Comparison of Track XI Fluorometric Immunoassay with Bio-EnzaBead Enzyme-Linked Immunosorbent Assay for Detection of Serum Antibody to Mouse Hepatitis Virus

MARIE C. LA REGINA,¹ JACK LONIGRO,¹ LUETHIA WOODS,¹ WILLIAM C. HALL,² AND RICHARD E. DOYLE³

Department of Comparative Medicine, St. Louis University Medical School, St. Louis, Missouri 63104,¹ and Pathology Associates, Ijamsville, Maryland 21754²

Received 10 August 1987/Accepted 21 November 1987

The Track XI system (Microbiological Associates, Bethesda, Md.) was compared with the Bio-EnzaBead assay (Organon Teknika, Durham, N.C.) for the detection of antibody to mouse hepatitis virus (MHV). Strain A/J mice were inoculated intranasally with MHV type 3. Sera were collected at 1, 2, 4, and 9 weeks postinoculation and tested. Individual serum samples were retested twice by each method. The results suggested that the Track XI system was more sensitive and reliable than the Bio-EnzaBead assay in detecting antibody to MHV type 3 in individual serum samples from A/J mice.

Mouse hepatitis is a common infectious disease of mice which is caused by a coronavirus. Several factors affect the clinical and pathologic manifestations of the disease, including the strain of the virus and the strain of mouse infected. The disease alters the immune response and can adversely affect experimental results (7). Since signs and lesions may be minimal or totally absent in resistant strains of mice (7-9), serologic testing is a primary method of diagnosis.

Two commercially produced serologic testing systems detect antibody to mouse hepatitis virus (MHV) in mouse serum. This study compared results of the Bio-EnzaBead enzyme-linked immunosorbent assay (ELISA) (Organon Teknika, Durham, N.C.) with results of the Track XI fluorometric immunoassay (FIA) (Microbiological Associates, Bethesda, Md.) in an experimental MHV infection in a strain of mice (A/J) resistant to MHV. We wished to determine to what extent results varied with time postinoculation and to determine which assay appeared more reliable when individual serum samples were retested.

A total of 26 90-day-old, adult female A/J mice (Jackson Laboratories, Bar Harbor, Maine) were housed in plastic shoe-box-type cages with filter bonnets. Mice inoculated with MHV were kept in a biological safety hood (Nu Aire, Inc., Plymouth, Minn.), while control mice were kept on an adjacent rack in the same room. Bedding consisted of ground corn cobs (Anderson, Maumee, Ohio), and all mice had free access to food (Purina, St. Louis, Mo.) and water. All bedding, food, cages, and filter bonnets were autoclaved before use. Caretakers wore gloves, caps, shoe covers, and sterile surgical gowns when working with the mice.

The virus strain used was MHV type 3 (MHV3) ATCC VR-262 (American Type Culture Collection, Rockville, Md.). This strain was chosen because previous work detailed its effects on A/J mice (3, 8, 9). Experimental mice were anesthetized lightly with methoxyflurane and inoculated intranasally with 10⁵ 50% lethal doses of MHV3 (titer determined by passage in BSVS mice).

Four to five mice inoculated with MHV3 were euthanized with CO₂ at 1, 2, 4, and 9 weeks postinoculation. Blood was collected by cardiac puncture in serum separator microtubes, allowed to clot, and centrifuged at 13,600 x g for 2 min. The sera were stored at −70°C. Two control mice were euthanatized before the experiment began to confirm negative MHV titers. Five control mice were euthanatized 9 weeks after the experiment began. Euthanasia and blood collection procedures for control mice were identical to those used for experimental mice. The nasal passages, sinuses, lungs, livers, and gastrointestinal tracts were collected from all mice. The tissues were fixed with 10% neutral buffered Formalin, embedded in paraffin, and stained with hematoxylin and eosin.

Immunoperoxidase staining to detect MHV antigen was performed by using the avidin-biotin technique for the horseradish peroxidase enzyme (5, 6). Noses, lungs, ilea, ceca, colons, and livers were processed through paraffin, sectioned at 5 μm, and stained for the virus at a 1:200 dilution of mouse anti-MHV antibody for 24 h. Controls consisted of Formalin-fixed MHV-infected nude mouse liver. Negative control procedures consisted of replacing the MHV antibody with normal mouse serum and staining each tissue section.

The Bio-EnzaBead test kit uses ferrous beads coated with antigen. The antigen is MHV A59. The beads are incubated with a 1:30 dilution of serum, washed, and placed in wells containing a conjugate which recognizes both immunoglobulin G (IgG) and IgM mouse antibodies. The appearance of color upon the addition of a substrate solution indicates the presence of antibody to MHV. Results were determined colorimetrically at 690 nm and compared with negative and positive control values. Results were reported as optical density units. All serum dilutions and pipetting of Enzabody and wash solutions were done with an automatic pipette-diluter.

The Track XI FIA employs an indirect immunofluorescence technique. The antigen (MHV A59), immobilized within a dried colloidal gel, reacts with coronavirus-specific antibody in the sample and is then reacted with fluorescein-labeled anti-mouse antibody. The amount of fluorescence is measured quantitatively in the Track XI MP fluorescence reader. Fluorescence values from unknown samples are compared against the curve calculated by the Track XI reader with low, high, and mid-range titers of control sera. Directions for both the ELISA and the FIA kits were

* Corresponding author.
followed exactly, with no substitutions or additional manipulations. The sera were retested twice with both kits at approximately 2- to 4-week intervals. Between tests, the sera were kept frozen at -70°C. Interpretation of the results was based on manufacturer recommendations and laboratory variability. Reliability was defined simply as consistent negative or positive results for any given sample over the three test runs. Statistical analysis of reliability data and number of positive results was performed by using the chi-square test.

Mild focal hepatitis associated with viral antigen in macrophages and occasional hepatocytes was noted in mice at 7 days postinoculation.

Table 1 summarizes the results of the serologic assays. Positive antibody titers were found in four of five mice by the FIA in the first test run at 1 week postinoculation, whereas the ELISA detected only one positive serum. Retesting of sera at 1 week postinoculation showed relatively high reproducibility for both the FIA and the ELISA. At 2, 4, and 9 weeks postinoculation, the FIA gave more positive results with a higher degree of reproducibility than the ELISA. A total of 19 sera were tested three times each. Although two of these sera showed poor reproducibility with the FIA, the lack of reproducibility or reliability with the ELISA (10 of 19 results) was significantly greater ($P < 0.01$). The FIA also detected a greater number of positive samples than the ELISA ($P < 0.01$). Samples from control mice remained negative for all three test runs with the FIA. With the ELISA, one control sample tested positive in two of the three test runs.

The presence of MHV infection after intranasal inoculation was confirmed by immunoperoxidase demonstration of antigen within cells and by typical hepatic lesions. These results were similar to those obtained by other investigators (1, 2). The serologic results suggest that the Track XI FIA was more sensitive and reproducible than the Bio-EnzaBead ELISA for A/J mice infected with MHV3. In a similar comparison, the Track XI assay for detection of antibody to Brucella abortus in cattle was found to be more sensitive than either the ELISA or the FIA (4).

The serious discrepancies between the ELISA and the FIA in our study were not apparent until positive sera were compared. There was no consistent change which would specifically suggest poor technique, reagent failure, or improper incubation times. Positive and negative control values for the ELISA were within normal expected ranges. Although technical error cannot be ruled out, it is not the only possible explanation for our data.

The results of a previous study comparing an immunofluorescence test and the Bio-EnzaBead ELISA (10) indicated that reactions of Bio-EnzaBead with MHV3 and MHV-JHM strains were weaker than reactions with other MHV strains tested. In earlier study (8), no neutralizing antibody was found in serum of A/J mice 15 days after intraperitoneal inoculation with MHV3. In fact, a general conclusion has been drawn from a review of several studies of MHV in mice (7) that the classic humoral immune response to MHV appears to be weak. The poor results obtained with the ELISA reported here might be explained by a weak humoral response, except that positive titers were consistently obtained with the FIA. Perhaps antigenic sites within the colloidal gel are more available to serum antibody than are sites on the ferrous beads or the magnitude of the fluorescence reaction is so much greater compared with the color change in the enzyme reaction that smaller amounts of antibody can be detected. For whatever reason, our results suggest that the Track XI FIA is sensitive and reliable in detecting antibody to MHV3 in A/J mice, despite the poor humoral response to this strain of virus.

### LITERATURE CITED

Anim. Sci. 34:261–263.