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Incidence of Anti HIV Antibodies and Viral Antigen in Standard and Control sera

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Summary: Most material used for control and calibration in a clinical laboratory is based on pool sera of human origin, guaranteed to be HBsAg-free. Since little information is available on the potential infectivity of HIV, the causative agent of acquired immunodeficiency syndrome (AIDS), 54 control and calibration sera, in routine use, were investigated for the incidence of antibodies to HIV by means of Elisa. Sixteen test specimens (= 30%) gave positive or borderline Elisa results and were further analysed by immunoblotting, resulting in 15 samples all recognizing gp 160 and partially the p 24, p 31, p 55, p 64 and gp 120 band. Only one sample with borderline Elisa result was negative by this assay. Furthermore, all sera were examined for the presence of viral antigen by a solid phase Elisa. All samples under investigation gave negative antigen Elisa results. Bearing in mind that the sensitivity of this assay is limited to 10 µg/l of viral antigen, no conclusion on infectivity should be drawn. The high incidence of HIV-antibodies in the sera investigated demands that this material should be handled with special care by laboratory personnel.

Introduction

The acquired immunodeficiency syndrome (AIDS) (1) is a rather recently described disease primarily affecting certain risk groups like promiscuous homosexuals, drug addicts and haemophiliacs who have received clotting concentrates, which have not been tested for HIV-antibodies or inactivated by heat, and the sexual partners of these groups (2–4). The causative agent has been described as a human retrovirus designated Human T lymphotropic virus type III/lymphadenopathy associated virus (HTLV III/LAV) (5, 6); in the new nomenclature it is called human immunodeficiency virus (HIV). All available evidence suggests that transmission of the virus occurs predominantly through contact with blood as well as blood derivatives. However, the virus has also been isolated from other body fluids like urine or saliva of infected persons (7).

Health workers and laboratory staff are caring for an increasing number of patients, infected with HIV, and there is concern regarding their risk of infection

from casual or percutaneous occasional exposure to the virus (8, 9). In spite of a low transmission rate of HIV infection documented within this group of persons (10, 11), the continuation of precautions regarding the handling of body fluids is recommended and emphasized. Therefore in Austria all specimens for laboratory investigations that are suspected to come from HIV-positive individuals have to be marked yellow/red, for handling with special care by laboratory staff.

For control and calibration purposes materials from human sources are generally preferred, in order to maintain the same matrix as the test specimen of interest. For this purpose most vendors provide preparations tested for HBsAg, to minimize the potential risk of hepatitis B infection. Only partial information is available on the incidence of antibodies to HIV in quality assurance sera routinely used in laboratories and their potential risk of HIV transmission. Therefore all control and calibration sera used in this laboratory were investigated for antibodies to HIV

by an enzyme linked immunosorbent assay (Elisa). Since testing for anti HIV antibodies by Elisa may give false positives, screening results have to be confirmed by immunoblot analysis. Because reactivity in these assays indicates antibody presence, but not viral infectivity, all sera were tested for the evidence of viral particles using an HIV antigen Elisa.

Materials and Methods

Quality assurance reagents

A total of 54 quality assurance reagents were obtained from the following companies:

- Abbott Laboratories, North Chicago, USA (n = 15),
- American Monitor Corp. Indianapolis, USA (n = 4),
- Beckman Instruments Inc., Brea, LA, USA (n = 5),
- Behringwerke, Marburg, FRG (n = 5),
- BIO RAD Laboratories, Richmond, LA, USA (n = 3),
- Boehringer Mannheim Diagnostics Inc., Mannheim, FRG (n = 11),
- Du Pont de Nemours Inc., Wilmington, USA (n = 5),
- Helena Laboratories, Beaumont, Texas, USA (n = 1),
- Hoffmann La Roche, Basel, Switzerland (n = 1),
- Immuno AG, Vienna, Austria (n = 2),
- Merck, Darmstadt, FRG (n = 2),
- Merz + Dade, Düringen, Switzerland (n = 2).

Elisa screening for anti HIV antibodies

All control sera and calibrators listed above were investigated by use of a Du Pont HIV Elisa kit (Du Pont). The assay was performed according to the manufacturers instructions. *o*-Phenylene-diamine was used as the chromogenic substrate, and absorbance (A) was read at 410 nm using a Dynatec Elisa reader within two hours after stopping the peroxidase reaction. All samples were tested in duplicate.

The cut off for reactivity was that recommended by the manufacturer, calculated as the mean absorbance of the positive controls divided by two (experiment 1: 0.680 A, experiment

2: 0.445 A), all values above were considered to be positive. Following the recommendations of the Paul Ehrlich Institute (Frankfurt, FRG), this range was extended, and all samples with absorbance values down to 50% beneath the suggested cut off (exp. 1: 0.340 A, exp. 2: 0.223 A) were also examined by the immunoblot technique.

Immunoblot analysis

All positive or borderline positive Elisa results were further investigated using an enzyme linked immuno electrotransfer western blot kit (Du Pont). Briefly, transblotted nitrocellulose strips were incubated overnight with the test samples diluted 1:100. Detection of anti HIV antibodies was performed with biotinylated anti human IgG H + L antibodies, followed by a further incubation step with an avidin-peroxidase conjugate. 2-Chloro-1-naphthol was used as the chromogen, giving a purple-blue reaction product.

Detection of HIV viral antigen

For the demonstration of HIV viral antigen, a sandwich solid phase enzyme immuno assay (Abbott) was performed, following the manufacturers instructions. The chromogenic substrate for the peroxidase reaction was *o*-phenylene-diamine. The cut off for reactivity was calculated by adding 0.050 to the mean absorbance value of the three negative controls as recommended by the manufacturer. All samples were tested in duplicate and absorbance values equal to or greater than the calculated cut off value were considered reactive for HIV antigen.

Results

In this study a total of 54 control sera and calibrators was tested for anti HIV antibody by means of Elisa. Sixteen sera (= 30%) gave positive or borderline positive Elisa results (experiment 1, cut off 0.680 A, extended range 0.340 A, experiment 2, cut off 0.445 A, extended range 0.222 A). Thirty eight sera

Tab. 1. Quality assurance sera with positive Elisa results by Du Pont HIV Elisa.

Manufacturer or distributor		Lot	Measured (A)	Cut off (A)	Lane no. in fig. 1
Beckman	Decision Level I	C506105	0.478	0.340	7
Beckman	Decision Level II	C506107	0.380	0.340	14
Beckman	Decision Level III	C506106	0.607	0.340	17
Behring	Kontrollogen LP	62321 OF	1.373	0.222	11
Bio-Rad	Lyphocheck II	C-370-5 11802	0.675	0.340	18
Bio-Rad	Lyphocheck III	C-370-5 11803	0.910	0.340	13
Boehringer	Precinorm U	151621-09	0.977	0.340	10
Boehringer	Precipath U	15080103-09	0.890	0.340	12
Boehringer	Precipath U	15294601-35	0.677	0.340	4
Boehringer	Precinorm U	15033803-06	1.592	0.340	6
Boehringer	Calibrator	15352702-09	1.050	0.340	15
Boehringer	Calibrator	15557303-11	0.232	0.222	19
Merz + Dade	Moni-trol IE	L + D 207	0.420	0.340	5
Merz + Dade	Moni-trol IIE	P + D 107	0.755	0.340	9
Am. Monitor	Qualify I	QN 21D	0.584	0.340	16
Am. Monitor	Qualify II	QA 30C	0.454	0.340	8

(= 70%) were considered nonreactive. Two of the 38 Elisa negative samples were equine-based but supplemented with human material. Absorbance values for positive reactive samples are shown in table 1, for nonreactive samples in table 2.

All these 16 positive or borderline positive Elisa results were further analysed by immunoblot. The results are demonstrated in figure 1. Lane 1 shows a negative control, lanes 2 and 20 positive controls, and lane 3 a weak reactive control sample. Lanes 4 to 19 show the 15 samples found to be Elisa-positive. The sera that were reactive in immunoblotting experiments can be identified in table 1 according to their lane number indicated in figure 1. Only one sample

giving a borderline Elisa result (0.232 A, cut off 0.222 A) was negative by immunoblot analysis (lane 19). All positive samples reacted strongly with the gp 160 band. Furthermore a weaker reactivity with p 24, p 55 and p 64 was observed. A considerable number of samples showed an additional p 31 band (lanes 4–6, 9, 10, 12, 13, 14, 15). All control sera and calibrators initially tested were further investigated for HIV viral antigen using the Abbott enzyme immuno assay, giving negative results for all test specimen. To investigate the sensitivity of this assay, viral antigen (Du Pont) was titrated from concentrations of 20 µg/l down to 5 µg/l. The lowest detectable concentration of viral antigen by this assay was 10 µg/l (see fig. 2).

Tab. 2. Quality assurance sera with negative Elisa results.

Manufacturer or distributor		Lot	Measured (A)	Cut off (A)
Abbott	Phenytoin H	9507-10 83-107AZ	0.103	0.340
Abbott	Phenytoin M	9507-10 83-107AZ	0.084	0.340
Abbott	Phenytoin L	9507-10 83-107AZ	0.072	0.340
Abbott	Phenobarbital H	9500-10 90-288AZ	0.065	0.340
Abbott	Phenobarbital M	9500-10 90-288AZ	0.060	0.340
Abbott	Phenobarbital L	9500-10 90-288AZ	0.064	0.340
Abbott	Digitoxin H	9535-10 86-659AZ	0.064	0.340
Abbott	Digitoxin M	9535-10 86-659AZ	0.070	0.340
Abbott	Digitoxin L	9535-10 86-659AZ	0.070	0.340
Abbott	Digoxin H	9511-10 87-353AZ	0.075	0.340
Abbott	Digoxin M	9511-10 87-353AZ	0.074	0.340
Abbott	Digoxin L	9511-10 87-353AZ	0.074	0.340
Abbott	Methotrexate H	9522-10 88-091SV	0.067	0.340
Abbott	Methotrexate M	9522-10 88-091SV	0.064	0.340
Abbott	Methotrexate L	9522-10 88-091SV	0.080	0.340
Am. Monitor	Standard 1	SL 1022 C	0.004	0.340
Am. Monitor	Standard 2	SH 1033 B	0.005	0.340
Behring	NT Protein Kontr.	06 5118	0.065	0.340
Behring	Pathoplasma I	502848 A	0.050	0.340
Behring	Pathoplasma II	502942 A	0.055	0.340
Behring	Kontrollplasma N	502788 C	0.056	0.340
Bio-Rad	Lyphocheck I	C 370-5 11801	0.170	0.340
Boehringer	Plasmacontr. Plus	175927/5269	0.050	0.340
Boehringer	Precinorm I	152287	0.010	0.222
Boehringer	Preciclot I	615382	0.012	0.222
Boehringer	Preciclot II	615382	0.005	0.222
Boehringer	Precichrom II	152419	0.006	0.222
Du Pont	Bilirubin Calibr. 1	01125 1A	0.002	0.222
Du Pont	Bilirubin Calibr. 2	01125 1B	0.003	0.222
Du Pont	Bilirubin Calibr. 3	01125 1C	0.003	0.222
Du Pont	ACA Normal	6DB621	0.002	0.222
Du Pont	ACA Elevated	6DB626	0.003	0.222
Helena Lab.	CK/LC Isoenzyme Ko	5131	0.012	0.340
Roche	Control Serum P	B06379705	0.004	0.340
Immuno	Konroll Pl. AK-D	AK 082	0.025	0.222
Immuno	Kontroll Pl. AK-R	AK 080	0.020	0.222
Merck	Seronorm TM	211025/74	0.004	0.222
Merck	Pathonorm L	212123/20	0.003	0.222

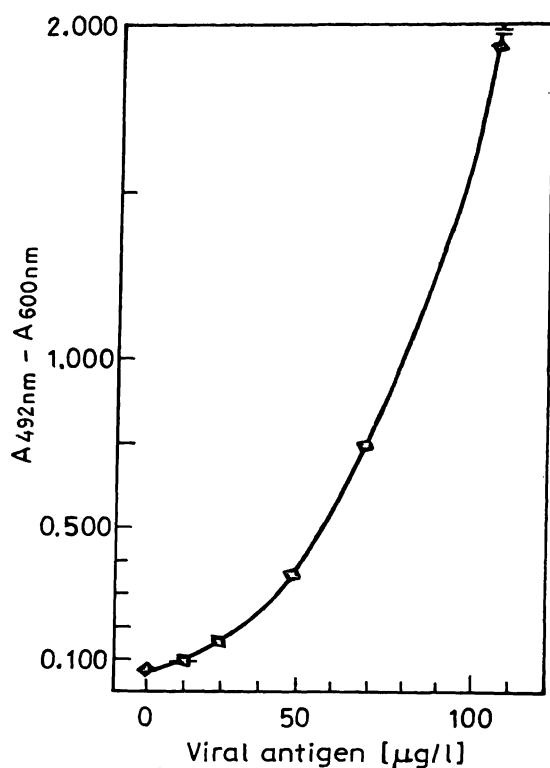


Fig. 1. Immunoblot reactivity of control and calibration sera tested in HIV antibody Elisa:
lane 1: negative control,
lanes 2, 20: positive control,
lane 3: weak positive control,
lanes 4–19: samples tested;
Individual samples may be identified in tab. 1.

Discussion

For quality assurance sera originating from human material, the number of donors may be 6000 or more. Although pools are tested for HBsAg, donors are often members of risk groups for HIV infection. Therefore 54 control sera and calibrators routinely used in this laboratory were tested for antibodies to HIV. Elisa screening gave about 30% reactive samples. These data are in agreement with *Howanitz et al.* (12), who reported the presence of HIV antibodies in about 31% of quality assurance sera on the basis of initial tests. However, 7 of 45 reactive samples in this study were nonreactive on repeated testing. In our study, 15 of the 16 positive or borderline Elisa results were confirmed by immunoblot analysis, indicating that the observed Elisa reactivity was not due to preparation artefacts. The one sample giving a negative immunoblot result had also shown absorbance values close to the extended cut off value. The fact that gp 41 could only be found in two of the 15 positively reactive sera investigated may be due to the dilution effect, since all these quality assurance materials are produced from pool sera, and this band is weaker than the others even in strongly positive cases. However, gp 160, which is known to be the

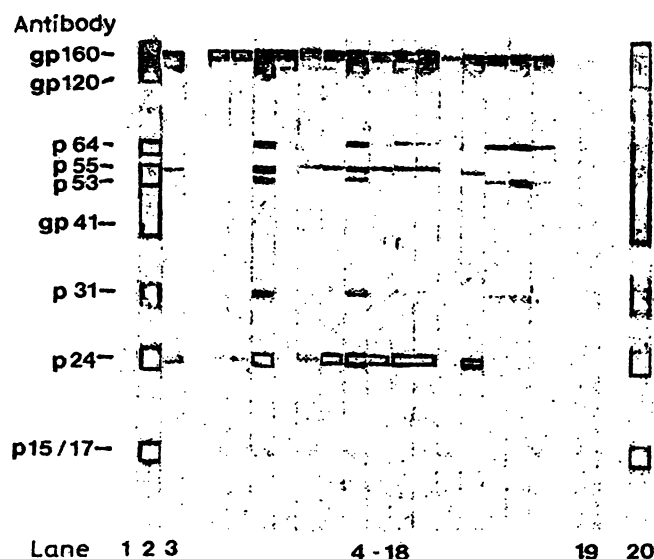


Fig. 2. Titration of viral antigen: cut off for sensitivity of the assay is 10 µg/l viral antigen. Absorbance without viral antigen 0.057.

viral protein to which the highest titers of antibodies are mounted, gave the strongest reactivity in all samples. Moreover, all 54 samples were tested for viral antigen, but no positivity was observed. However, titration of viral antigen using this Elisa procedure showed sensitivity down to concentrations of 10 µg antigen per l diluent only. Therefore no conclusions can be drawn regarding the infectivity of the samples tested, because the amount of infectious agent may be beneath the quantities detectable by the antigen Elisa.

In high percentages of haemophiliacs receiving commercial clotting concentrates which have not been tested for anti-HIV antibodies and heat inactivated, transmission of the virus has been described, resulting in the development of antibodies to HIV and exacerbation of AIDS (13, 14, 15). HIV has been isolated from peripheral blood and other body fluids of most patients with pre-AIDS, and from a considerable number of patients with the fully developed picture of AIDS. The virus has been isolated from lyophilized factor VIII concentrates (16), indicating that the virus is resistant to the lyophilization procedure.

These results strengthen the case for handling all control sera with special care, and according to the safety precautions against hepatitis B infection, which will also protect against HIV infection (17). Since methods are now available for testing sera for the presence of anti HIV antibodies, and, furthermore, rather mild procedures for inactivating the virus have been established (18), vendors should be obliged to provide control and calibration materials which are anti HIV antibody-negative, in order to lower the potential risk for laboratory personnel.

References

1. Curran, J. W. (1983) *New Engl. J. Med.* 309, 609–610.
2. Gottlieb, M. S., Schroff, R., Schanker, H. M., Weisman, J. D., Thiem Fan, P., Wolf, R. A. & Saxon, A. (1981) *New Engl. J. Med.* 305, 1425.
3. Masur, H., Michelis, M. A., Greene, J. B., Onorato, I., Van Stouwe, R. A., Holzman, R. S., Wormser, G., Brettmann, L., Lange, M., Murray, J. W. & Wuningham-Rundles, S. (1981) *N. Engl. J. Med.* 305, 1431–17435.
4. Davis, K. G., Horsburgh, C. R., Hasiba, U., Scholket, A. L. & Kirckpatrick, C. H. (1985) *Ann. Int. Med.* 98, 284–286.
5. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984) *Science* 224, 497–500.
6. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler Blin., Verzinet-Brun, F., Rouzioux, C., Rosenbaum, W. & Montagnier, L. (1983) *Science* 220, 868–871.
7. Goedert, J. J., Blattner, W. A. (1985) The epidemiology of AIDS and related conditions, in: *AIDS: Ethiology diagnosis, treatment and prevention* (De Vita, V. T., Hellmann, S. & Rosenberg, S. A., eds.) Philadelphia J. B. Lippincott Co, pp. 1–30.
8. Centers for disease control (1982) *Morbid. Mortal. Weekly Rep.* 31, 577–580.
9. Centers for disease control (1983) *Morbid. Mortal. Weekly Rep.* 32, 450–451.
10. Hirsch, M., Wormser, G. P. & Schooley, R. T. (1985) *N. Engl. J. Med.* 313, 1–4.
11. Weiss, S. H., Saxinger, W. C., Rechtman, D., Grieco, M. H., Nadler, J., Holman, S., Grinzburg, H. M., Groopman, J. E., Goedert, J. J., Markham, P. D., Gallo, R. C. & Blattner, A. W. (1985) *J. Amer. Med. Ass.* 254, 2089–2093.
12. Howanitz, P. J., McBride, J. H., Kliewer, K. E. & Rodger-son, D. O. (1986) *Clin. Chem.* 32, 773–777.
13. Evatt, B. L., Ramsey, R. B., Lawrence, D. N., Zila, L. D. & Curran, J. W. (1984) *Ann. Intern. Med.* 100, 499–504.
14. Ledermann, M. M., Ratnoff, O. D., Evatt, B. L. & Mc Dougal, J. S. (1985) *Ann. Intern. Med.* 102, 753–757.
15. Smolen, J. S., Bettelheim, P., Köller, U., Mc Dougal, S., Graininger, W., Luger, T. A., Knapp, W. & Lechner, K. (1985) *J. Clin. Invest.* 75, 1828–1833.
16. Levy, J. A., Mitra, G. & Mozen, M. M. (1984) *Lancet II*, 722–723.
17. Bericht der Kommission für Fragen der Virusdesinfektion in der Humanmedizin der Deutschen Vereinigung zur Bekämpfung der Viruskrankheiten (DVV) and des Bundesgesundheitsamtes (BGA) (1987) *Deutsches Ärzteblatt* 84/18, 1217–1226.
18. Heinburger, N., Schwinn, H., Gratz, P., Luben, G., Kumpe, G. & Herchenhan, B. (1981) *Drug. Res.* 31, 619–622.

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