Dot Immunobinding Assay for Simultaneous Detection of Specific Immunoglobulin G Antibodies to Measles Virus, Mumps Virus, and Rubella Virus

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A dot immunobinding assay was used to detect antibodies to measles virus, mumps virus, and rubella virus antigens. Filter paper soaked with serum or whole blood was directly applied to the antigen-coated nitrocellulose sheets. The test was easy to perform, and its results agreed very well with those obtained by standard enzyme immunoassay.

In certain parts of the world measles continuously represents a serious public health problem (12). Vaccination programs using measles-mumps-rubella vaccine have significantly reduced the incidence of measles, mumps, and rubella in only a few years (9); however, during a recent measles outbreak in the United States about half of the cases occurred in previously vaccinated individuals (11). Serological surveys will be needed in the future to identify susceptible individuals and to monitor vaccine efficacy. Dot immunobinding assays (DIA) have found wide application in the detection of both viral antigens and antibodies (4–6, 8, 13). They have been thoroughly evaluated in the screening for antibodies to human immunodeficiency viruses, and they were found to be as sensitive and specific as standard enzyme immunoassays (EIA). DIA can be performed under field conditions because sophisticated laboratory equipment is not required, and the test is highly cost-effective (8, 13). Serum or whole blood collected on filter paper is a convenient sample for serological assays (1, 5, 10, 14). We used serum or whole blood collected on filter paper for the simultaneous detection of immunoglobulin G-class antibodies to measles virus, mumps virus, and rubella virus by DIA in order to discriminate between seropositive and seronegative individuals.

The following specimens were included in this study: (i) 210 randomly selected serum samples from the specimen library of our routine diagnostic laboratory; (ii) pairs of serum samples taken from patients during the acute and convalescent phases of infection: nine with measles, seven with rubella, and four with mumps virus infection; and (iii) pairs of venous blood and finger stick blood samples simultaneously taken from 16 healthy staff members of the Department of Virology, University of Turku. Venous blood was allowed to clot at room temperature, and the serum was removed after low-speed centrifugation. Fingertips were punctured with a lancet, and 10 μl of blood was collected in a heparinized capillary pipette and immediately applied to precut filter paper (Whatman International, Maidstone, United Kingdom; catalog no. 3030917). The filters were allowed to dry at room temperature and then stored at 4°C before being used.

Standard indirect EIA was carried out as described by Meurman et al. (7). Briefly, microtiter wells were coated with 0.25 μg of measles virus or rubella virus antigen or with 0.5 μg of mumps virus crude lysate antigen prepared in our laboratory as described earlier (7). A control antigen prepared from uninfected cultured human diploid fibroblasts was used at a concentration of 0.5 μg per well. Sera were tested at a dilution of 1:100 in duplicate wells. Known positive and negative sera were included in each test. Results were expressed as ratios calculated by dividing the mean of the two optical density values obtained from the virus antigen by the mean value obtained from the control antigen. A ratio greater than 2.5 was regarded as a positive result. For sera tested in serial dilutions, the highest serum dilution was regarded as the end-point titer when the optical density value obtained from the virus antigen was three times the value obtained from the control antigen and was at least 0.150.

For DIA, nitrocellulose filters (Schleicher & Schuell, Dassel, Germany; no. 402099) were cut in squares (20 by 20 mm) and coated with virus and control antigens, and then 2 μl of the three virus antigens and the control antigen was spotted to each filter square. Measles virus and rubella virus antigens were used at a concentration of 0.5 μg/μl, and mumps virus and fibroblast control antigens were used at a concentration of 1 μg/μl. Spots were allowed to dry for 15 min at room temperature, and then the filters were soaked in phosphate-buffered saline (PBS) containing 10% nonfat dry milk powder (PBS-M) at 4°C overnight to saturate the remaining binding sites. Before being used, the filters were washed four times in distilled water. One hundred microliters of human serum diluted 1:20 in fetal bovine serum was added to precut pieces of Whatman filter paper (20 by 20 mm). Filters were air dried at room temperature, sealed in plastic bags, and stored at 4°C. Before testing, the serum soaked filters were prewetted with PBS-M, then put directly on the antigen-coated nitrocellulose filters, and incubated at room temperature for 30 min. Filters with dried blood were soaked in 100 μl of fetal bovine serum, resulting in a serum dilution of approximately 1:20, and incubated with the antigen-coated nitrocellulose filters. Nitrocellulose filters were then washed twice for 5 min in PBS–0.1% Tween 20 and incubated with peroxidase-labeled rabbit antibodies to

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human immunoglobulin G (Dakopatts, Glostrup, Denmark) at room temperature for another 30 min. As a chromogen 20 mg of 3-amino-9-ethylcarbazole was dissolved in 5 ml of dimethylformamide, and sodium-acetate buffer was added to obtain a final volume of 1 liter. The solution was filtered through a 0.22-μm-pore-size filter and stored in aliquots of 10 ml at −20°C. Prior to use, 10 μl of 3% H₂O₂ was added and the filters were incubated in this solution for 30 min. A visible red spot was considered a positive result. Known positive and negative control sera were included in all experiments.

Plaque reduction neutralization for the detection of antibodies to measles virus was carried out with some selected sera as described by Albrecht et al. (2).

The results obtained by DIA and by EIA from the 210 serum samples are shown in Table 1. All sera giving a positive result by EIA were also found positive by DIA. A few sera in each test gave a positive result by DIA but remained negative by EIA. Of the nine serum specimens from the acute phase of the measles virus infection, two were positive by EIA and eight were positive by DIA. All seven serum samples from acute-phase rubella virus infection were positive by EIA, but only six of them gave a positive result by DIA. Two of the four mumps virus infection serum samples were positive by EIA, and all four were positive by DIA. Paired samples of serum and whole blood from 16 healthy individuals were tested. All three samples contained antibodies to measles virus and rubella virus as determined by both assays, while one of the samples was negative for mumps virus by both tests and another was negative by EIA but positive by DIA. None of the samples contained detectable antibodies to human diploid fibroblasts.

Nine serum samples which were negative by EIA but positive by DIA for measles virus antibodies were tested by plaque reduction neutralization test in serial twofold dilutions starting from 1:5, and all sera reduced the number of plaques by at least 50% at the lowest dilution, indicating that they contained neutralizing antibodies to measles virus and that DIA yielded a true positive result. The negative control serum included in all tests did not show any plaque reduction at a dilution of 1:5.

DIA is a sensitive method for the simultaneous detection of antibodies to multiple antigens. In our study, DIA detected more positive sera than the indirect EIA, the standard reference test. This observation has also been made by others who have compared DIA with EIA for the detection of antibodies to human immunodeficiency virus (8). This increased sensitivity could be due to the more efficient binding of the antigen to the nitrocellulose filter than to the polystyrene microtiter plates. Another factor might be the higher serum dilution we used in our EIA. Four serum samples which were positive for measles virus by DIA and by plaque reduction neutralization test but negative by EIA were retested by EIA at a dilution of 1:40, but only one of them could be regarded as positive by EIA at this lower dilution. For the identification of nonimmune candidates who would benefit from vaccination, this increased sensitivity may be a disadvantage because of the possibility of detecting seropositive individuals with nonprotective titers (3). The sensitivity could, however, be lowered by increasing the serum dilution or by shortening the duration of the serum incubation.

Although none of the sera tested contained measurable antibodies to human diploid fibroblasts, it was useful to have the control antigen included for the interpretation of the test result.

The test is easy to perform, the antigen-coated nitrocellulose filters can be stored at 4°C without any loss of activity for several weeks, and the possibility of using whole blood collected on filter paper makes this test suitable for large studies in unfavorable conditions.

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