Dot Immunobinding Assay Compared with Enzyme-Linked Immunosorbent Assay for Rapid and Specific Detection of Retrovirus Antibody Induced by Human or Simian Acquired Immunodeficiency Syndrome

RICHARD L. HEBERLING,1* S. S. KALTER,1 PRESTON A. MARX,2 JAMES K. LOWRY,3 AND ARTHUR R. RODRIGUEZ4

National Institutes of Health–World Health Organization Collaborating Center for Reference and Research in Simian Viruses, Department of Virology and Immunology, Southwest Foundation for Biomedical Research, P.O. Box 28147, San Antonio, Texas 78284;1 California Primate Research Center, Davis, California 95616;2 and Department of Pathology, Santa Rosa Hospital, Santa Antonio, Texas 782853

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A rapid, specific, and sensitive modification of the dot immunobinding assay was compared with the standard enzyme immunoassay as a screening procedure for the detection of antibody in human or simian acquired immunodeficiency syndrome. Comparative testing with the available enzyme immunoassay procedures, either in commercial kit form or as provided by diagnostic laboratories, indicated excellent correlation. Ease of operation and cost are key features of the dot immunobinding assay procedure.

It is now recognized that a similar acquired immunodeficiency syndrome (AIDS) occurs in human and nonhuman primates (principally Macaca spp.) with common clinical and pathological manifestations (6, 14). Several viruses, including simian immunodeficiency virus (SIV), simian retrovirus serotype 1 (SRV-1), and SRV-2, are known to be associated with simian AIDS (SAIDS). A number of serological procedures have detected antibodies to these viruses in various primate populations (7, 14, 17, 19). Similarly, the same procedures have been used successfully to detect antibody to the human immunodeficiency virus (HIV) (3).

The procedures used for serological evidence of infection include enzyme-linked immunosorbent assay (ELISA), immunofluorescence, serum neutralization, and protein or immunoblotting (Western blot). These procedures all have advantages and disadvantages. The major disadvantages are the need for expensive laboratory equipment and inability to use these tests under field conditions. A simpler and less expensive test for the serodiagnosis of AIDS and SAIDS would therefore be advantageous. Recently, a dot immunobinding assay (DIA) was described as an alternative for current procedures in viral diagnosis because of its simplicity, rapidity, cost-effectiveness, sensitivity, and specificity (8–11). This report provides data on testing human and nonhuman primate sera by the DIA, a commercial ELISA kit for the AIDS virus (human T-cell lymphotropic virus [HTLV-III]) serology (Abbott Laboratories), and ELISA as performed at the California Primate Research Center (CPRC), Davis, Calif., for detection of HIV and SRV-1 and SRV-2 antibody.

Human sera were obtained from a variety of patients at the Santa Rosa Hospital, San Antonio, Tex. This group included a number being evaluated for AIDS, hemophiliacs, and dialysis patients. Sera were collected principally for virological studies. These samples were split and examined independently at Santa Rosa with a commercial HTLV-III ELISA kit (Abbott Laboratories, North Chicago, III.) and at the Southwest Foundation for Biomedical Research (SFBFR) with the DIA. Monkey (Macaca mulatta) sera were provided by the CPRC for comparative studies with the ELISA test used at that center. These latter sera were derived from two groups of animals: a colony with endemic SAIDS, and juveniles from a SAIDS-free group which were used for experimental SRV-1 transmission studies. Pre- and postinfection sera from the latter group were tested.

Two isolates of SRV-1 were compared in the DIA. One of these was provided by M. D. Daniel (New England Regional Primate Research Center Southboro, Mass.) as a persistently infected Raji cell line (4). The second strain came from the CPRC and was also adapted to grow in Raji cells (15). These two viruses were comparable in their reactivity, and the CPRC strain was selected as the test antigen. SRV-2, a second serotype, was isolated and grown in Raji cells at the CPRC (17). SIV (STLV-III), a rhesus monkey (Macaca mulatta) isolate (5) obtained from Dr. Daniel, and HIV (HIV strain B), obtained from R. Gallo, were cultivated in the HUT78 human T-cell line. The Raji and HUT78 cell cultures were grown in RPMI 1640 medium with 10% fetal bovine serum and 50 μg of gentamicin per ml. Virus-containing culture fluids were harvested 3 to 4 days following a 1:10 split of the cells. Cells were pelleted at 1,500 rpm for 15 min, and the supernatant virus suspension was treated with 8% polyethylene glycol 6000 and 0.5 M sodium chloride. After 5 h of stirring at 4°C, the suspension was allowed to stand overnight. The virus was then pelleted by centrifugation at 5,000 rpm for 15 min. The supernatant fluid was discarded, and the pellet was suspended in 1/100 volume of TNE (0.01 M Tris buffer, 0.15 M NaCl, 0.002 M EDTA, pH 9.0). This was used undiluted as the DIA antigen. The suitability of using undiluted antigen was determined by testing twofold serial dilutions against known positive and negative sera. Uninfected cell cultures were treated similarly to provide a control antigen. HIV antigen was inactivated with psoralen as previously described (8, 9).

* Corresponding author.
The DIA was used as previously described (8, 9). Controls consisted of uninfected cell culture antigens as well as positive and negative sera. Readings are dependent on the intensity of the staining reaction and are graded from 1+ to 4+, the latter being the greatest positive reaction. Positive reactions are any detectable red-colored spot of greater intensity than the cell control and negative serum. The control and negative serum dots are generally colorless or barely perceptible. The HTLV-III ELISA (Abbott Laboratories) test kit was used and interpreted as directed by the manufacturer. The procedure used at the CPRC has been described previously (18).

Sera tested by the DIA and the Abbott ELISA procedures for detecting HIV antibody resulted in 93% agreement (Table 1). In addition, there were five sera positive by DIA and negative by ELISA and one serum positive by ELISA and negative by DIA. Of the five DIA-positive sera, four were 1+ and one was 2+ in staining intensity.

Thirty-nine of the human sera were also tested in the DIA for HIV and SIV antibody. Of the 39 sera examined, 15 had HIV antibody; of these, 8 were also positive for SIV. HIV-negative sera were also SIV negative.

Of the 68 serum samples obtained from the endemic SAIDS group of macaques living in a corral (group 1), agreement in SRV-1 antibody detection was obtained for all but one serum tested by the two laboratories. There were 38 (56%) positive and 29 (43%) negative samples detected; agreement was 99% (Table 2). One animal was positive in the ELISA and negative in the DIA. Of the 46 juvenile experimental animals, good correlation was again obtained by the two laboratories. In group 2, 17 (37%) of the animal sera were found with antibody and 27 (59%) of the sera were antibody free; agreement was 98%. Twenty-two of the 27 negative sera were preinoculation sera; 2 sera were positive by the ELISA and negative by DIA.

A number of procedures are in use for detecting AIDS and SAIDS antibody in human and nonhuman primate sera. Commercial kits are available and are used principally for detecting HIV antibody in human sera. Although these kits may be used for the evaluation of monkey sera, most laboratories generally develop their own test procedures.

Inasmuch as there is a need to evaluate simians for antibody to type D SAIDS viruses (SRV-1 and SRV-2), because of their usefulness for AIDS and SAIDS investigations and for colony health practices, a simple, rapid, sensitive, specific, and inexpensive assay procedure would be of value. The DIA is such a procedure (8, 9), and studies on herpesvirus simiae (B virus) (10) and rabies virus (11) emphasize the effectiveness of the method for detecting viral antibodies. This DIA provides data well within the acceptable range obtained by commercial and other laboratory methods. A somewhat similar procedure (a dot ELISA) has been reported for the detection of antibody to HIV (12).

The study reported herein was not undertaken to assay sera for antibody to the indicated viruses. The primary purpose was to ascertain the effectiveness of the procedure by comparing the DIA with methods performed in other laboratories on the same test sera. Therefore, monkey sera were independently examined for SRV-1 antibody at the CPRC and SFBR. Human sera similarly were tested at the Santa Rosa Hospital and at SFBR for HIV antibody. It was of interest that 8 of 15 human sera positive for HIV were also positive for SIV, an antigenically related virus (14), by the DIA. Additionally, 14 of the monkey sera which showed a 3 to 4+ reaction to SRV-1 were also weakly reactive to SRV-2. This was attributed to cross-reactivity between these two antigenically related viruses (18). One serum from an SRV-2-infected monkey showed a similar cross-reactivity, with a more intense reaction against the SRV-2 antigen.

Differences obtained in this study cannot be explained without recourse to extensive testing by other procedures, such as Western blots. As with all methods, variability exists, particularly with low-titered sera. This was clearly demonstrated by finding that those sera for which differences in results were observed were generally weakly positive (± or 1+) DIA reactors. However, for a screening test, the data as obtained by this DIA suggest its consideration when a simple, rapid, and sensitive technique is desired. Its applicability to field work has already been suggested, as little in the way of specialized equipment is required. Preliminary field studies do indicate the suitability of the procedure (manuscript in preparation).

Studies to compare various laboratory methods have used sera from suspected positive or control negative individuals. In the study performed here, the human sera were not generally from patients known to have AIDS, but rather from hospital patients receiving blood or blood products and outpatients on dialysis therapy. The monkey sera, on the other hand, were from selected groups of animals: a group of animals with endemic SAIDS (group 1) and a SAIDS-free group of animals (group 2). Closer agreement was obtained with the nonhuman primate groups of sera.

A word of caution needs to be added about nonspecific reactions that have been described when monkey sera from normal populations were tested by ELISA. Although not applicable to the present study, in which the intent was to compare the DIA and ELISA procedures, monkey sera have been reported in several studies to show a high degree of nonspecific antibody-binding reactivities (13, 19). Human sera have also been found to react nonspecifically when tested for retrovirus antibody, including HIV (1, 3, 21). Therefore, inclusion of appropriate controls as well as confirmation by other procedures is necessary in certain instances. However, for mass surveys, particularly in the field, the results obtained by the DIA indicate its usefulness.

### Table 1. Results obtained by testing human sera for HIV (HTLV-III) antibody by the DIA and Abbott ELISA

<table>
<thead>
<tr>
<th>Result</th>
<th>DIA</th>
<th>ELISA</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>22 (25.6)</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>58 (67.4)</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>+</td>
<td>5 (5.8)</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1 (1.2)</td>
</tr>
</tbody>
</table>

*Concordance was 93%.

### Table 2. Testing of monkey (M. mulatta) sera by ELISA (at CPRC) and DIA (at SFBR) with SRV-1 (type D retrovirus) antigen

<table>
<thead>
<tr>
<th>Result</th>
<th>No. (%) of sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPRC</td>
<td>SFBR</td>
</tr>
<tr>
<td>Group 1</td>
<td>Group 2</td>
</tr>
<tr>
<td>+</td>
<td>+ 38 (56)</td>
</tr>
<tr>
<td>-</td>
<td>- 29 (43)</td>
</tr>
<tr>
<td>+</td>
<td>- 1 (1)</td>
</tr>
<tr>
<td>-</td>
<td>+ 0</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

*Group 1, Correlated animals with endemic SAIDS; group 2, experimental group of juvenile animals inoculated with SRV-1. Twenty-two of the 27 sera were collected prior to inoculation. Concordance was 99% for group 1 and 98% for group 2.
LITERATURE CITED


