

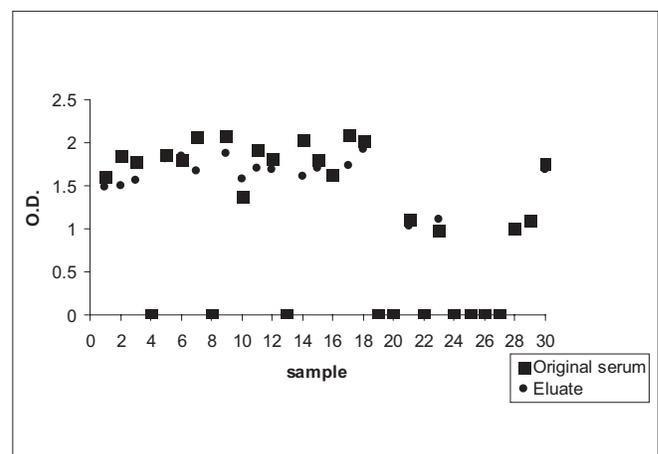
## Stability of human immunodeficiency virus antibodies in filter paper-spotted serum

Sir,

Acquired immune deficiency syndrome (AIDS) resulting from human immunodeficiency virus (HIV) infection has become a major global health concern in the last few years, with the gravity of the problem being more serious in the developing and the underdeveloped countries. In addition to suffering and death, the disease perpetuates poverty through work loss, school drop-out, decreased financial investment and increased social instability.<sup>[1]</sup> Providing diagnostic support is essential for ensuring quality health care in the fight against the HIV/AIDS epidemic. Correct laboratory protocols are important not only in the diagnosis but also in the screening of donated blood, surveillance, prevention of mother-to-child transmission, voluntary counseling and testing and laboratory monitoring of HIV antiretroviral therapy. It is important to make use of established and tested protocols for collection and transport of the samples from the field conditions to the lab as it is not practical to have diagnostic facilities everywhere. Use of filter paper as a transport medium for blood and serum was suggested as early as 1966.<sup>[2]</sup> Subsequently, many researchers have tried filter papers for the transport of sera and whole blood. In 1987, it was reported that results of an enzyme-linked immunosorbent assay (ELISA) and the immunoblotting test for HIV antibodies from the eluates from sera-impregnated filter paper discs agreed with the results obtained by ordinary serum testing.<sup>[3]</sup> In another study, 1020 specimens were tested using five different commercial enzyme-linked immunoassay systems for the detection of HIV type 1 antibody from filter paper discs impregnated with whole blood.<sup>[4]</sup> All five systems performed adequately, with specificities in excess of 99%. Some other studies have tried to simulate tropical field conditions for storage purpose for the filter-spotted samples.<sup>[5]</sup>

With the objective of testing the stability of diagnostically important serum antibodies against HIV antigens (p24, gp160, gp120) on the filter paper using ELISA and Western Blot (WB), we tested 20 samples of HIV-positive subjects and 10 samples of HIV-negative subjects. To avoid any bias, the testing was carried out by a double blind method. Results from the fresh sera were used as baseline results for comparison with the results obtained from sera spotted on the filter paper (Whatman filter paper 3). We used small strips of the paper to spot 100  $\mu$ l of serum, which was then dried and packed in separate polythene bags and stored at room temperature for 10 days. On the 11<sup>th</sup> day, the serum was eluted from the filter paper. The elution was carried out by incubation of the pieces of the strip in 100  $\mu$ l of elution buffer (phosphate-buffered saline pH 7.4 with 0.05% Tween and 1% bovine serum albumin). The eluted sample was used for ELISA and WB.

It was observed that all the samples that were positive for ELISA in fresh sera were positive with the filter paper eluate obtained after a storage period of 10 days at room temperature. All the negative samples were negative, giving a 100% concordance as expected. Furthermore, we observed that the optical densities (ODs) of fresh sera matched well with the sera eluted from the filter paper [Figure 1]. The correlation coefficient between the OD values was 0.988. Also, it was observed that in WB all the HIV antigen-specific bands were present in the filter paper eluate sample. Intensities of the band for core antigen p24 and envelope antigens (gp120 and gp160) in the WB for the filter paper eluate (when measured as pixel densities using a gel documentation system) were similar to that obtained from fresh sera.



**Figure 1: Enzyme-linked immunosorbent assay results of the fresh sera and the eluate from the filter paper**

Both ELISA and WB results were consistent for different storage periods (up to 10 days), suggesting that antibodies from sera collected on filter paper can remain stable. Stability of antibodies reactive to viral antigens of diagnostic importance was further tested with two of these samples for a 15-days storage period. The ELISA and WB results were consistent over the period of 15-days storage of the filter paper. All diagnostically important serum antibodies against HIV antigens (p24, gp160, gp120) were detected in the WB results. Antibodies against other antigens (p55, p31, p51, p56 and gp41) were also found to be stable.

Unlike previously reported studies, we looked at actual ODs and intensities of the band rather than just qualitatively positive ELISA results or the presence of the band. The results clearly show that filter paper can be used as a potential tool for the transport of serum samples from the field site to the testing lab as the antibodies of diagnostic importance for HIV remain stable on it.

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