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EVALUATION OF ENZYME-LINKED IMMUNOSORBENT AND ALTERNATIVE ASSAYS FOR DETECTION OF HIV ANTIBODIES USING PANELS OF BRAZILIAN SERA

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SUMMARY

Sera from 472 Brazilian subjects, confirmed to be either positive or negative for HIV antibodies and comprising the total clinical spectrum of HIV infection, were utilized in the evaluation of six commercially available enzyme-linked immunosorbent assays (ELISA), as well as of four alternative assays, namely indirect immunofluorescence (IIF), passive hemagglutination (PHA), dot blot and Karpas AIDS cell test. The sensitivities ranged from 100% (Abbott and Roche ELISA) to 84.2% (PHA) and the specificities ranged from 99.3% (IIF) to 80.2% (PHA). The sensitivity and specificity of the PHA and the sensitivity of the Karpas cell test were significantly lower than those of the other tests.

Although the IFF and dot blot had good sensitivities and specificities, the six ELISA were more attractive than those tests when other parameters such as ease of reading and duration of assay were considered.

KEY WORDS: Quality control; HIV antibody tests; Brazilian sera.

INTRODUCTION

Infection with human immunodeficiency virus (HTV) has a worldwide distribution and great efforts are being made to restrict its dissemination. These efforts are met by the development of laboratory tests for detection of antibodies to HIV, which are particularly important in the screening of samples in blood banks. Therefore, the quality of the tests needs to be continuously assessed, as it has been done for some of them, in most cases disclosing, acceptable sensitivities and specificities[2, 8, 10]. However, there are no data available on the quality control of the assays utilized in Brazil, although the use of reliable kits is of great importance due to the high proportion of AIDS cases transmitted by blood or blood products in Brazil (819 out of a total of 83907 AIDS cases until September 1989[7]).

In this paper we compared the sensitivities, specificities and ease of performance of different serologic tests for HIV, some of which that still have not been subjected to similar evaluations, in panels of Brazilian sera.

MATERIAL AND METHODS

Sera. Two panels comprising the total clinical spectrum of HIV infection were utilized. The first panel (A) comprised 292 sera including 27

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sera from AIDS patients, 17 sera from patients with AIDS-related complex, 35 sera from patients with persistent generalized lymphoadenopathy, 68 HIV-antibody positive sera from asymptomatic persons and 145 negative sera from healthy individuals. The second panel (B) comprised 180 sera including 90 positive sera from asymptomatic blood donors and 90 negative sera from healthy individuals. The clinical forms of HIV infection were determined in accordance with CDC's criteria[1]. All positive sera produced positive reactions in both indirect immunofluorescence[11] and Western blot[12] tests. Negative sera did not produce positive reactions in any of the two tests. The panels were maintained at -20°C unitl tests were performed.

Indirect immunofluorescence. This test was carried out using both infected and uninfected H9 cells, kindly provided by Dr. R. C. Gallo (National Institute of Health, Bethesda, USA). An HIV-1 infected cell suspension was mixed with an equal part of an uninfected cell suspension. Glass slides containing the acetone-fixed cells were incubated with the test sera at a 1:8 dilution for 60 minutes at 37°C. After three washings with 0.15 M phosphate buffered saline, pH 7.2, (PBS) the slides were incubated at room temperature with a fluorescein-labelled anti-human IgG (Biolab-Merieux, Rio de Janeiro, Brazil) for 60 minutes. Slides were then rinsed three times, five minutes each, with PBS, and read on a Leitz epifluorescence microscope. Results were regarded as positive when approximately 50% of the cells showed a fluorescence pattern specific for HIV-1[11].

Western blot. A commercially available Western blot assay (Du Pont Company, Wilmington, Delaware, USA), licensed by USA Food and Drugs Administration, was used in accordance with manufacturer's instructions. Briefly, sera were considered positive when they reacted with at least to one band corresponding to each one of the structural genes (env, pol and gag). This criterium is recommended by the World Health Organization[14] and it has been previously demonstrated to be suitable for Brazilian AIDS patients[5].

Kits. The following kits were evaluated: ELISA, Abbott Diagnostic Products (Irving, Texas, USA), Roche Diagnostica (Basel, Switzerland), Hoechst Enzygnostic (São Paulo, São Paulo, Brazil), Virgo Electronucleonics (Columbia, MD, USA), Organon Teknika (Boxtel, The Netherlands), Salck Indústria e Comércio de Produtos Biológicos (São Paulo, São Paulo, Brazil); passive hemagglutination, PHA, Salck Indústria e Comércio de Produtos Biológicos (São Paulo, São Paulo, Brasil); indirect immunofluorescence, IIF, Virgo Electronucleonics (Columbia, MD, USA); dot blot, Embrabio, Empresa Brasileira de Biotecnologia Ltda. (São Paulo, São Paulo, Brazil); and Karpas AIDS cell test, Fujichemical Industries Ltd. (Chokeiji, Takaoka, Japan). The tests were done according to manufacturers' instructions and the parameters evaluated were the sensitivity, specificity, duration of the assay, equipment requirements and serum dilutions.

Statistical analysis. For panel A and B it was calculated the sensitivity (true positives/true positives plus false negatives), specificity (true negatives/true negatives plus false positives) and their 95% confidence intervals. The chi-square test was used to calculate the differences in frequencies of false-positive (false-negative) test results between the kits. Goodman statistic was used in multiple comparisons of proportions.

RESULTS

As shown in table 1 and 2, respectively, the sensitivities ranged from 100% (Abbott ELISA and Roche ELISA) to 84.2% (Salck PHA) and specificities ranged from 99.3% (Virgo Electronucleonics IIF) to 80.2% (Salck PHA). There were no significant differences in the sensitivities and

TABLE 1
Sensitivities and specificities of seven kits for detection of HIV antibodies using panel A.

Kit	95% confidence interval			
	Sensitivity		Specificity	
ELISA Roche	100%	(0.97-1.00)	94.6%	(0.89-0.98)
ELISA Abbott	100%	(0.97-1.00)	96.0%	(0.91-0.99)
ELISA Hoechst	99.3%	(0.95-1.00)	98.0%	(0.94-1.00)
ELISA Organon	99.3%	(0.95-1.00)	98.6%	(0.94-1.00)
ELISA Virgo	98.0%	(0.94-1.00)	98.0%	(0.94-1.00)
IIF* Virgo	99.3%	(0.95-1.00)	99.3%	(0.95-1.00)
Dot blot Embrabio	98.6%	(0.95-1.00)	98.0%	(0.94-1.00)

*IIF = indirect immunofluorescence.

TABLE 2
Sensitivities and specificities of three kits for detection of HIV antibodies using panel B.

Kit	95% confidence interval			
	Sensitivity		Specificity	
ELISA Salck	98.0%	(0.92-1.00)	97.2%	(0.92-1.00)
Karpas cell test	91.2%	(0.84-0.97)	85.7%	(0.77-0.93)
PHA* Salck	84.2%	(0.75-0.91)	80.2%	(0.71-1.00)

*PHA = Passive hemagglutination assay.

specificities among the ELISA, dot blot and IIF when assessed in panel A (chi-square = 6.05, P = 0.4179). On the other hand, the sensitivity of the PHA was significantly lower than those of the ELISA Salck and Karpas AIDS cell test as assessed in panel B (Goodman test = 21.53, P < 0.05). The specificities of the PHA and Karpas AIDS cell test were also significantly lower than that of the ELISA (Goodman test = 21.53, P < 0.05 and Goodman test = 11.78, P < 0.05, respectively). The convenience and ease of performance for each assay is shown in table 3. Although costs were approximately the same for all kits (data not shown), there were differences in the equipment requirements amongst the assays, such as for incubators, shakers, washers, readers, etc. In this respect, the simplest tests evaluated by us were the dot blot assay, PHA and Karpas AIDS cell test. The Hoechst ELISA has the advantage of utilizing undiluted serum samples.

TABLE 3
Convenience and ease of performance of the kits.

Kits	Time required +	Equipment	Serum dilution
ELISA Roche	180 min.	1, 2, 3, 7	1:100
ELISA Abbott	150 min.	2, 3, 7, 8	1:100
ELISA Hoechst	120 min.	1, 2, 3, 7	—
ELISA Virgo	120 min.	1, 2, 3, 7	1:100
ELISA Organon	120 min.	1, 2, 3, 7	1:100
IIF* Virgo	120 min.	1, 4, 5	1:20
Dot blot Embrabio	16-18 h.	4, 7	1:100
ELISA Salck	120 min.	1, 2, 3, 7	1:100
PHA** Salck	90 min.	1	1:12
Karpas cell test	90 min.	4, 6	1:20

1. Incubator, 2. Washer, 3. Reader, 4. Shaker, 5. Fluorescence microscope, 6. Bright field microscope, 7. Aspirator device, 8. Water bath.
+ Time required for carrying out the assay (not for interpretation of results)
*IIF = indirect immunofluorescence.
**PHA = passive hemmaglutination.

DISCUSSION

The most desirable characteristic of an assay for detection of HIV antibodies in screening of blood or organ donations is to have a sensitivity high enough to detect all true positive sera. The Western blot technique for HIV-antibody detection has been considered a gold standard[13, 14]. In addition it has been demonstrated that IIF has very good sensitivity and specificity[3, 11]. Therefore, in the panels used in the present work, we considered as true positive those sera that were concomitantly positive in both Western blot and IIF. All assays tested herein, with exception of the PHA and Karpas cell test, have appropriate sensitivities. Minor differences observed amongst these assays were probably due to faults of the operators or of the instruments utilized. On the other hand, the specificity is also important, since an assay leading to a high number of false-positive sera unduly prevents the use of some donated blood samples, therefore increasing the cost of the transfusion. In addition, costly confirmatory tests would be used unnecessarily.

The Abbott ELISA, which utilizes an **Escherichia coli** recombinant antigen, was not more specific than other assays which utilizes sucrose-gradient purified viruses as antigens such as Virgo Electronucleonics, Hoechst or Organon ELISA. This fact is in disagreement with a previous report of higher sensitivity and specificity of the recombinant assays[9].

In this study, some alternative assays in relation to the conventional ELISA such as PHA and Karpas AIDS cell test, that may have great potential, were also evaluated. They showed low sensitivities and specificities and need to be improved.

For countries with few resources, the other important parameters which should be taken into account in an assay for routine use are the costs and the equipments required for performance of the assay. In this respect, the dot blot assay, although utilizing the simplest methodology for performance, reading and interpretation of the results, requires a relatively long time to be performed, which precludes their routine use in the screening of blood samples, but we think

that the time required for its performance could be reduced. Indeed, we developed a similar dot enzyme immunoassay which could be performed in four hours[6].

The IIF had very good sensitivity and specificity values, but this assay has some constraints, such as need for fluorescence microscope, long time for evaluation, hard labour as well as subjective interpretation. However, in countries where IIF tests are already used in government blood transfusion centres to avoid transfusional Chagas' disease and malaria (e.g. Brasil), this assay has an inestimable value. Indeed, in 1985 we carried out a serological survey, in which 11.000 sera from Brazilian donors were tested by IIF, finding a very good correlation of this assay and the Western blot assay[4]. Unfortunately, due to several reasons, mainly the lack of a political decision, a good opportunity to produce IIF tests in large scale to be used in blood banks, which could have saved important Brazilian government resource was lost.

For a better evaluation of the sensitivity of assays it is necessary to increase the number of sera in the panels as well as to include borderline and early conversion sera. Because of the high incidence in Brazil of tropical endemic diseases such as Chagas' disease, malaria, leishmaniasis, hepatitis and leprosy, some of them inducing polyclonal B cell activation, it is also necessary to test the assays with panels constituted by such sera in order to make a more detailed evaluation of the specificity of the kits. However, in a preliminary evaluation employing 50 sera, we did not observed any interference using sera from patients with these diseases (IVO-DOS-SANTOS et al., unpublished data). Other parameters which need to be evaluated is the lot-to-lot variation of the kits as well as the proficiency. Finally, to facilitate and expand routine HIV-antibody screening in blood banks of developing countries it is still necessary to develop tests which would be at the same time quick, stable and reliable.

RESUMO

Avaliação de testes sorológicos para a detecção de anticorpos anti-HIV em painéis de soros de brasileiros.

Os soros de 472 brasileiros, confirmados como sendo positivos ou negativos em relação à presença de anticorpos anti-HIV e compreendendo todo o espectro clínico da infecção, foram utilizados na avaliação de seis ensaios imunoenzimáticos comerciais (ELISA), bem como de quatro testes alternativos tais como imunofluorescência indireta (IFI), hemaglutinação passiva (HP), dot blot e Karpas AIDS cell test. As sensibilidades variaram de 100% (ELISA Abbott e Roche) a 84,2% (HP) e as especificidades variaram de 99,3% (IFI) a 80,2% (HP). A sensibilidade e especificidade da HP e a sensibilidade do Karpas AIDS cell test foram significativamente menores que os outros ensaios.

Embora a IFI e o dot blot tivessem apresentado uma boa sensibilidade e especificidade, os ensaios imunoenzimáticos (ELISA) foram mais adequados para serem utilizados em triagem quando outros parâmetros tais como facilidade de leitura e interpretação dos resultados e duração dos ensaios foram considerados.

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