Detection of Antibodies to Human Immunodeficiency Virus Type 1 in Whole Blood and Saliva by Using a Passive Hemagglutination Test

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A passive hemagglutination test (PHA) for detecting human immunodeficiency virus type 1 antibodies in serum samples by using envelope glycoprotein (gp160)-coupled sheep erythrocytes was described earlier (M. B. Vasudevchari, K. U. Uffelman, T. C. Mast, R. L. Dewar, V. Natarajan, H. C. Lane, and N. P. Salzman, J. Clin. Microbiol. 27:179-181, 1989). In the study reported here, the applicability of the PHA test to the detection of antibodies in whole-blood and saliva samples has been investigated. We observed a 100% correlation between PHA and commercial enzyme-linked immunosorbent assay in 101 whole-blood samples and 98% correlation between PHA and reactivity to envelope proteins in Western blots (immunoblots) of 53 saliva samples. Furthermore, salivary antibodies could be detected in 19 of the 22 seropositive individuals. As in serum, antibodies to envelope proteins were widely prevalent in all the Western blot-reactive saliva samples.

Although difficult to collect and process, serum is the only body fluid regularly used in tests for human immunodeficiency virus type 1 (HIV) antibodies. Whole blood and fluids such as saliva have been proposed as alternatives for HIV antibody testing. The tests that have been tried for saliva, immunoglobulin G capture immunassays (5), radioimmuno-precipitation (1, 3), and Western blots (immunoblot assays) (2), require difficult and lengthy procedures and are economically unsuited for widescale use. Recently, a rapid autologous erythrocyte agglutination test which involves a monoclonal antibody to human erythrocytes coupled to a synthetic peptide corresponding to envelope protein of HIV has been reported (4). We had earlier described a rapid and sensitive passive hemagglutination (PHA) test for detecting antibodies to HIV in serum samples (6). We have since extended the applicability of PHA for detecting antibodies to HIV in whole-blood and saliva samples, making it a test of broader general value.

Whole-saliva, serum, and whole-blood (heparinized) samples were collected from acquired immunodeficiency syndrome patients. For 22 individuals, paired saliva and serum samples were collected at the same time. The saliva samples were diluted 1:2 in phosphate-buffered saline, mixed by vortexing, frozen and thawed once, and centrifuged at 2,500 × g for 5 min to remove debris. The samples were then digested with N-acetyl-L-cysteine by mixing 1 ml of saliva with 0.5 ml of 0.1 M sodium citrate - 2H2O and 0.5 ml of N-acetyl-L-cysteine (10 mg/ml). The saliva thus digested was preadsorbed for 1 h at room temperature with bovine serum albumin-coupled erythrocytes (final concentration, 50% erythrocytes). These treatments resulted in a 1:8 dilution of the saliva samples. All further dilutions for PHA were done in phosphate-buffered saline containing 0.5% bovine serum albumin. Enzyme-linked immunosorbent assays (ELISA) and Western blot assays were performed as specified in the instructions of the manufacturer (Du Pont Co., Wilmington, Del.).

Of the 101 whole-blood samples tested, 71 gave a positive reaction in PHA for HIV antibodies at 1:1,000 and 1:2,000 dilutions. The plasma obtained from each of these blood samples was also tested for HIV antibodies by ELISA, and a 100% correlation between two tests was observed (data not shown). The relationship between the PHA test that we have devised and other commercial tests has already been described (6). Although whole blood collected by venipuncture was used in the experiment described above, small volumes (10 to 25 μl) of blood obtained by a pin-prick and immediately diluted in phosphate-buffered saline to prevent clotting were also suitable for testing, greatly facilitating the acquisition of test samples.

A total of 53 saliva samples were titrated by PHA at twofold dilutions. Positive reactivity by PHA was scored in 46 saliva samples, of which 3 (6%) gave titers of 1:8, 5 (11%) gave titers of 1:16, and 38 (83%) gave titers of 1:32 or higher dilutions. Seven samples showed agglutination even at a dilution of 1:512. When the samples were analyzed by Western blots, 47 showed reactivity to HIV proteins; of these, 13 (28%) had antibodies reactive to gag/pol/env gene products of HIV and 34 (72%) had antibodies reactive to pol/env or only env gene products. The Western blot patterns of six saliva samples are shown in Fig. 1. Positive ELISA readings above the calculated cutoff absorbancy value (0.300) were obtained in only 24 samples. A good correlation was found between the intensity of envelope reactive bands in Western blots and the PHA titers (Fig. 2). Two samples exhibited a prozone effect at dilutions of 1:16 and 1:32, but at higher dilutions both showed clear agglutination patterns. Thus, it is recommended that saliva samples be titrated at dilutions from 1:8 to 1:64 to score PHA results. The sensitivity of PHA in saliva samples could not be compared with sensitivities of other tests for antibody detection, since quantitation of antibodies to HIV in saliva has not been reported in the literature. However, the results of PHA presented in this report indicated that PHA is quite sensitive in detecting HIV antibodies in saliva.

The results obtained with saliva were compared with those

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obtained with 22 matched serum samples (Table 1). All 22 serum samples were scored positive for HIV antibodies by PHA, ELISA, and Western blot assays. Of the 22 matched saliva samples, 19 (86%) showed various degrees of reactivity to different HIV proteins in Western blots, and all 19 were positive by PHA (PHA titer, >1:8) while only 11 (50%) were positive by ELISA. The three samples that were nonreactive to any HIV proteins in Western blots were also negative by PHA and ELISA. Differences in serum and salivary antibody status have been observed in previous studies (3) and may reflect either the classes of antibodies involved in the assay methods or the low level of immunoglobulin G in saliva. When saliva was used directly, nonspecific agglutination was frequently observed, resulting in false-positive reactions. However, after preabsorption of saliva samples with bovine serum albumin-coupled erythrocytes, such nonspecific agglutination did not occur, and this procedure is used routinely prior to PHA using saliva.

A switch from serum to whole-blood or saliva specimens for testing HIV antibodies, especially in children, has obvious advantages (5). Although saliva samples showed reactivities to a variety of HIV proteins in Western blots, antibodies to envelope proteins were invariably present in all of them. For this reason, use of envelope (gp160)-coated sheep erythrocytes in our PHA test favors successful detection of antibodies in saliva. In our test, the need to separate serum is obviated by using whole blood, and the same high levels of sensitivity and accuracy that we previously reported for serum are preserved, both being as high as those observed with more elaborate commercial test kits.

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LITERATURE CITED


