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Stability of Human Immunodeficiency Virus (HIV) Antibodies in Postmortem Samples

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ABSTRACT: The stability of human immunodeficiency virus (HIV) antibodies was studied for samples of sera, vitreous fluid and bile obtained from eight HIV-positive autopsy cases. The autopsy delay was on average 5 days. The samples were stored at room temperature (20°C) for 51 to 314 days and tested repeatedly. In Western blotting on fresh postmortem samples, the antibodies detected most of the proteins of the virus. Antibodies against all major envelope, core and transmembrane proteins, although weakened, were also detected in stored sera. In stored vitreous fluid and bile the envelope protein gp 160, the transmembrane protein gp 41 and in half of the cases also the major core protein p 24 could still be detected. The disappearance of p 24 was associated with AIDS, but was detected in all samples from patients with early infection. Of screening tests, the enzyme-linked immunosorbent assay applying synthetic peptide as an antigen detected antibodies from all serum samples, but was less applicable to vitreous fluid or bile. Another immunoassay, applying recombinant antigen, succeeded in vitreous fluid and bile but not in sera. The rapid visually read assay detected antibodies in most samples of fresh whole blood, bile and in most of the vitreous samples, but was less useful on stored specimens.

KEYWORDS: pathology and biology, AIDS, HIV, epidemiology, homosexuality, autopsy, vitreous fluid, bile

Testing for human immunodeficiency virus (HIV) antibodies in autopsies has been useful for epidemiological purposes [*1–5*]. Reliable tests may also be needed for reducing occupational health hazards in the mortuary, because the HI virus may survive for several days in postmortem samples [*6–8*]. The applicability of the tests for detecting HIV antibodies in autopsy samples depends on the postmortem stability of the antibodies as well as on the sensitivity of the screening tests. Immunoglobulins are considered relatively resistant to autolysis [*9*]. HIV-1 antibodies have been detected in decomposed bodies and in blood samples stored months or years in cool conditions [*7*], and in blood-

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stains up to 6 months [*10*]. Klatt et al. [*11*] considered vitreous humor reliable for HIV antibody testing only up to 34 hours postmortem.

We report that HIV antibodies can be verified in samples of sera, whole blood, vitreous fluid, or bile that were stored for months at room temperature. There were considerable differences in the applicability of the screening tests to different postmortem samples.

Material and Methods

Sera, vitreous fluid, and bile were obtained from eight HIV-positive autopsy cases. Five of the cases were patients from the Aurora Hospital, where medical treatment of HIV-infected patients in Helsinki is centered. In three cases the samples were obtained from medicolegal autopsies performed at the Department of Forensic Medicine, University of Helsinki. The autopsy delay was 5 days on average (range 3–7 days). Of the cadavers, one was severely decomposed.

The samples were stored at room temperature (20°C) in a sealed plastic container. Western blotting (Organon Teknika), two commercial enzyme immunoassays (Combi 1-2 test, PharmaciaR, Wellcozyme-HIV-Recombinant, WellcomeR) and a visually read rapid assay (TestPackR HIV-1/HIV-2, Abbott) were applied to fresh and stored samples. Different test methods, antigen types, proteins detected and final patient serum dilutions used are depicted in Table 1. The storage time varied between 51 and 314 days. For comparison, additional samples were stored in a refrigerator at +4°C and tested repeatedly. HIV-negative sera, vitreous fluid and bile served as controls.

Results

Fresh postmortem sera reacted with all major gene products of HIV in Western Blot (gp 160, 120, 41 and p 24, 31, 55 and 65) (Fig. 1). Samples stored at room temperature for longer periods gave distinctly weaker bands although the different gene products were still clearly visible. The p 24 antigen was not visible with sera derived from AIDS patients.

None of the tests gave a positive result with all samples (Table 2). The Combi 1-2-test revealed antibodies in all serum samples, fresh and stored, but failed with a number of vitreous and bile samples. In order to study whether vitreous contains substances that might interfere with the EIA test, fresh vitreous from an uninfected case was used as a diluent for a weak positive serum sample. No effect was seen to the signal/noise ratio suggesting that the negative results were not due to inhibitory substances in the vitreous.

TABLE 1—*Comparison of used methods for antibody tests.*

Test	Method	Type of antigen	Specificity of antigen	Final serum dilution
Combi-1-2 test Pharmacia^{R}	ELISA	Synthetic peptide	gp41	1:11
Wellcozyme-HIV-recombinant Wellcome^{R}	ELISA	Recombinant protein	gp41, Core	1:2.5
TestPack^{R} HIV-1/HIV-2 Abbott	Rapid assay, visually reading	Recombinant protein	Envelope, Core	5–10 μL to 5 drops
Organon teknika	Western blot	Virus lysate	All virus proteins	1:50

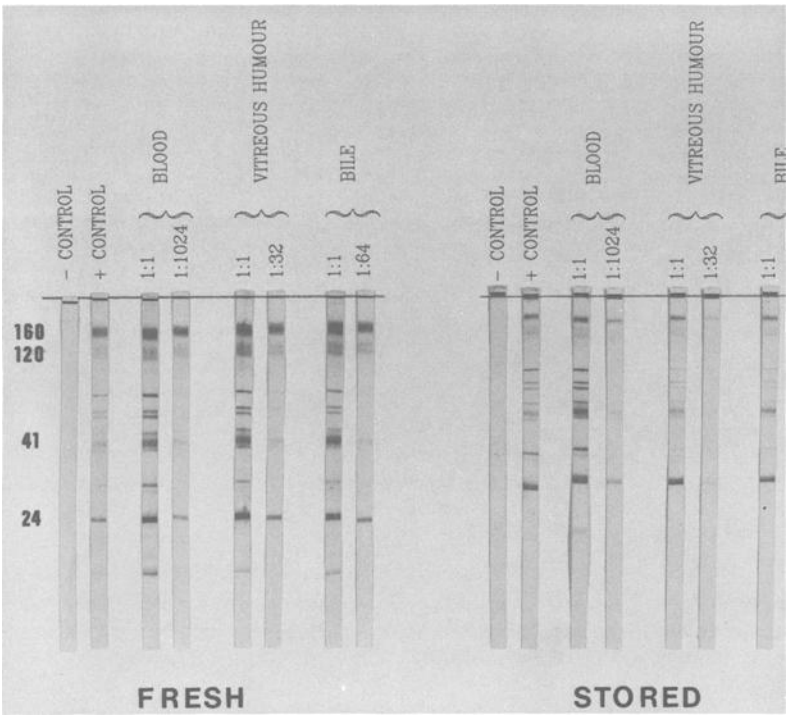

FIG. 1—*Western blotting of fresh and stored sera (storage time 51 days at room temperature). Most envelope and core proteins still visible although weakened.*

TABLE 2—*Applicability of screening tests in detecting HIV antibodies in fresh samples and samples stored 51–266 days at room temperature.*

	Test					
	Combi 1-2		Wellcozyme-HIV-Recombinant		TestPack HIV-1/HIV2	
	Fresh (n = 8)	Stored (n = 8)	Fresh (n = 8)	Stored (n = 8)	Fresh (n = 8)	Stored (n = 8)
Positive test/positive sample						
Sera[a]	8/8	8/8	4/8	3/8	7[b]/8	?[c]/8
Vitreous	4/8	3/8	8/8	6/8	6/8	6/8
Bile	7/8	5/8	8/8	8/8	8/8	6[b]/8

[a]Whole blood tested by visually read rapid assay.
[b]Other samples inconclusive, see text.
[c]All inconclusive, see text.

The Wellcozyme test based on competitive EIA failed to detect several of the serum samples but was better for vitreous and bile samples. This may have been partly due to the decomposition of the serum samples. Some cases where serum samples were initially negative with this test were retested diluted (usually 1/10) and became positive. The Abbott test failed to detect antibodies in any of the stored serum samples but was sensitive in fresh serum samples and both fresh and stored bile and vitrous samples. The problem was probably also due to the decomposition of the stored serum samples, the clotted and decomposed blood was not absorbed effectively into the membrane of the test kit. Similar problems were encountered with two of the bile samples. The result was not compatible with a negative result as defined by the manufacturer but rather "inconclusive." In two vitreous samples the test failed to detect the antibodies and gave a clearcut negative result.

The effect of storage is shown in Fig. 2. Particularly with Wellcozyme-test samples gave different results before and after storage. Also in some vitreous and bile samples the result became negative after storage reflecting perhaps the small amount of initial antibodies in the samples.

Discussion

Since 1986 medicolegal autopsies have been screened in Helsinki as a back-up for surveillance programs based on voluntary tests [*1,4*]. In a population with low HIV prevalence refusals from tests by people with risk behavior may strongly bias the seroprevalence figures. Autopsy screening efficiently reveals the true incidence in groups where refusal may be common. By the end of the year 1992, 15 HIV-positive cases had been identified among approximately 16 000 autopsies. Four of them were previously unknown cases not reported before. At the same time the seroprevalence estimated from other surveillance programs was less than 0.05%. These figures, although still too small

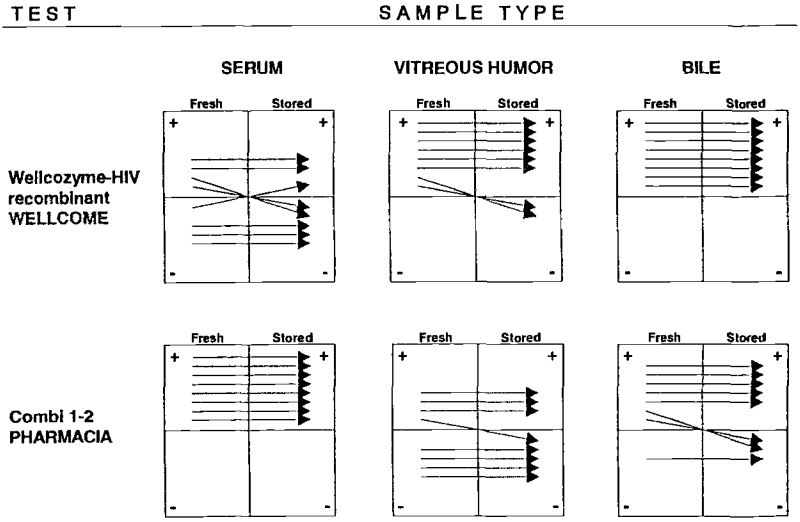

FIG. 2—*Comparison of applicability of two enzyme immunoassays (Wellcozyme-HIV-Recombinant, WellcomeR, Combi 1-2 test, PharmaciaR) on fresh and stored samples from eight HIV-positive autopsies. The cut off level is indicated by a horizontal line in each box. Arrows above cut off line considered positive (+) and below it negative (−). In some of the cases changes in the sample reactivity after a storage can be seen from positive to negative or vice versa.*

for statistical significance suggest that screening of medicolegal autopsies for HIV antibodies is effective in finding both previously known HIV-cases as cases in whom the infection had not been detected during lifetime.

Testing for HIV may also be desired for safety reasons in mortuaries. Viable HIV could be isolated from blood at autopsy up to 21 hours after the patient's death under conditions of a proper body storage in a refrigerated vault [*8,11*]. Recently Nyberg et al. [*6*] were able to isolate viable HI-virus up to 14 days postmortem from spleen specimens. These findings strongly underscore the need for caution during the handling of postmortem specimens and body fluids. Unfortunately, testing for HIV antibodies only reveals part of the infected individuals. Particularly during the early phase of infection the amount of virus in blood and other body fluids may be very high in the absence of detectable antibodies. This emphasizes the need for efficient protection of autopsy personnel during all autopsies.

Problems with postmortem laboratory testing of blood and body fluids include autolysis, hemolysis, bacterial contamination, desiccation and loss from decomposition. These problems are enhanced by prolonged postmortem intervals [*11,13*]. However, proteins, like immunoglobulins, are considered less likely to be affected by decomposition or interference from hemolysis or bacterial growth when measured in the vitreous humor of the eye [*13,14*]. Our results indicate that HIV antibodies can be detected in biological samples even if stored for months at room temperature. In Western blotting, all major proteins of the virus could be detected in all fresh samples and also in stored sera. The tests used in this study demonstrated that wide variation between different test formats may be expected when samples from medicolegal autopsies are tested. It has been demonstrated that tests based entirely on synthetic HIV-specific peptides are not as sensitive as tests employing both synthetic peptides and recombinant antigens [*15*]. This is probably the reason why the Combi 1-2 test failed to detect antibodies in several vitreous and bile samples. The test using a competitive test format (Wellcozyme) is very sensitive to the initial condition of the sample since undiluted sample is directly mixed with immunologically active test reagents. Decomposed blood and bile samples were clearly problematic and the results turned out to be quite unreliable. The rapid test worked well with the bile samples but could not be used for serum samples where inconclusive results were often obtained.

For practical purposes the ideal test to be used for autopsies should be robust against variation in the sample quality. Since serum samples may be so badly decomposed that other samples must be used as surrogates and these often have lower amounts of antibodies than sera, also the sensitivity must be rather high. Our experiment suggests that a solid phase EIA employing a combination of antigens that gives it a high sensitivity would be the method of choice. If a rapid test is used, availability of a regular EIA as a back up is essential [*16*]. Western blot is useful for vitreous and bile as well as for sera and should be used to confirm the initially positive result.

Bile turned out to be useful for HIV antibody testing. Bile is easy to obtain in putrefied autopsies from which blood or vitreous cannot be aspirated for testing.

To obtain data on the effect of storage temperature on the EIA test results, samples were also stored at +4°C in a refrigerator. In sera and bile we observed no effect, but vitreous fluid turned more rapidly inconclusive or negative in samples stored at room temperature.

Klatt et al. [*11*] assessed the reliability of postmortem enzyme immunoassay testing for antibody to HIV using blood and vitreous humor in AIDS patients. They found that blood tested at least 58 days postmortem was consistently repeatedly positive for HIV antibody, as also was confirmed by the present results. However, Klatt et al. [*11*] considered that HIV serology on vitreous fluid was reliable only 34 hours postmortem. They reported that 5 of the 16 vitreous specimens taken 34 to 384 hours postmortem were

negative whereas blood gave positive results in enzyme immunoassay. They, however, did not test the positive vitreous samples repeatedly to obtain data on the stability of the antibodies in the vitreous. In our experience, vitreous humor tested repeatedly positive for several months postmortem in specimens stored at room temperature or at +4°C.

In conclusion, our results suggest that HIV antibodies can be detected for weeks to months in postmortem specimens, even if stored at room temperature. Postmortem testing for HIV antibodies in autopsies seems thus to be a reliable method for screening for safety purposes or for monitoring the prevalence of asymptomatic carriers in autopsy series. There were significant differences in the usefulness of different tests. Obviously, several tests must be available that can be applied depending on the quality and condition of the sample.

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