Haematology and Blood Transfusion Vol. 31
Modern Trends in Human Leukemia VII
Edited by Neth, Gallo, Greaves, and Kabisch

Improvement of HIV Serodiagnosis

I. Wendler [1], J. Schneider [1], F. Guillot [1], A. F. Fleming [2], and G. Hunsmann [1]

Summary

Experience is described with four different assays to detect antibodies against the HIV. ELISA tests using HTLV-III from two different cell lines or the bacterially synthesized envelope peptide ENV(80)-DHFR were compared with the confirmatory immunoprecipitation assay. A total of 831 sera – 678 from Germany and 155 from Zambia – were examined. The specificity of the ENV(80) ELISA was found to be superior to the two virus ELISAs and equivalent to the immunoprecipitation. The diagnostic value of the ENV(80) ELISA test was confirmed with European and African sera.

Serological assays for the detection of antibodies to the human immunodeficiency virus (HIV), earlier named LAV, human T-lymphotrophic virus (HTLV)-III, or ARV, are important for identifying individuals infected with such a virus and for epidemiological research on the acquired immunodeficiency syndrome (AIDS) and related diseases. Four different methods are widely used to determine HIV antibodies.

In the enzyme-linked immunosorbent assay (ELISA), antigen adsorbed to beads or wells of microtitre plates is incubated with sera to be tested. Bound immunoglobulins are detected by a second enzyme-linked anti-antibody. The amount of first antibodies bound is quantitated by the colour-producing enzyme reaction. Numerous commercial ELISAs are available.

For Western blotting, viral proteins are separated electrophoretically in the presence of sodium dodecyl sulfate (polycrylamide gel electrophoresis) (PAGE) and transferred to a nitrocellulose membrane. Antibodies binding to viral proteins on the membrane are visualized with an anti-antibody linked to an enzyme catalysing a colour reaction.

In the immunofluorescence assay, virus-producing or non-infected cells as negative controls are fixed onto slides and then incubated with patients' sera. Subsequently, antibodies recognizing viral proteins are visualized under a fluorescence microscope with a second fluorescein-labelled anti-antibody.

For immunoprecipitation (IP), lysates of virus-producing cells labeled with radioactive amino acids are incubated with patients' sera. The immunocomplexes are collected by adsorption to protein A sepharose and subsequently separated by PAGE. Precipitated radiolabelled proteins are identified by autoradiography. IP is an expensive and sophisticated assay. On the other hand, it is highly specific because native viral polypeptides are selected out of a large excess of cellular proteins and characterized by PAGE according to their molecular weight (Fig. 1).

The ELISA is most suitable for screening large numbers of sera for HIV antibodies. However, our own experience and interlaboratory tests regularly performed among German blood banks have shown that the ELISA gives a substantial number of false positive results which have to be sorted out by time-consuming confirmatory assays [1]. More seriously, up to 5% of positives may

[1] Deutsches Primatenzentrum Göttingen, Kellnerweg 4, D-3400 Göttingen, FRG
[2] Tropical Diseases Research Centre, 9, Ndola, Zambia

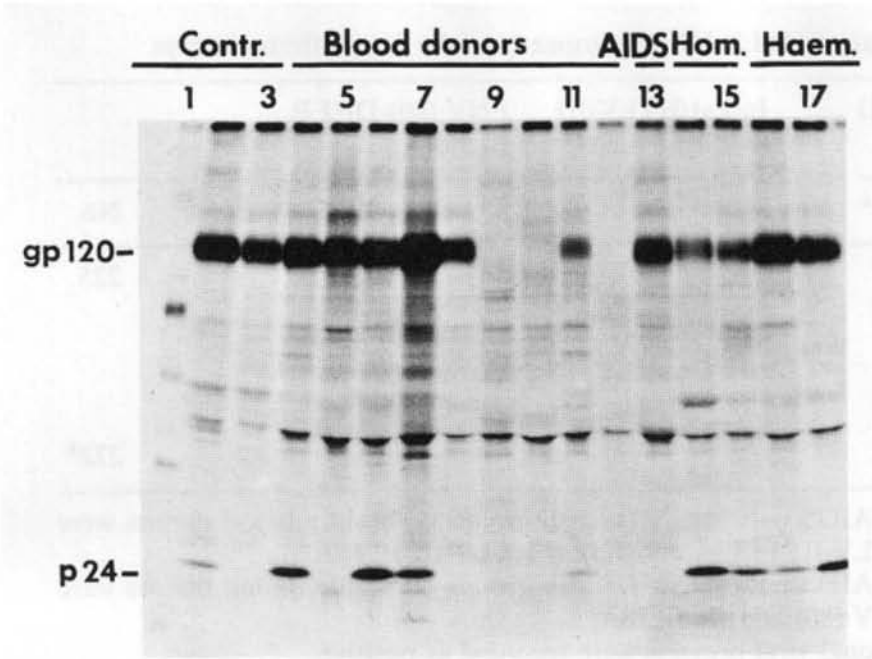

Fig. 1. Immunoprecipitations of HIV polypeptides confirm results of the ELISA. Cell extracts of H9/HTLV-III cells labelled for 4 h with ^{35}S-cysteine were reacted with ELISA-positive sera from blood donors (*4–12*), AIDS patients (*13*), homosexuals (*14, 15*) and haemophiliacs (*16, 17*). Gp120 and p24 were detected as the predominant immunoreactive polypeptides in HTLV-III infected cells

be missed in the primary ELISA screening [2]. False-positive results occur when antibodies bind to cellular antigens, e.g., histocompatibility antigens [3], or proteins derived from medium contaminating the antigen preparation. False-negative results are probably due to the low concentration of the viral membrane protein gp120 in the virus preparation. Gp120 is mainly detected by serum antibodies in the IP assay [4]. However, it is lost considerably during virus preparation and density gradient purification [5]. Therefore, sera with low titres or sera which recognize only gp120 may be missed in the screening ELISA.

To obtain a highly specific and sensitive ELISA, the essential antigenic determinants have to be presented in high concentrations free of non-viral proteins. The antigen for the ELISA and other HIV tests is most commonly derived from the H9/HTLV-IIIB cell line [6]. In our experience, virus harvested from these cultures by two sedimentation steps contains a great excess of contaminating proteins from cells and medium. We have established a cell line permanently producing HTLV-IIIB by infecting the human Jurkat mature T-cell line [7]. Jurkat/HTLV-III cells release 10–15 times more virus than the H9/HTLV-III line. Moreover, such virus preparations are less contaminated with non-viral proteins, as shown by PAGE analyses and determination of specific RT activity. To normalize ELISAs performed with different antigen stocks, the relative optical density (OD) of each serum was calculated by dividing its OD through the OD of a positive control serum run on the same plate.

A comparison of sera tested in the ELISA with antigen derived from H9/HTLV-III or Jurkat/HTLV-III has shown that the relatively pure virus from Jurkat cells allows a clearer differentiation between positive and negative sera (Table 1). ELISA results of all sera were compared with those obtained by IP. In our experience, IP is the most specific and sensitive HTLV-III antibody assay (Fig. 1). IP positive and negative sera overlap in their relative OD in the ELISA. This range extended from 0.1 to 0.25 with antigen from Jurkat/HTLV-III cells, but from 0.1 to 0.6 when H9/HTLV-III cultures were used as antigen source. Sera attaining OD values within the range of uncertainty were classified as +/−.

A pronounced enhancement of specificity and sensitivity is expected from second generation ELISA tests, which use bacterially produced antigens. In these tests, the essential antigenic determinants can be offered in great quantity and purity.

In our laboratory, the diagnostic potential of a bacterially produced peptide of the HIV *env* gene was tested. A synthetic DNA fragment of 240 base pairs coding for a peptide homologous to a conserved region of the gp41 transmembrane glycoprotein of HIV was expressed in *E. coli* as a N-terminal fu-

Table 1. Detection of antibodies against HTLV-III in European sera by four different assays

IP[a]		H9/HTLV-III ELISA[a]		Jurkat/HTLV-III ELISA[a]		ENV (80)-DHFR ELISA[b]		IP[b]	
Score[c]	No.	Score[d]	%[e]	Score[f]	%	Score[g]	%	Score	No.
+	111	+	57	+	77	+	95	+	225
		+/−	42	+/−	23	+/−	2		
		−	1	−	0	−	3		
		+	3	+	0	+	3		
		+/−	40	+/−	3	+/−	1		
−	68[h]	−	57	−	97	−	96	−	272[h]

[a] The same 179 sera derived from AIDS patients, AIDS risk groups or healthy blood donors were tested comparatively by IP, H9/HTLV-III ELISA and Jurkat/HTLV-III ELISA.
[b] The same 497 seca derived from AIDS patients, AIDS risk groups or healthy blood donors were tested comparatively by IP and ENV (80)-DHFR ELISA.
[c] Sera precipitating gp120 or additional viral proteins were recorded as positive.
[d] Sera with a relative OD below 0.1 were scored as negative. Positivity was recorded when a serum gave a relative OD of at least 0.6 (see text).
[e] Percent of IP + or − sera.
[f] Sera with a relative OD below 0.1 were scored as negative. Positivity was recorded when a serum gave a relative OD of at least 0.25 (see text).
[g] Sera with OD below 0.15 were scored as negative. Positivity was recorded when a serum gave an OD of at least 0.25.
[h] Including sera which were not tested by IP, since they gave clear negative results in other different screening assays.

Table 2. Detection of antibodies against HTLV-III in African sera by three different assays

IP		Jurkat/HTLV-III ELISA			ENV (80)-DHFR ELISA		
Score[c]	No.	Score[f]	No.	%[g]	Score[g]	No.	%
+	27	+	27	100	+	26	96
		+/−	0	0	+/−	0	0
		−	0	0	−	1	4
−	128[h]	+	2	2	+	0	0
		+/−	44	34	+/−	3	2
		−	82	64	−	125	98

For footnotes see Table 1.

sion protein to dihydrofolate reductase [8]. The resulting ENV(80)-DHFR peptide was enriched to high purity by affinity chromatography and used as antigen in an ELISA [9]. A total of 497 sera from German individuals with AIDS, from AIDS risk groups and from healthy blood donors were evaluated by IP and ELISA, using the ENV(80)-DHFR peptide as antigen. The results show that the ENV(80) ELISA is a valuable screening assay for HTLV-III antibodies (Table 1). The results of the ENV(80) ELISA and IP agreed in 95% of the sera. However, the Jurkat/HTLV-III ELISA agreed with IP in only 77%. No clear statement was possible for 2% of the sera tested with the ENV(80) ELISA. Accordingly, the latter was ten times more accurate than the Jurkat/HTLV-III ELISA. Even more uncertain and false-positive results were obtained in the H9/HTLV-III ELISA. The specificity of the ENV(80) ELISA is comparable to

that of the IP. Three per cent of the sera scoring as positive in either test were missed in the other assay. This discrepancy between ENV(80) ELISA and IP may be explained by the genomic heterogenicity found in LAV/HTLV-III variants [10–13].

The high specificity of the ENV(80) ELISA compared to the HTLV-III ELISA was also confirmed in a study comprising 155 sera from tropical Africa (Table 2). In conventional ELISAs, reactions of African sera are often difficult to interpret. The presence of high titres of antimalarial and other antibodies and immunocomplexes in these sera leads to a high frequency of false-positive and uncertain results [14, 15]. As summarized in Table 2, the ENV(80) ELISA is 96%–98% specific when applied to African sera. However, early seroconversion was obviously not detected in one patient, who had antibodies in the IP against gp120 but not gp41. HIV antibody tests which work reliably in Africa are urgently needed to assess the prevalence of viral infection and the spread of the AIDS epidemic through this continent.

Further improvement of the ENV(80) ELISA should be possible when the antigen is supplemented with peptides corresponding to other conserved regions of the viral envelope and/or core protein.

Acknowledgements. We thank H. Buss, R. Jung, A. Jurdzinski, S. Mader, D. Schreiner and S. Sievert for expert technical assistance, and C. Schalt for typing the manuscript. The gift of the ENV(80) peptide from Hoffmann-La Roche is gratefully acknowledged.

References

1. Hunsmann G, Wendler J, Schneider J (1986) The LAV/HTLV-III antibody test: methods, scoring and implications. AIFO 1:22–25
2. Schneider J, Wendler I, Bayer H, Hunsmann G (1987) Zur Bedeutung der Serumantikörperbestimmung gegen humane lymphotrope Retroviren. Ärztliches Laboratorium 33:45–47
3. Kühnl P, Seidl S, Holzberger G (1985) HLA DR4 antibodies cause positive HTLV-III antibody ELISA results. Lancet 1:122–1223
4. Schneider J, Bayer H, Bienzle U, Hunsmann G (1985) A glycopolypeptide (gp100) the main antigen detected by HTLV-III antisera. Med Microbiol Immunol 134:39–42
5. Montagnier L, Clavel F, Krust B, Chamaret S, Rey F, Barre-Sinoussi F, Chermann JC (1985) Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy-associated virus. Virology 144:283–289
6. Popovic M, Sarngadharan MG, Read E, Gallo RC (1984) Detection, isolation and continous production of cytopathic retroviruses (HTLV-III) from patient with AIDS and pre AIDS. Science 224:497–500
7. Wendler I, Jentsch KD, Schneider J, Hunsmann G (1987) Efficient replication of HTLV-III and STLV-III mac in human Jurkat T-cell cultures. Med Microbiol Immunol 176:273–280
8. Certa U, Bannwarth W, Stüber D et al. (1986) Subregions of a conserved part of the HIV gp41 transmembrane protein are differentially recognized by antibodies of infected individuals EMBO J 5:3051–3056
9. Schneider J, Wendler I, Guillot F, Hunsmann G, Galatti H, Schoenfeld H-J, Stüber D, Mous J (1987) A new ELISA test for HTLV-III antibodies using a bacterially produced viral env gene product. Med Microbiol Immunol 176:47–51
10. Wain-Hobson S, Sonigo P, Danos O, Cole S, Alizon M (1985) Nucleotide sequence of the AIDS virus. Cell 40:9–17
11. Ratner L, Haseltine W, Patarca R et al. (1985) Complete nucleotide sequence of the AIDS virus, HTLV-III. Nature 131:277–284
12. Sanchez-Pescador R, Power MD, Barr PJ, Steimer KS, Stempien MM, Brown-Shimer SL, Gee WW, Renard A, Randolph A, Levy JA, Dina D, Luciw PA (1985) Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science 227:484–492
13. Muesing MA, Smith DH, Cabradilla CD, Benton CV, Lasky DJ, Capon DJ (1985) Nucleic acid structure and expression of the human AIDS/lymphadenopathy retrovirus. Nature 313:450–485
14. Hunsmann G, Schneider J, Wendler I, Fleming AF (1985) HTLV positivity in Africans. Lancet 26:952–953
15. Wendler I, Schneider J, Gras B, Fleming AF, Hunsmann G (1986) Seroepidemiology of human immuno deficiency virus in Africa. British Med J 293:782–785