Detection and Titration of Measles Virus Antibody by Hemagglutination Inhibition and by Dot Immunobinding

S. S. KALTER,* R. L. HEBERLING, AND J. D. BARRY

Virus Reference Laboratory, Inc., 7540 Louis Pasteur, San Antonio, Texas 78229

Received 31 May 1990/Accepted 25 September 1990

Measles continues to be a major disease of both human and nonhuman primates. The dot immunobinding assay, a modified enzyme immunoassay, permits the detection of measles virus antibody in the nonlaboratory setting with either serum or whole blood collected on filter paper.

Measles is a significant disease of both human and nonhuman primates. Since the introduction of human vaccine programs, the incidence of measles in humans has sharply decreased. Outbreaks continue to occur, however, and the determination of the presence or absence of antibody continues to be the method of choice for detection of immunity. Measles is also an important disease in nonhuman primates, particularly among young animals. Species variation in susceptibility to measles virus infection is recognized. Subclinical infection is the most common manifestation, but fatalities do occur, particularly in New World species. Monkeys in the wild apparently are free of measles, contracting infection only after contact with humans (21).

A number of diagnostic tests for detecting antibody response to measles virus are available. Of these, the hemagglutination inhibition (HI) test has been most extensively used (19). The specificity and sensitivity of the HI test are equal to those of the serum neutralization test; moreover, the HI test is more sensitive than the complement fixation test, but it is reportedly less sensitive than the enzyme immunoassay (3, 15). The HI test has the disadvantage that erythrocytes are required for the test and for the treatment of test sera to remove nonspecific agglutinins. The dot immunobinding assay (DIA), a modified enzyme immunoassay, has been shown to equal enzyme immunoassays in the detection of antibodies to a wide variety of viral antigens, and it is easier to perform (2, 10, 25). The use of filter paper to apply serum or blood directly to the nitrocellulose sheet permits the direct testing of whole blood, thus eliminating the need for venipuncture and the separation of serum from the cells (11, 17). Another advantage of the DIA over the HI test is that the DIA can be used in the field or office setting, as no laboratory equipment is needed.

In this report, the HI test and the DIA are compared for their abilities to detect measles virus antibody in human sera and whole blood and in nonhuman primate sera and whole blood. Preliminary studies comparing the DIA with the HI test indicated an excellent correlation (11). Other investigators have demonstrated the use of filter paper for the collection and testing of measles virus antibody (4, 6, 22, 27). It also was of interest to ascertain how the DIA compares with the HI test for detecting and titrating measles virus antibody.

Serum samples from humans or from nonhuman primates (*Macaca mulatta*) were obtained after venipuncture in the usual manner. Whole blood was collected on precut filter

paper after finger puncture and either was used directly in the DIA or, after being dried, was stored at 4°C until it was used (17). A variety of filter papers have been used in the DIA; for this study, Whatman filter paper (grade 4CHR, catalog no. 3004633) in 1-in. (ca. 2.5-cm)-wide rolls was cut into 3- to 5-mm strips.

Measles virus antigen was used in the HI test and the DIA as previously described (11). The DIA procedure has been reported in detail (11-14). Sera were tested either undiluted or at various dilutions by dipping precut filter paper strips into the serum or serum dilution and draining excess fluid off by carefully passing the strip over the lip of the tube. After being dried, blood-saturated strips were applied directly to the nitrocellulose sheet, care being taken that the entire strip was in contact with the nitrocellulose sheet, as evidenced by the strip becoming moist. If titrations were done on the whole-blood strips, two strips were placed in 0.5 ml of phosphate-buffered saline and allowed to stand overnight at 4°C, and twofold dilutions of this eluate were made after an initial dilution of 1:10. All results reported here are based on initial dilutions.

Fifty-two human specimens were tested by both the HI test and the DIA either as whole blood (with or without elution) or as serum. Thirty-two of these specimens were titrated for antibody endpoint. Sixteen of the 32 serum samples had titers of less than 1:8 (negative) by the two procedures; 8 showed a one-tube difference, 1 indicated a two-tube difference, and the remaining 7 had the same titer by both the HI test and the DIA (Table 1). Of the 20 blood specimens tested as whole blood and tested after elution, 18 were in complete agreement, with the numbers of positives and negatives the same for both the whole blood and the eluate. For the remaining two samples, the eluates were positive at a 1:10 (negative at 1:20) dilution and equivocal results were obtained with the corresponding whole blood.

A total of 325 rhesus monkey (*M. mulatta*) serum samples were individually tested at different times and at different dilutions by both the DIA and the HI test. A total of 258 serum samples were positive by both the HI test and the DIA (79.0%), and 275 were positive by the DIA (85.0%). Another 78 serum samples were then simultaneously tested at the same dilution (1:10), and 52 (67.0%) were positive by both procedures. The remaining 26 serum samples (33.0%) were negative (<1:10) by both the DIA and the HI test.

In a vaccine study, 60 animals whose serum samples had titers of <1:10 all seroconverted (fourfold) after vaccination, as determined by both procedures. It is of interest that only a one-tube difference in serum titers (with one exception) was observed between the HI test and the DIA results. In

* Corresponding author.

TABLE 1. Comparison of measles virus antibody titers of human sera by DIA and HI test

Serum no.	Titer by:	
	DIA	HI test
1	1:8	1:16
2	1:8	1:32
3	1:8	1:16
4	1:128	1:256
5	1:128	1:128
6	1:8	1:16
7	1:8	1:8
8	1:64	1:64
9	1:128	1:128
10	1:64	1:64
11	1:512	1:256
12	<1:8	1:8
13	1:128	1:128
14	1:16	1:16
15	1:16	1:8
16	1:16	1:8
17-32	<1:8	<1:8

Table 2, 25 representative serum samples are listed, showing the pre- and postvaccination titers obtained by the DIA and the HI assay.

The development of a viral diagnostic test that is rapid, sensitive, specific, and cost-effective has merit, particularly if it requires little laboratory equipment and needs no highly specialized, trained personnel and therefore can be done in the office setting or in the field. An added advantage, particularly when individuals are being screened for the presence or absence of antibody, is that whole blood can be applied directly to filter paper in the DIA. A number of

well-documented reports indicate the suitability of using filter paper to collect whole blood for detecting antibody, including measles virus antibody (1, 4, 5, 7-9, 16-20, 22-29.) As shown herein, there is excellent agreement between the DIA and the HI test for detecting measles virus antibody. Excellent reproducibility was also shown when pre- and postvaccination sera were compared by the DIA and the HI test. Preliminary data comparing human patient sera following measles virus infection indicate the appropriateness of the DIA for detecting an antibody increase when acute-phase and convalescent-phase sera are tested. These results suggest that the DIA may serve for serodiagnosis of measles virus infection as well as for evaluation of vaccination programs and epidemiological surveillance. This comparison of the DIA with such standard procedures as the HI test and the immunofluorescence assay (data not shown) for detecting measles virus antibody, as well as its applicability to other test antigens, suggests wider employment of the DIA in the laboratory setting.

The technical assistance of Melisande Meyers and Frances Pfluger is gratefully acknowledged.

Portions of this study were supported by Small Business Innovation Research Program grants 1R43 AI29317-01 and 1R43 AI128604-01.

REFERENCES

- Adams, E., and R. P. Hanson. 1956. A procedure for adsorbing virus neutralizing antibodies on paper disks. *J. Bacteriol.* **72**: 572.
- Bode, L., L. Beutin, and K. Kohler. 1984. Nitrocellulose enzyme-linked immunosorbent assay (NC-ELISA)—a sensitive technique for the rapid visual detection of both viral antigens and antibodies. *J. Virol. Methods* **8**:111-121.
- Boteler, W. L., P. M. Luipersbeck, D. A. Fuccillo, and A. J. O'Beirne. 1983. Enzyme-linked immunosorbent assay for detection of measles antibody. *J. Clin. Microbiol.* **17**:814-818.
- Brody, J. A., R. McAlister, R. Haseley, and P. Lee. 1964. Use of dried whole blood collected on filter paper disks in adenovirus complement fixation and measles hemagglutination inhibition tests. *J. Immunol.* **92**:854-857.
- Chapman, O. D. 1924. The complement fixation test for syphilis. Use of patient's whole blood dried on filter paper. *Arch. Dermatol. Syphilol.* **9**:607-611.
- Draper, C. C., and A. Kelly. 1969. Experience with the use of blood collected on discs of absorbent paper for measuring rubella hemagglutination-inhibiting antibodies. *Symp. Ser. Immunobiol. Stand.* **11**:105-106.
- Farzadegan, H., K. H. Noori, and F. Ala. 1978. Detection of hepatitis B surface antigen in blood and blood products on filter paper. *Lancet* **i**:362-363.
- Farzadegan, H., T. Quinn, and B. F. Polk. 1987. Detecting antibodies to human immunodeficiency virus in dried blood on filter paper. *J. Infect. Dis.* **155**:1073-1074.
- Fuchs-Beraud, F., and M. Aymard. 1989. The use of a nitrocellulose enzyme immunoassay for the rapid screening of monoclonal antibodies to human enteroviruses. *J. Biol. Stand.* **17**: 1-7.
- Hawkes, R., E. Niday, and J. Gordon. 1982. A dot-immunobinding assay for monoclonal and other antibodies. *Anal. Biochem.* **119**:142-147.
- Heberling, R. L., and S. S. Kalter. 1986. Rapid dot-immunobinding assay on nitrocellulose for viral antibodies. *J. Clin. Microbiol.* **23**:109-113.
- Heberling, R. L., and S. S. Kalter. 1987. A dot-immunobinding assay on nitrocellulose with psoralen inactivated *Herpesvirus simiae* (B virus). *Lab. Anim. Sci.* **37**:304-308.
- Heberling, R. L., S. S. Kalter, P. A. Marx, J. K. Lowry, and A. R. Rodriguez. 1988. Dot immunobinding assay compared with enzyme-linked immunosorbent assay for rapid and specific detection of retrovirus antibody induced by human or simian

TABLE 2. Comparison of measles virus antibody titers of monkey sera by HI test and DIA

Serum no.	Prevaccine titer by:		Postvaccine titer by:	
	HI test	DIA	HI test	DIA
1	20	40	320	640
2	20	40	320	640
3	80	160	320	640
4	<10	<10	320	640
5	320	320	320	640
6	<10	<10	160	320
7	<10	<10	320	640
8	<10	<10	160	320
9	<10	<10	320	640
10	80	160	320	640
11	<10	<10	320	640
12	<10	<10	160	320
13	<10	<10	320	160
14	<10	<10	320	160
15	<10	<10	320	320
16	<10	<10	320	640
17	<10	<10	320	320
18	<10	<10	320	320
19	<10	<10	40	80
20	<10	<10	80	80
21	<10	<10	320	320
22	<10	<10	320	320
23	<10	<10	320	640
24	<10	<10	320	640
25	<10	<10	320	640

- acquired immunodeficiency syndrome. *J. Clin. Microbiol.* **26**: 765-767.
14. Heberling, R. L., S. S. Kalter, J. S. Smith, and D. G. Hildebrand. 1987. Serodiagnosis of rabies by dot immunobinding assay. *J. Clin. Microbiol.* **25**:1262-1264.
 15. Kahane, S., V. Goldstein, and I. Sarov. 1979. Detection of IgG antibodies specific for measles virus by enzyme-linked immunosorbent assay (ELISA). *Intervirology* **12**:39-46.
 16. Kalter, S. S. 1963. Disc method for identification and titration of cytopathic viruses and detection of antibodies resulting from their infections. *J. Lab. Clin. Med.* **62**:525-534.
 17. Kalter, S. S., R. L. Heberling, C. L. Coe, and A. R. Rodriguez. 1988. The collection of blood on filter paper and its use in the dot-immunobinding assay (DIA) for detection of antibody. *J. Virol. Methods* **20**:181-183.
 18. Kumar, S., A. H. Band, J. C. Samantaray, N. Doug, and G. P. Talwar. 1985. A dot enzyme-linked immunosorbent assay for detection of antibodies against *Entamoeba histolytica*. *J. Immunol. Methods* **83**:125-133.
 19. Lennette, E. H., and N. J. Schmidt (ed.). 1979. Diagnostic procedures for viral, rickettsial, and chlamydial infections, 5th ed. American Public Health Association, Washington, D.C.
 20. Low, P. C., M. A. Dow, and R. S. Fujioka. 1985. Use of the NC-immunosorbent assay for the rapid, sensitive, and quantitative detection of human enteroviruses. *J. Virol. Methods* **12**:225-234.
 21. Meyer, H. M., B. E. Brooks, R. D. Douglas, and N. G. Rogers. 1962. Ecology of measles in monkeys. *Am. J. Dis. Child.* **103**:307-313.
 22. Nakano, J. H., D. L. Miller, S. O. Foster, and E. W. Brink. 1983. Microtiter determination of measles hemagglutination inhibition antibody with filter papers. *J. Clin. Microbiol.* **17**:860-863.
 23. Oprandy, J. J., J. G. Olson, and T. W. Scott. 1988. A rapid dot immunoassay for the detection of serum antibodies to Eastern equine encephalomyelitis and St. Louis encephalitis viruses in sentinel chickens. *Am. J. Trop. Med. Hyg.* **38**:181-186.
 24. Top, F. H., Jr., P. Gunakasem, C. Chantarasri, and J. Supavadee. 1975. Serologic diagnosis of dengue hemorrhagic fever using filter paper discs and one dengue antigen. *Southeast Asian J. Trop. Med. Public Health* **6**:18-24.
 25. Towbin, H., and J. Gordon. 1984. Immunoblotting and dot immunobinding. Current status and outlook. *J. Immunol. Methods* **72**:313-340.
 26. Urakami, H., S. Yamamoto, T. Tsuruhara, N. Ohashi, and A. Tamura. 1989. Serodiagnosis of scrub typhus with antigens immobilized on nitrocellulose sheet. *J. Clin. Microbiol.* **27**: 1841-1846.
 27. Wassilak, S. G. F., R. H. Bernier, K. L. Herrman, W. A. Orenstein, K. J. Bart, and R. Amber. 1984. Measles seroconfirmation using dried blood specimens in filter paper. *Pediatr. Infect. Dis. J.* **3**:117-121.
 28. Worth, R. M. 1964. The filter disc blood collection method adapted for a mumps HAI serologic survey. *Am. J. Hyg.* **79**:245-249.
 29. Zhuang, H., A. G. Couplepis, S. A. Locarnini, and I. D. Gust. 1982. Detection of markers of hepatitis B infection in serum dried on to filter paper: an application to field studies. *Bull. W.H.O.* **60**:783-787.