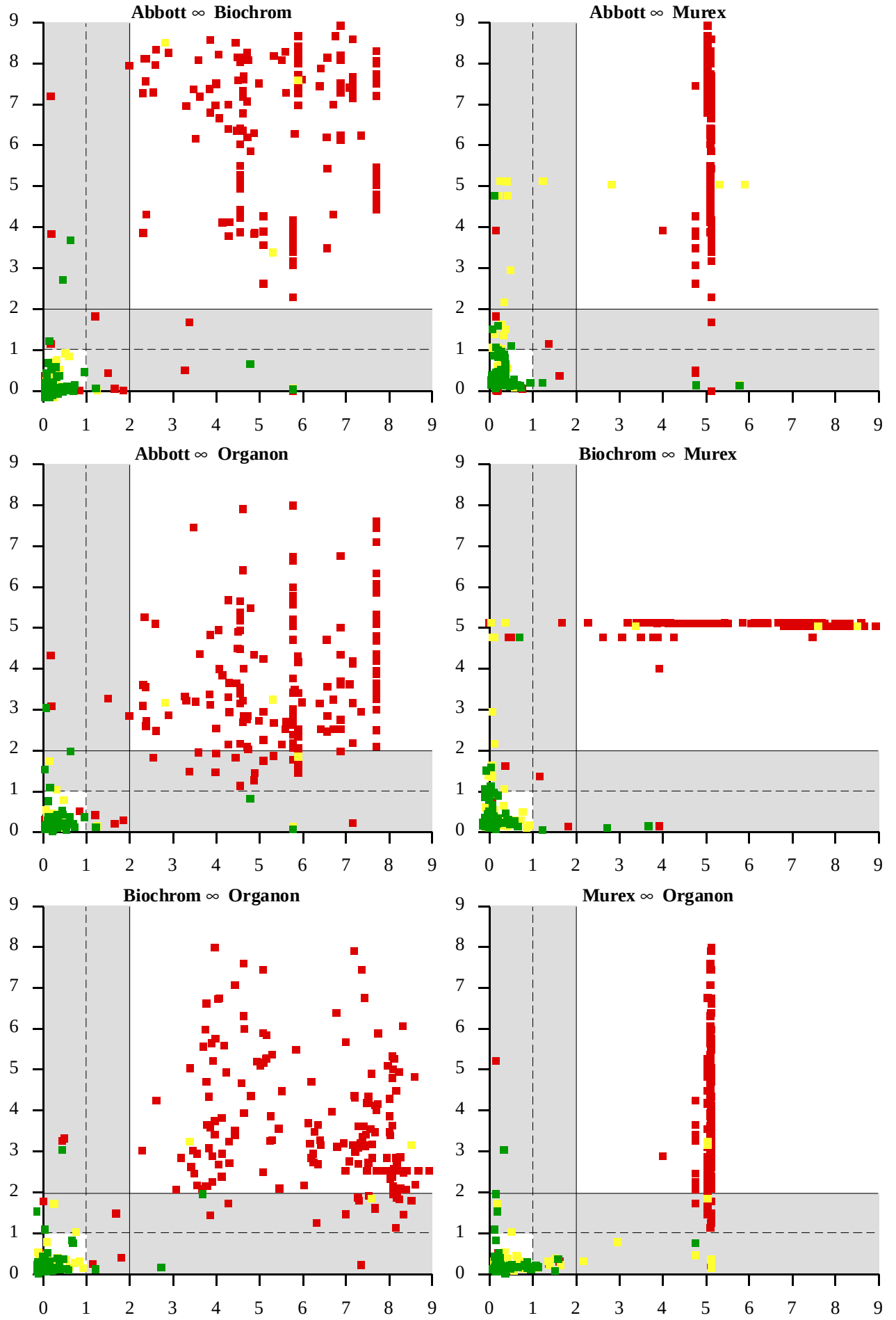

Figure 1: Paired comparison of the signal/cut-off ratios for each HIV test combination (n=255). Only the sera which express a signal/ cut-off ratio greater than 1.0 and smaller than 2.0 (grey zone) must be confirmed with HIV Western blot. All sera exceeding the threshold of 2.0 in both assays were also positive in HIV WB o at least indeterminat.
■ = WB positive, ■ =WB negative, ■ = WB indeterminat, graphics are titled x ∞ y

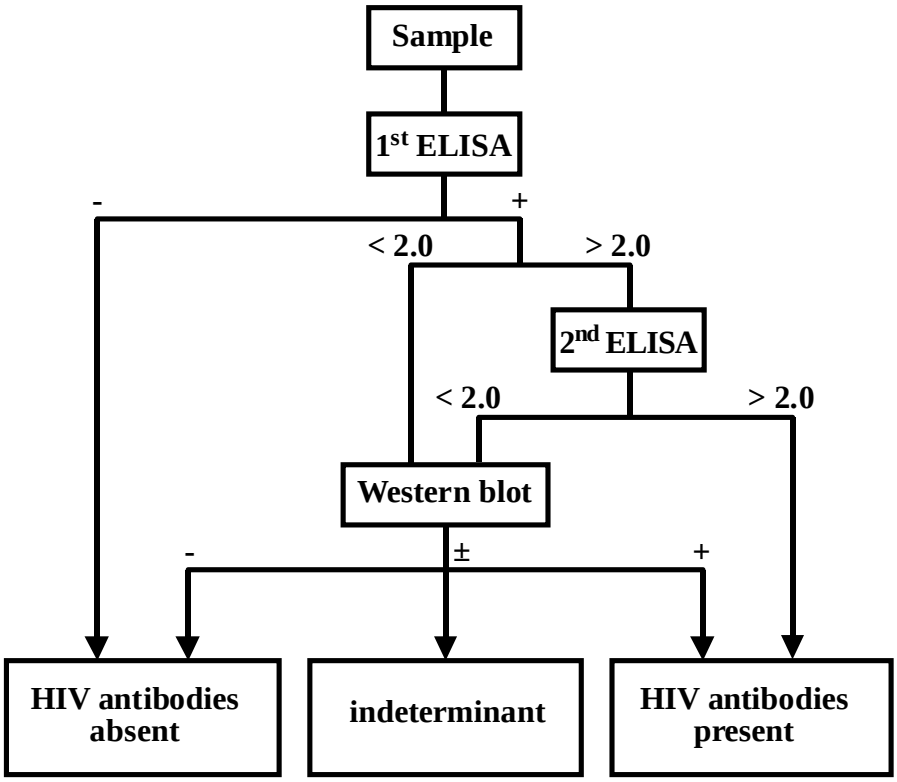

Figure 2

Proposed HIV testing strategy using quantitative EIA results.

results shown here are in good agreement with the results described by Hou et al. [5]. Furthermore, I propose similar investigations with other pairs of ELISAs, especially with other combination tests for HIV-1 and HIV-2, in order to establish less expensive standardized testing strategies for all assays commonly used. The use of HIV-1/-2 assays with high sensitivity as the first assay, followed by a second assay with high specificity but different from the first assay (e.g. other antigens, other test principle) is recommended.

Disclaimer

The views expressed in this report are those of the authors and are not necessarily also held by the institute. Use of trade names is for identification only and does not imply endorsement by the INCQS.

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