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EVALUATION OF EIA KITS FOR THE DETECTION OF HIV ANTIBODIES

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Summary. — We compared four commercially available enzyme immunoassay (EIA) kits for the detection of HIV antibodies using immunoblot analysis as a confirmatory test. The kits gave satisfactory results as far as sensitivity and specificity are concerned as required for the use in the laboratories of blood banks. For the sera of patients on haemodialysis, haemophiliacs, patients "under observation" for AIDS and homosexuals the results obtained by Behring and Organon kits were less satisfactory, as the number of false positive results was much higher than with kits produced by Genetic and Wellcome. The frequency of false negative results was small in the tests using Organon and Genetic kits, while using Behring and Wellcome kits no false negative results were found.

Key words: HIV, laboratory diagnosis of AIDS, enzyme immunoassay

Introduction

Commercial enzyme immunoassay (EIA) kits for the detection of antibodies against human immunodeficiency virus (HIV) are available since March 1985. They were developed in the shortest possible time, mostly to be used at blood banks in the USA and Europe. At the beginning, the standards of quality have not been clear, especielly as different groups of serum samples from risk groups and blood donors were tested.

We compared four commercial kits for EIA in six different groups of sera with concomitant immunoblot analysis as a confirmatory test. The tests have been performed in routine virus laboratory having some experiences in EIA for diagnosis of viral infections.

Materials and Methods

The sera tested were divided into following groups: sera of the healthy personnel tested routinely as a prophylactic measure since the AIDS cases started to appear (435), sera from persons belonging to high risk groups* (130), sera of haemofiliacs (57), sera from patients treated regularly at the haemodialysis unit of the hospital (277), sera of patients without diagnosis of AIDS but tested as "under observation" (126) and some sera of patients with AIDS.

* homosexuals, drug addicts and their sex partners

Table 1. False results obtained with commercial kits as related to immunoblot analysis

Serum group	BEHRING		ORGANON		GENETIC		WELLCOME	
	false pos. %	false neg. %	false pos. %	false neg. %	false pos. %	false neg. %	false pos. %	false neg. %
Health personnel	0.23	—	—	—	—	—	—	—
High risk persons	3.15	—	6.92	0.77	—	—	—	—
Haemophiliacs	7.02	—	10.53	1.75	3.50	—	—	—
Patients on dialysis	18.41	—	13.72	—	6.86	1.08	—	—
Patients "under observation"	9.52	—	13.49	—	7.14	—	—	—
patients with AIDS	—	—	—	—	—	—	—	—
Total of sera	7.1	0	6.8	0.19	3.0	0.29	0	0

EIA. We used four commercial test kits: Behring Enzygnost-Anti-HTLV-III (Behring Werke AG), Genetic Systems LAV EIA™ (Genetic System Corporation), Vironostika Anti-HTLV-III Microelisa System (Organon Teknika), and Wellcozyme Anti-HTLV-III (Wellcome Diagnostics), as these were the most easily available for us in 1985 and 1986.

All the tests were carried out according to the instructions of the procedurs. Among the kits tested only the Wellcome test was a competitive EIA. In this test the viral antigen is captured by human anti-HIV antibodies coated on the solid phase. In all other EIAs, virus antigen is immobilized on the solid phase. Bound anti-HIV antibodies are detected by means of enzyme labelled antibodies against human immunoglobulin.

The serum samples were not heat inactivated.

Immunoblot technique. We used the method of electrophoresis of viral antigens in polyacrylamide gel developed by Laemmli with transfer to nitrocellulose paper (Johnstone and Thorpe, 1985), an incubation with tested serum and visualization of the reaction by the immunoenzyme method. Proteins from purified, disrupted HIV grown in the H9 cell lines, which were kindly provided by Dr. Gallo, served as antigens for the immunoblot analysis. The serum samples were regarded positive for anti-HIV antibodies if a precipitation line was observed with $p24^{gag}$ and/or gp 41^{env} accompanied by other bands previously associated with the virus (Esteban, 1985).

Results

Table 1 shows that there were no false negative reactions with Behring and Wellcome tests. Organon kit failed to detect one sample in the high risk group and one sample in the group of haemophiliacs. Genetic kit missed three samples from patients on dialysis.

Significantly more false positive results were found for the sera of patients on haemodialysis, patients "under observation" and haemophiliacs with

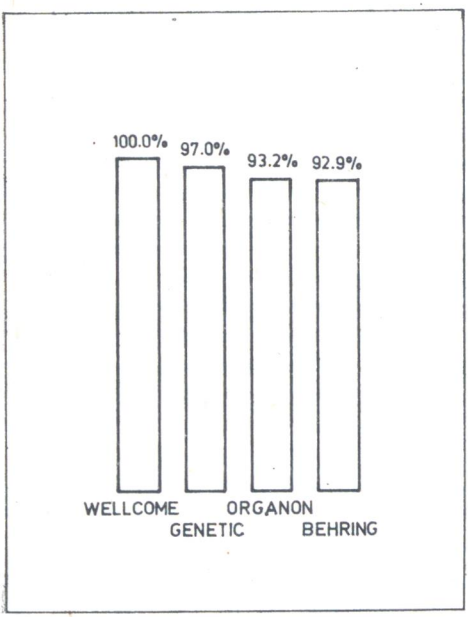

Fig. 1.
Evaluation of the specificity of commercial EIA among all sera tested.

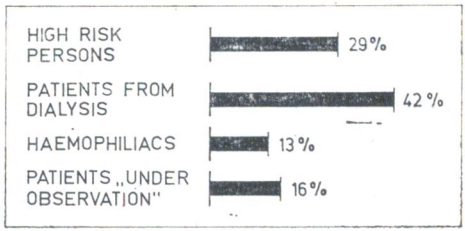

Fig. 2.
Confirmation by immunoblotting analysis of inconclusive anti-HIV antibody results in the sera of tested groups of patients.

Behring and Organon kits than with the Genetic kit. Wellcome tests scored no false positive reactions.

On the basis of these results in the sera of all groups tested we calculated the sensitivity (true positives/true positives plus false negatives) and specificity (true negatives/true negatives plus false positives) of the kits used (Reesink *et al.*, 1986). Evaluation of specificity of commercial EIA is shown on the Fig. 1. The sensitivities ranged from 99.7% for Genetic and 99.8% for Organon to 100.0% for Behring and Wellcome kits.

Immunoblot analysis was used as a confirmatory test for some clearly negative serum samples with EIA from each serum group for the confirmation of clearly positive serum samples with EIA and for the serum samples which have given previously inconclusive results by EIA. Fig. 2 shows the contribution of immunoblot analysis for the confirmation of inconclusive or false positive results obtained by EIA for individual groups of the serum samples.

Immunoblot analysis did not give positive results in two cases which have been found positive by EIA. The first was a serum of a haemophiliac and the second a serum of patient with the symptoms of AIDS.

Discussion

Our results are in agreement with recently published study of an evaluation of commercial EIA kits (Reesink *et al.*, 1986). The difference is that we detected somewhat higher frequencies of false positive results for the group of serum samples belonging to patients treated on dialysis, haemophiliacs, patients "under observation" and persons from the high risk groups. The difference could be explained by a greater number of serum samples which were sent to our laboratory for confirmation as they had been reactive already at least in one EIA.

It is well known that in the groups tested, the antigenic relationship between HLA-DR4 and HIV stresses the importance of the final interpretation of positive results obtained by EIA (Kühln *et al.*, 1985; Mortimer *et al.*, 1985) as the sera of persons positive for DR4 and, with a greater probability, even the sera of members of risk groups also show antibodies for HIV. It is, therefore, necessary to include a confirmatory test such as immunoblot analysis or other tests with a higher specificity as that of EIA (Weiss *et al.*, 1985).

On very rare occasion also immunoblot analysis can miss the presence of HIV antibodies. In the study of Ward *et al.* (1986) in which 67 190 sera have been tested, two sera were found positive by EIA, but negative in immunoblot analysis although HIV had been isolated. Biberfield *et al.* (1986) discovered among the blood donors three false positive sera obtained by immunoblot analysis.

Our results indicate that all the commercial kits for EIA are of good quality, but Behring and Organon kits are less reliable as are the kits produced by Genetic and Wellcome (in this case as competitive EIA).

We believe that for the confirmatory tests at least two different EIA kits should be used, first a non competitive and then a competitive test in addition to immunoblot analysis.

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