TECHNICAL NOTE

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Evaluation of a Rapid Assay System, HIV 1/HIV 2 Testpack, Abbott, to Detect Human Immunodeficiency Virus Antibodies in Postmortem Blood

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ABSTRACT: For evaluating the HIV 1 / HIV 2 Testpack (Abbott, Chicago, IL) to detect antibodies to human immunodeficiency virus (HIV) in whole postmortem blood 456 samples were collected prior forensic autopsies. All samples were tested using the enzyme-linked immunoassay (ELISA) and the Testpack; positively reactive samples and samples with equivocal results were confirmed by Western blot. Of the 456 samples 21 (4.6 per cent) proved to be reactive in both systems (confirmed by Western blot). In 17 cases (3.7 percent) interpretation of the result was difficult, but no serious misinterpretations occurred.

It is concluded that the HIV-Testpack provides accurate results in testing whole postmortem blood for HIV antibodies.

KEYWORDS: toxicology, HIV-Testpack, rapid test, post-mortem blood, HIV-testing

Prevalence of human immunodeficiency virus (HIV) is considered to be higher in material from autopsies performed in forensic science departments than expected from health statistics. In 1992 2.7 per cent of all corpses investigated in the Institute of Legal Medicine, Frankfurt, Germany, proved to be HIV positive. On the other hand HIV prevalence in the German population was estimated to be less than 0.1 per cent [*1*]. This discrepancy can be explained by the fact that in these departments a high number of corpses of drug addicts with a generally higher rate of HIV-Infection are examined [*2*]. For instance, in 1992 160 autopsies of drug-addicts (12.5 per cent of all autopsies) were performed in Frankfurt. In 13.1 per cent of these cases HIV-antibodies were detected in the blood samples by ELISA-technique. In contrast to this observation only 1.2 per cent of 1125 corpses not associated with drug-abuse were found to be HIV-positive. However, these data indicate that the risk of HIV-transmission for the medical staff is great enough to cause concern. Therefore HIV-testing of blood samples before autopsy is recommended. In a HIV positive case necessary precautions should be taken.

The ELISA—HIV test is a standard procedure [*3*] used also in some forensic sciences institutions. But when this test is not available, such as in cases where the dead body is examined on spot or the autopsy is performed outside the institute, a rapid, simple and still reliable test for the detection of HIV—antibodies would be most helpful.

An immunoassay for the qualitative detection of HIV-antibodies (SUDS) has been developed by Murex Corporation (Norcoss, GA) and was successfully tested in forensic autopsy cases [*4*]. The mobile test-kit "HIV 1 / HIV 2 Testpack," developed by Abbott (Chicago, IL) has been found to meet the requirements of providing rapid and reliable results [*5–7*]. However, this kit has been tested on blood samples from living persons only, no data of samples from corpses have been published.

Postmortem-blood decomposes quite rapidly. Haemolysis and putrefaction may influence the HIV-testing, although studies of Penning et al. [*8,9*] using Abbotts Second Generation-ELISA indicate that the test results regarding this material are still accurate and reliable. Over the last two years applying Abbotts Third Generation-ELISA a similar experience has been made in our laboratory (personal observations).

In this study the data obtained by HIV-testing of whole postmortem blood samples using the HIV 1/HIV 2 Testpack are compared to those obtained by using the Recombinant HIV 1/HIV 2, 3rd Generation EIA, both manufactured by Abbott.

Materials and Methods

A total of 456 blood samples of cases examined in the Department of Legal Medicine (Zentrum der Rechtsmedizin), University of Frankfurt were collected by puncture of V. subclavia or from V. femoralis before autopsy.

The samples were simultaneously screened for HIV-1 and HIV-2—antibodies by using the standard—ELISA (Abbott Recombinant HIV-1/HIV-2, 3rd Generation EIA, Abbott, Chicago, IL) and the mobile test-kit (Abbott, HIV-1/HIV-2 Testpack).

To examine the rapid-test so called Reaction Discs are applied, which are fixed on top of a small rectangular fluid-container and which are covered by a filter (Fig. 1). In contrast to the procedure recommended by the manufacturer to use serum or plasma whole

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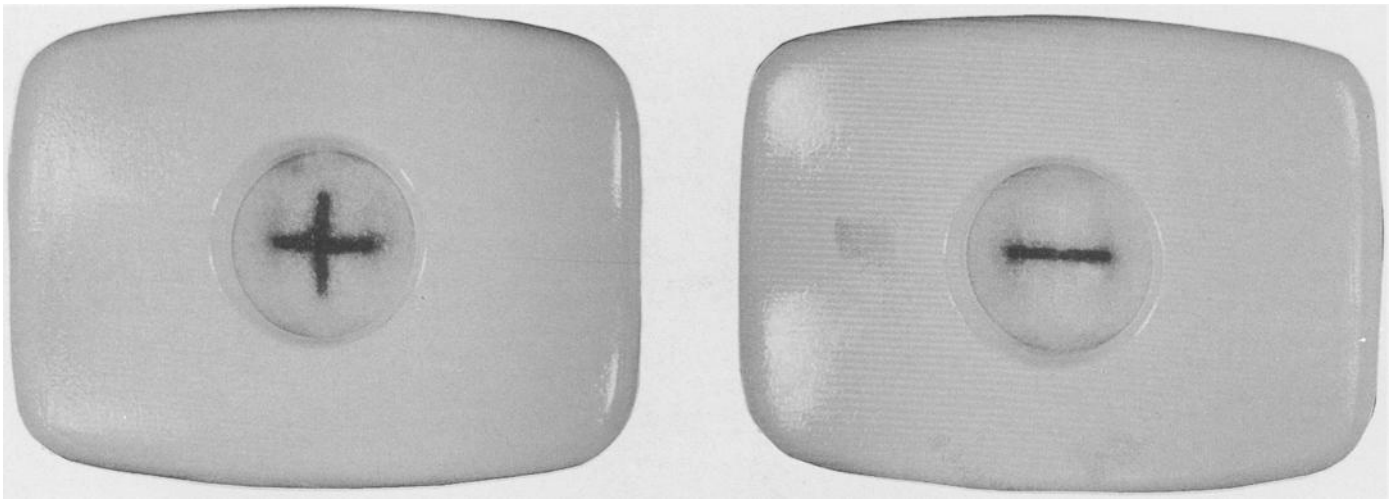

FIG. 1—*Typical HIV-positive and HIV-negative test-results.*

blood was applied in the case of the test-kit to mimic autopsy circumstances occurring outside the institute, where a centrifuge is not available to separate serum or plasma. One drop of blood was mixed with five drops of dilution-buffer in a small plastic-tube and applied to the prewetted filter covering the Reaction Disc. Particles such as small blood clots are separated and the fluid passes through the filter to the Reaction Disc, where HIV-antibodies bind to the fixed antigen. In this Testpack three different recombinant antigens: HIV-1-core and -envelope and HIV-2 envelope are applied. The use of recombinant antigens reduces the possibility of cross-reactions with human antibodies other than anti-HIV, e.g. antibodies against cells or media, in which the HIV was grown to produce native HIV-antigen. After the diluted blood has passed the filter, other, non-specific antibodies are removed by washing the filter and the Reaction Disc with buffer. Phosphatase labeled anti human-IgG (goat) reagent is added on top of the filter and incubated for 3 minutes. After removing the filter an additional washing step is performed to remove unbound conjugates and the chromogen (enzyme substrate) is added. Results are usually obtained after 10–15 seconds (color-development), although the manufacturer recommends to wait two minutes until reading the results to achieve maximum sensitivity. In case of a negative result "−" appears on the Reaction Disc, in a positive case "+".

To reduce background color, the Reaction Disc is finally washed with buffer.

The signal "+" occurs, because anti-HIV-IgG from blood binds only to the recombinant HIV-antigen which is placed on a vertical bar on the Reaction Disc. On the horizontal bar human antibodies other than anti-HIV will bind when all reagents are added in proper sequence and are functionally active. Therefore in case of serostatus "negative" only the horizontal bar will be colored, resulting in "−", whereas in case of serostatus "positive" also the vertical bar will appear, producing a "+" signal (Fig. 1).

All blood samples which exhibited positive or results different from ELISA were further tested by Western blot analysis.

Results

Of the 456 samples tested, HIV-antibodies were unequivocally detected in 21 samples (4.6%) by the Testpack. The results were confirmed by ELISA and Western-Blot analysis.

In addition to these samples 17 other Testpack results (3.7 per cent) could have been considered to be positive, that is, false positive (negative in Western Blot). Usually the color-development, positive as well as negative reaction, is completed within 10–15 seconds, which is much less than the recommended two minutes. But in these 17 cases a faint vertical bar occurred beside the strong horizontal ("negative") bar after a period of 1–2 minutes. In this way of reading a HIV-*positive* result may be suggested. However, these results were considered to be HIV-negative, which was confirmed by Western Blot.

Of these 17 samples only two and 12 others (together 3.1 percent) were found to be reactive in ELISA (the optical density reading was only slightly higher than the cutoff), but could not be confirmed by Western Blot.

Discussion

In screening postmortem blood for HIV-antibodies the present study indicates, that the Abbott HIV-1/HIV-2 Testpack is a very useful tool, even when in contrast to the manufacturers recommendation whole, even haemolytic blood, not serum or plasma, is used.

Problems to read the results occurred in 17 cases (3.7 per cent) and were found to be associated with a weak positive reaction after a reaction time which was prolonged, compared to the time, in which the reaction normally takes place. This may be due to nonspecific antibodies, which remained on the filter after the washing-steps. On the other hand, in some of these blood-samples involved, the time to pass the filter and the Reaction Disc was found to be much longer. Precipitates or small fibrin-clots may have blocked the filter and the Reaction Disc pores leading to a longer contact of the sample with the fixed antigens. However, the low intensity of the faint vertical bar combined with the delay of its occurrence is a good indication that this test had failed to give conclusive results. Although these samples could be rather considered as negative, further testing by Western blot is recommended.

The comparison with the ELISA—results show, that the number of potentially false positive results were similar (3.7 and 3.1 percent, respectively). However, in most cases the Testpack recognizes other samples as false positive than ELISA. In our opinion, a number of 3.7 per cent inconclusive and potentially false positive Testpack results (as well as 3.1 per cent in ELISA) is acceptable, since the consequence of false positive or inconclusive results in

autopsies would lead only to special precautions of the medical staff.

In cases where an autopsy is performed outside the department or when an urgent (whole-) blood-test has to be performed on a patient, this HIV-1/HIV-2 Testpack is a useful and reliable tool providing results in a rather short time.

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