A RECOMBINANT IMMUNODOT ASSAY FOR DETERMINATION OF HIV-1 GP41 ANTIBODY TITRES

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The dot immunobinding assay (dot system) has been advised as an alternative method for the detection of specific antibodies against the human immunodeficiency virus (HIV)(*1*). The use of recombinant antigens reduces significantly the false reactivity rate (*2, 3*). A recombinant peptide with antigenic determinant of the HIV transmembrane gp41 (rAg) with molecular weight of 21 kD produced in *E. coli* was described recently (*4*).

The test utilizes immobilized antigen on nitrocellulose paper membrane BA85 (NCP) (Schleicher and Schuell) using the Minifold-Vakuum-Filtrationsystem apparatus (Schleicher and Schuell). The optimal rAg concentration was estimated in box titrations with affinity purified anti-HIV human IgG. The presence of antibody bound to the rAg was detected by peroxidase labelled anti-human IgG antibodies. We have examined 10 human sera, diluted in 2-fold steps starting from 1:2, containing specific antibodies against HIV-1 envelope and core proteins, as previously shown in a commercial recombinant enzyme immunoassay (ENVACOR HIV-1, Abbott). From these 5 sera gave strong signals (with titres ranging 1:32 - 1:128) with the control antigen masking the HIV-1 reactivity (*5*), which was removed by absorption to a dense bacterial suspension (5×10^9 bacteria/ml). The differences between the signals obtained with the rAg and control antigen (Table) became thus more pronounced ($P<0.001$), but specific antibody titres were not decreased. The human sera analyzed by the dot immunobinding assay were further evaluated in indirect immunofluorescence test (IF). The antibody titres observed in the dot system and IF correlated ($r=0.72$).

The sensitivity of the described dot system seems as comparable with reported sensitivity of commercially available ELISA (*5, 6*) but slightly less sensitive than e. g. the Bio-Enzabead (Organon-technika) (*5*). The described simple and sensitive method has an additionall advantage that the NCP with rAg can be prepared and stored more than 6 month with no appreciable deterioration of immunoreactivity (*7*).

Table. Levels of gp41 antibodies in human sera estimated by immunodot assay

n	gp41 recombinant antigen		non-recombinant *E. coli* antigen	
	non-absorbed	absorbed	non-absorbed	absorbed
10	337.8[a]	548.7[b]	26.0[c]	5.3[d]

Each value represents the geometric mean of antibody titres.
$P<0.001$: a vs c; b vs d; $P<0.05$: c vs d; $P>0.05$: a vs b (Student's test).
For absorption the suspension of non-recombinant *E. coli* bacteria was used.

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