

Detection Methods of Anti-human Immunodeficiency Virus Antibody — Comparative study —

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INTRODUCTION

For the screening of human immunodeficiency virus (HIV)-infected individuals, a number of the enzyme immunoassay (EIA) kits have been developed. These EIA with indirect method (the first generation kit) are used for the detection of anti-HIV antibodies.

Recently, Hoechst Co. developed a new EIA kit, Enzygnost Anti-HIV micro (the second generation kit). The assay is based on the competitive activities of a serum sample with the enzyme-labeled anti-HIV antibodies reactive to the HIV antigens fixed on the solid phase. The presence of the antibodies in the sample will result in the lower optical density (OD) value. On the other hand, Fujirebio Co. developed a gelatin-agglutination method, Serodia-HIV¹⁾. The gelatin particles coated with the solubilized HIV antigens are simply added into the serum sample, and then the pattern of the agglutination is macroscopically examined.

We report the specificity and the reproducibility of these two methods²⁾ in comparison with the indirect immunofluorescent (IF) assay.

MATERIALS AND METHODS

The donors of serum samples are listed in the Table 1 and 2. It has to be mentioned that the healthy carriers of human T-lymphotropic virus type I and the adult T-cell leukemia patients are included in the "virus-infected patients" and the "malignancy patients", respectively. The sera were used without the heat-

Table 1. Detection of Anti-HIV Antibodies by Enzygnost Anti-HIV micro

Sample from	IF	Enzygnost Anti-HIV micro
Hemophiliacs	34/62*	34/62
Hemodialysis pts.	0/76	0/76
Blood donors	0/126	0/126
Virus-infected pts.	0/80	0/80
Jaundice pts.	0/9	0/9
Malignancy pts.	0/20	0/20
SLE pts.	0/32	0/32
RA pts.	0/15	0/15
Others	0/8	0/8
Total	34/428	34/428

* No. positive/No. tested

Table 2. Detection of Anti-HIV Antibodies by Serodia-HIV

Sample from	IF	Serodia-HIV
Hemophiliacs	22/48*	22/48
Hemodialysis pts.	0/77	0/77
Blood donors	0/135	1/135
Virus-infected pts.	0/93	0/93
Jaundice pts.	0/9	0/9
Malignancy pts.	0/56	0/56
SLE pts.	0/18	0/18
Total	22/436	23/436

* No. positive/No. tested

inactivation unless specified.

The IF assay was performed as described by Katamine et al³⁾. Briefly, CD4⁺ T-cells infected with lymphadenopathy associated virus⁴⁾, a strain of HIV, were smeared on a glass slide, dried at room temperature (RT)

and fixed in acetone. Fixed cells were treated with serum samples, diluted in phosphate-buffered saline to 1/10, at 37°C for 30 min. After washing, cells were stained with rabbit anti-human IgG conjugated with fluorescein isothiocyanate.

Enzygnost Anti-HIV micro kit was used according to the manual (Fig. 1). 25 μ l of serum sample and 100 μ l of peroxidase-labeled anti-HIV antibodies were put together into the antigen-fixed wells and incubated at 37°C for 60 min. After washing, 100 μ l of chromogen/substrate buffer were added, reacted at RT for 30 min, and the reaction was stopped by adding 100 μ l of 0.5N H₂SO₄. Thus, the value at OD₄₅₀ was measured. If the sample contains the specific antibodies, the reaction of the enzyme-labeled antibodies with HIV antigens is competitively inhibited, thus the OD value will become lower.

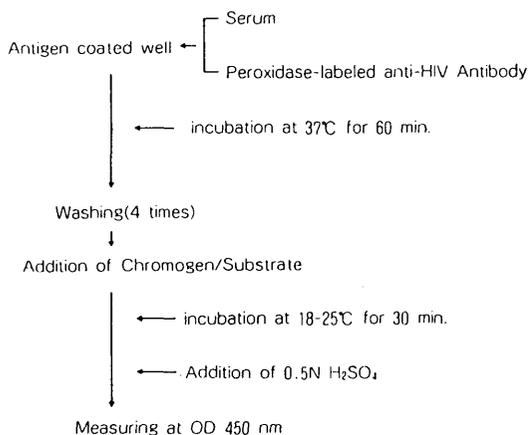


Fig. 1 Procedure of Enzygnost Anti-HIV micro

Serodia-HIV kit was also used according to the manual (Fig. 2). 75, 25 and 25 μ l of the serum-dilution buffer were poured into the 1st to the 3rd well of U-plate, respectively. 25 μ l of a serum sample were added into the 1st well and two-fold dilution by 25 μ l was done by using the 2nd and the 3rd wells to make the serum dilution of 1:8 and 1:16 in the 2nd and the 3rd well, respectively. Each 25 μ l of the non-sensitized and the sensitized (antigen-coated) gelatin-particles was added into the 2nd and the 3rd wells, respectively. After shaking to make a homogeneous suspension, the plate was left for 2hrs at RT, and then the ap-

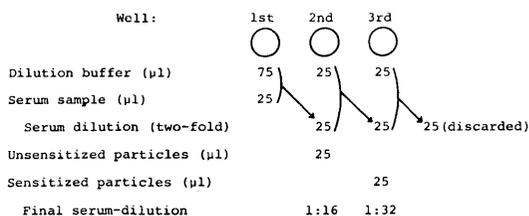


Fig. 2 Procedure of Serodia-HIV

pearance of the agglutination-pattern was examined.

RESULTS

The results are shown in the Table 1 and 2. In Japanese hemophiliacs, 34 out of 62 in Table 1 and 22 out of 48 in Table 2 were positive for anti-HIV antibodies by IF assay. These were confirmed by the radioimmunoprecipitation⁵⁾. The remaining samples which included those from patients with systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) as the autoimmune diseases were seronegative.

As shown in Table 1, the results obtained by the Enzygnost Anti-HIV micro kit were completely coincident with those of IF assay. None of false positive or false negative case was observed. The results by Serodia-HIV kit were also coincident with those of IF (Table 2). Only one case of healthy blood donor, however, was judged as false positive.

The distribution of the cut off index (COI: OD value/cut off value) measured by Enzygnost Anti-HIV micro was shown in Fig. 3 and 4. COIs in the almost all seropositive cases were lower than 0.06 (Fig. 3) and those in the seronegative cases were higher than 1.50 (Fig. 4). There was no case which gave COI around 1.0, thus indicating the clearness of the obtained results.

The simultaneous reproducibility was examined by measuring the same samples at the same time by two independent researchers. The reproducibility at an interval of several weeks was also tested. Since the results in the both kits were within the normal range of variation (less than twice in the antibody titer in Serodia-HIV kit and less than 0.45 of COI in Enzygnost Anti-HIV micro), it was concluded that

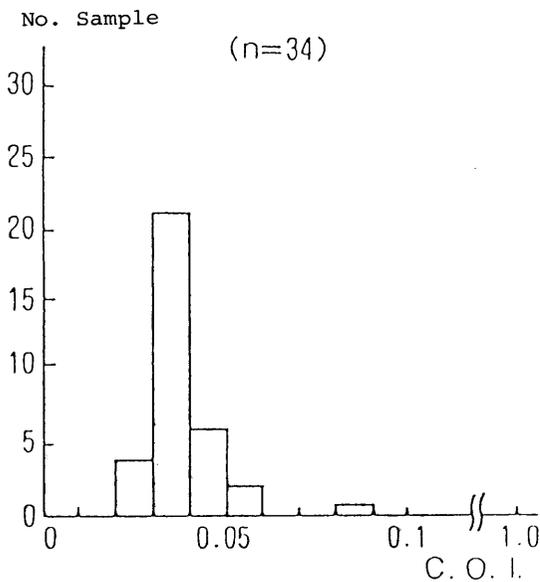


Fig. 3 COI Distribution of Seropositive Samples

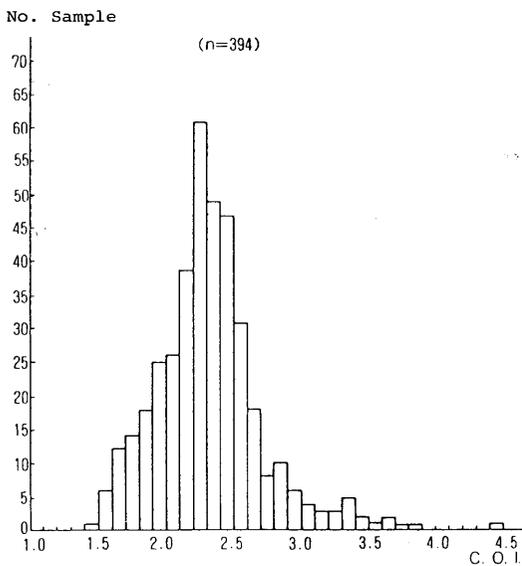


Fig. 4 COI Distribution of Seronegative Samples

those were satisfactorily reproducible.

In order to test a possible influence of the heat-inactivation of the sera, the sero-positive and -negative samples were examined by both kits after heating them at 56°C 30min, because there has been the seroconversion from negative to positive after heating in other EIA kits. In Enzygnost Anti-HIV micro, the results were

not significantly different before and after heat-inactivation. In Serodia-HIV, however, in many seropositive samples, the antibody titers after heating became more than 64 times higher than those before treatment. There was no conversion from seronegative to seropositive case by the treatment.

DISCUSSION

Although the number of samples was limited, both kits were very specific and useful for detecting anti-HIV antibody as far as we tested Japanese sera. We experienced in the past by the other assays that the sera from patients with autoimmune diseases frequently showed false positivity. But there was no such a case in these new methods.

The agglutination method (Serodia-HIV) showed the increased antibody titers after heating the seropositive samples. Therefore, it is necessary to take this matter into consideration when not only the inactivated sera are tested, but also the antibody titers of different samples are compared.

The procedure of Serodia-HIV kit is very simple and this method is thrifty. We can carry out the test wherever we want, but it takes 2hrs for judgement. The procedure of Enzygnost Anti-HIV micro kit is also very simple and the result is very clear-cut. It takes only 1.5hr for judgement, because one step of the reaction is omitted as compared with the 1st generation method. Therefore, we can recommend these two kits for screening of anti-HIV antibodies.

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