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Short Communication

Performance of HTLV-1 Screening Assays in Brazil

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Summary

In recent years a variety of studies have been carried out to compare the accuracy (generally expressed in terms of sensitivity and specificity) of commercially available anti-HTLV tests. None of these studies were performed in Brazil or in any other South American country. During the characterization of our Brazilian reference panel we evaluated the sensitivities and specificities of the Abbott HTLV EIA (100%; 89.7%) and the Biochrom HTLV-1/-2 ELISA (100%; 42.4%). Our conclusion was that both assays may be problematic in terms of correctly identifying HTLV-negative sera. We therefore adjusted the cut-off values using receiver operating characteristics (ROC). ROC analysis, which involves calculating sensitivity and specificity for several cut-off values, can be used to ascertain the co-variation in the specificity and sensitivity of any assay giving quantitative results. The optimum cut-off value for the assay in a given study population is the point that gives highest possible sensitivity in conjunction with a small false-positive fraction. Using the HTLV-1/-2 Western blot as the "gold standard", we were able to improve the specificity of the Biochrom HTLV-1/-2 assay to 95% without affecting its sensitivity of 100%. However, it seems that when using the Biochrom HTLV-1/-2 ELISA, there may be problems in separating positive and negative sera. In the case of the Abbott HTLV EIA, our ROC analysis revealed that the cut-off value suggested by the manufacturer was nearly identical to the optimum cut-off value. Adjustment will affect neither sensitivity nor specificity. However, a slight adjustment of the cut-off value result in a clearer separation of the positive and negative populations. Furthermore, we assume that this adjustment will help to avoid false-positive results when larger serum panels are investigated. Further investigations will show whether or not this problem is linked to the geographical regions where the test is performed (e. g. polyclonal stimulation due to parasitic infections in tropical countries).

Abbreviations

Accuracy: The ability of a test to classify samples correctly = (TP + TN)/(TP + FN + TN + FP) × 100.
δ-value: $\overline{\lg\ (\text{Ratio})}/\sigma_{\lg\ (\text{Ratio})}$.
FPF: False-positive fraction = 1 – specificity = (1 – TN/(TN + FP)) × 100.
NPV: Negative predictive value = TN/(TN + FN) × 100.
PPV: Positive predictive value = TP/(TP + FP) × 100.
ROC: Receiver operating characteristics.
Sensitivity: 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.
TP, FP: True positive, False positive
TN, FN: True negative, False negative

In the last few years, a number of groups have assessed the performance of commercially available HTLV screening assays (3, 6, 9). One of the most recently published reports (5) has provided a comparison of nine different commercially available anti-HTLV-1 assays. They evaluated sensitivity, specificity and δ-values (4, 7) for all nine assays, using a serum panel consisting of 225 sera. All these studies have reported high sensitivities and specificities (≥ 90%).

Our group recently characterized a reference panel for HTLV, HCV and HIV (1, 2). In our HTLV tests, we screened 600 Brazilian sera (taken from blood donors in Rio de Janeiro and Salvador, Bahia in 1988–1991) with the Biochrom HTLV-1/-2-ELISA (peptides from p19 and gp46; Biochrom KG, Berlin, D). The reactive sera were also tested with the Abbott HTLV-1 EIA (Abbott Laboratories, Chicago, USA). For confirmation we used the HTLV Blot 2.3 (HTLV-1/-2; Diagnostic Biotechnology Ltd., Singapore, Singapore). All the tests were performed and evaluated according to the manufacturer's instructions. Initially reactive sera were retested. Sera with discrepant ELISA results were tested for a third time. If two results were in agreement, they were interpreted as the final result and used for further analysis. Western blots showing bands against Gag (p19 or p24) and Env (gp46 or rgp46-1/-2 or rgp21) were classified as HTLV-1/-2 positive; those without any bands, as HTLV-1/-2 negative; and other reaction patterns, as "indeterminate".

In the light of previous experiments in Brazil with other immunological tests that employed different antigens and gave high false-positive ratios (i. e. Chagas-ELISAs, unpublished observations by W. Oelemann, and different HIV-ELISAs (2)), we compared the frequency distribution of the $\log_{10}$ (O.D./cut-off) ratio obtained from Western blot-confirmed results in order to ascertain the capacity of the tests to separate the negative and positive populations (4, 7). The operational characteristics (such as sensitivities and specificities) and the receiver operating characteristics (ROC) were calculated for each assay (8). This method analyzes the co-variation of sensitivity and the false-positive fraction by calculating and comparing these two variables using different cut-off values. The optimum cut-off value can easily be derived by plotting sensitivity and the false-positive fraction against each other. The point that gives the highest possible sensitivity in conjunction with the smallest false-positive fraction is the optimum cut-off value for the assay when it is employed to test the population under investigation. This method can be used to show whether or not the cut-off value recom-

mended by the manufacturer is the same as with the optimum cut-off value, which maximizes sensitivity and specificity in a given population.

In our screening assay, 18% of the sera were ELISA-reactive; however, in Western blot tests only 4% could be confirmed as positive, while 6% gave a negative Western blot result. The 48 HTLV Western blot indeterminate sera could be separated into three major groups: (1) these expressing a single band against rgp21 (45%), (2) rgp46-II in combination with another virus specific band, without fulfilling the criteria for HTLV-1 or HTLV-2 positivity (24%) and (3) a single band against p24 (10%). The other sera (21%) expressed single bands against other viral proteins.

The results of the $\log_{10}$-ratio transformation and the ROC analysis are shown in Fig. 2. Using the Western blot as the "gold standard", both assays exhibited a high level of false-positives (Abbott, 10.3% and Biochrom, 58%). The absolute values may change when a larger serum panel of HTLV-1/-2-confirmed positive and negative sera is investigated, but it seems reasonable to estimate that, in Brazil, false-positive fractions of 10–20% (specificities of 80–90%) will be obtained with commercially available immunoassays. The calculation of sensitivity in our study was biased by the fact that we investigated ELISA positive sera only. Since we have had no evidence of high levels of false-negative results in HTLV ELISAs or other immunological tests (Chagas, HIV), we estimated a "virtual" sensitivity of 100% for the HTLV ELISAs used in this study.

For comparison, in a 439-sample study of sensitivities and false-positive fractions of four different HIV-ELISAs (Abbott HIV EIA, Biochrom HIV-1/-2 ELISA, Organon HTLV-III ELISA, Wellcozyme HIV-1 + 2), we found sensitivities of 95.9 ± 1.2% (94.7 –97.4%) and specificities of 87.6 ± 6.9% (81.3–97.2%) when we compared Western blot results (2).

The frequency distribution of the serum panel tested is shown in Fig. 1. Most of the sera that gave false-positive reactions in our HTLV screening assay were low-reactive

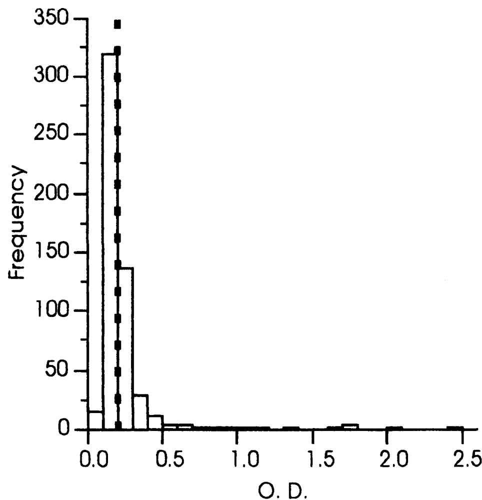

Fig. 1. Frequency distribution of the optical densities of the entire serum panel (Biochrom HTLV-1/-2 ELISA). The dashed line indicates the minimum cut-off value calculated according to the manufacturer's instructions (0.2 + mean of negative control).

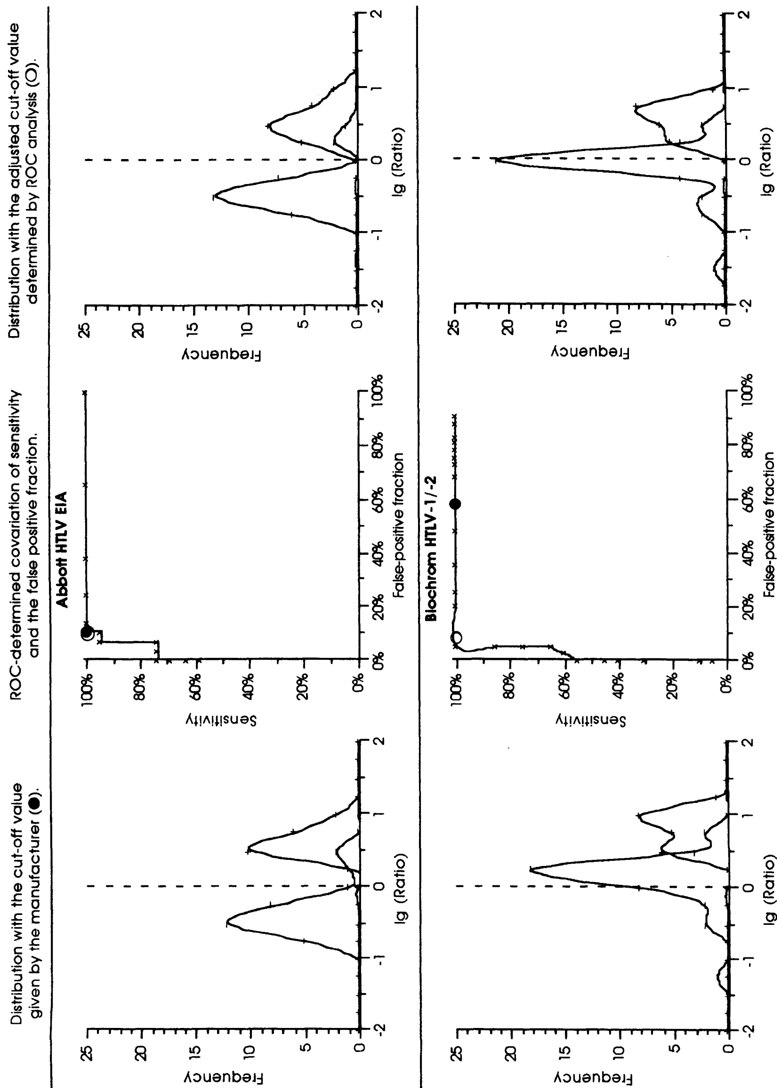

Fig. 2. The graphs on the left side show the frequency distributions of the $\log_{10}$-transformed signal/cut-off ratios calculated using the cut-off value suggested in the manufacturer's instructions for the Abbott HTLV EIA (top) and the Biochrom HTLV-1/-2 ELISA (bottom). The curve of the frequency distribution of the $\log_{10}$-transformed signal/cut-off ratios for the Western blot negative sera is marked with a minus (–), the one for the Western blot positive sera with a plus sign (+). The part of the curve from the Western blot negative sera on the right side of the dashed line symbolizes the fraction of false-positive reactions in the ELISA and vice versa. In the centre, the ROC curves are shown for each test. The cut-off values suggested by the manufacturers (corresponding to the frequency distributions shown by the graphs on the left side) are marked with an closed circle (•), our proposed cut-off values with an open circle (o). The graphs on the right side show the frequency distributions of the $\log_{10}$-transformed signal/cut-off ratios calculated with cut-off value estimated by ROC analysis, corresponding to the open circle (o) in the graphs in the centre.

Table 1. Performance of HTLV-1 assays (n = 60)

	Abbott HTLV EIA		Biochrom HTLV-1/-2 ELISA	
Type of Analysis[a]	Normal	ROC	Normal	ROC
Cut-off formula[b]	NC × K		NC + K	
K	4.5	5.0	0.2	0.35
Sensitivity	100.0%	100.0%	100.0%	100.0%
Specificity	89.7%	89.7%	42.5%	92.0%
FPF[c]	10.3%	10.3%	57.5%	8.0%
Accuracy	93.4%	93.4%	71.7%	87.5%
NPV[c]	82.6%	82.6%	54.1%	86.2%
PPV[c]	100.0%	100.0%	100.0%	100.0%
δ^-	− 1.62	− 1.77	− 0.01	− 0.64
δ^+	2.18	1.97	2.89	1.93

[a] normal: Cut-off value calculated according to manufacturer's instructions; ROC: Cut-off value calculated by ROC analysis;
[b] NC: Mean of negative controls
[c] FPF: False-positive fraction; NPV: Negative predictive value; PPV: Positive predictive value

sera with ratios of 1.63 ± 0.94. The $\log_{10}$-transformed ratios and the ROC analysis (Fig. 2) demonstrated that the specificity could be increased (without affecting sensitivity) by adjusting the cut-off value. If the cut-off value of the Biochrom HTLV-1/-2 ELISA is calculated by adding 0.35 instead of 0.2 to the mean of the negative control, the specificity will increase from 42% to 92% and the false-positive fraction will decrease from 58% to 8% (Tab. 1), but it seems that this assay may still pose a problem of separating positive and negative sera (Fig. 2). The Abbott EIA separates negative and positive populations very well and the cut-off point (4.5 × mean of negative control) coincides almost exactly with the optimum point as calculated by ROC analysis (Tab. 1, Fig. 2). A slight adjustment of the cut-off value, to the exact value of the calculated optimum point (5 × mean of negative control), will affect neither sensitivity nor specificity, but it will ensure that the cut-off value corresponds exactly to the minimum of the distribution curve between the positive and negative populations. All sera which had false-positive results in the Abbott EIA also showed false-positive reactions in the Biochrom EIA, but not vice versa.

Our results are in contrast to the high specificities of HTLV immunoassays reported elsewhere (3, 5, 6). One possible explanation for these differences could be the relatively low number of Western blot-confirmed sera analyzed in our study and/or the fact that only EIA reactive sera were investigated with the aid of Western blot. Working conditions in our Brazilian laboratory, however, cannot be cited as an explanation, given that independent tests in a state-of-the-art laboratory in Germany gave similar results. In our view, the most likely explanation is that the Brazilian sera (and perhaps sera from other tropical countries as well) generally produce higher backgrounds in commercial immunoassays, thus resulting in more false-positive reactions. We recommend a re-calcualtion of the cut-off value by laboratories in different countries using

the methods described above. The requisite investigations can be carried out easily, without performing further experiments, and the results will show whether or not adjustments need to be made for a given population in a particular geographical region.

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