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Validation of an Enzyme-linked Immunosorbent Assay for Detection of Mouse Parvovirus Infection in Laboratory Mice

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***Purpose*: Parvoviruses are among the most prevalent infectious agents in mouse colonies. Infection in laboratory mice is confirmed by detection of serum antibodies to these agents, and most diagnostic tests cannot distinguish serogroup of the infecting agent. The principal objective of the research reported here was to develop and validate a sensitive, serogroup-specific diagnostic test that will distinguish between mouse parvovirus (MPV) and minute virus of mice (MVM) infection.**

***Methods*: The MPV VP2 protein was expressed in bacteria, purified by use of metal-chelation chromatography, and used as antigen in an ELISA. More than 580 sera from uninfected mice and experimentally or naturally infected mice were screened by MPV indirect fluorescent antibody (IFA) test, then were re-tested using the MPV ELISA to define test sensitivity and specificity. An additional 3,700 sera were screened using a variety of tests, including the MPV ELISA and recombinant NS1 ELISA (rNS1 ELISA).**

***Results*: Using MPV IFA test results as a benchmark, the MPV ELISA had sensitivity of 92.3% and specificity of 99.8%. In addition, the MPV ELISA detected anti-viral antibodies at a higher dilution of serum than did the IFA test, and confirmed the infecting agent as MPV or MVM. When compared directly in a commercial laboratory, the MPV ELISA had higher sensitivity (90.3% versus 65%) than and similar specificity (98.3% versus 99.6%) to the rNS1 ELISA.**

***Conclusion*: The MPV VP2 ELISA provides a sensitive, serogroup-specific alternative for diagnosis and classification of parvovirus infection in laboratory mice.**

Parvoviruses are among the most common infectious agents of laboratory rodents. In a 1996 survey of 72 of the top 100 institutional recipients of NIH funds, approximately 45% of the respondent institutions had parvovirus infection in specific-pathogen -free (SPF) or non-SPF mice (1). Although parvoviruses can cause clinical disease, particularly in young animals, most of these infections are clinically silent. It is the latter that constitute a substantial threat to experimentation involving rapidly dividing cells, particularly those examining rodent immune responses. In addition, parvoviruses are stable, which increases the risk of environmental persistence and spread when undiagnosed cases exist in a colony.

Parvoviruses are small, non-enveloped, single-stranded DNA viruses that replicate through a double-stranded DNA intermediate (2). The two nonstructural proteins, NS1 and NS2, serve several functions during viral replication and are well conserved among the rodent parvoviruses. Amino acid identities for NS1 are > 90% when comparing minute virus of mice (MVM), mouse parvovirus strain 1a (MPV1a), rat virus (RV-Y; a strain of Kilham rat virus [KRV] isolated at Yale University), and H-1 virus (3). Two structural or capsid (VP) proteins comprise the viral coat, and the amino acid sequence of the major capsid protein, VP2, is contained within the minor, but larger VP1 protein. Through use of alternative splicing of the capsid coding transcript, VP1 protein has an additional 142 N-terminal amino acids. The third protein, VP3, comprising full virions, is present in variable amounts and is generated by cleavage of VP2. The capsid proteins are more divergent among parvoviruses and determine the serogroup for a particular strain (3).

Historically, rodent parvoviruses were assigned to one of three serogroups: minute virus of mice (MVM), rat virus (RV), or H-1 virus. Recently, the prototype virus for a new murine serogroup was detected in laboratory animal colonies. Testing of serum from infected animals yielded conflicting diagnostic results: parvovirus positive by use of generic diagnostic tests (i.e., MVM immunofluorescence testing), but negative in serogroup-specific tests, such as hemagglutination-inhibition (HAI) testing (4, 5). The agent was originally referred to as mouse orphan parvovirus, but it has been renamed mouse parvovirus 1 (MPV-1) (3).

The in vitro replication, genomic sequence, and pathogenesis of MPV-1a have been determined (3, 6). In vitro replication of MPV-1a was similar to that for MVM, MPV and MVM NS1 amino acid sequences were almost identical, but VP1 amino acid sequences were only 77% identical. Unlike MVM infection of mice (7), MPV-1 can establish persistent infection in immunocompetent adults (6). Extended viral infection and shedding make it difficult to eliminate MPV from mouse colonies (5), and the risk to research involving laboratory mice is high because

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MPV is lymphocytotropic and alters the host immune response. Two additional strains in the MPV-1 serogroup have been isolated, and the genomic sequence of both is similar to that of MPV-1a (8).

Testing for parvovirus infection in animals relies heavily on serologic detection of anti-viral antibodies in the host. In the early days of testing, HAI was the principal diagnostic method used to detect antibodies to parvovirus capsid proteins and confirm viral serogroup. More recently, an indirect fluorescent antibody (IFA) test, using infected cells as the source of antigen, has been used for serologic screening. Although it is sensitive, it detects antibodies to conserved (NS) and virus-specific (VP) proteins, and therefore, it cannot easily determine serogroup of the infecting parvovirus. Further, MPV-1a productively infects only L3 cells, a T-cell clone that is difficult and expensive to propagate. The ELISA, using purified virions as antigen, also has been used to detect murine parvovirus infection. Neither the virion ELISA or HAI is a practical screening test for MPV because they require large quantities of purified MPV. An alternative ELISA, using baculovirus-expressed MVM NS1 protein as antigen (rNS1 ELISA), was recently described (9). Because of high homology of NS1 among parvoviruses, the test is not serogroup specific, although it should detect infection with unidentified parvoviruses. Furthermore, it appears that not all MPV-infected mice seroconvert to NS proteins. Besselsen (10) examined seroconversion patterns of experimentally infected mice of various ages and strains. The rate of seroconversion of 12-week-old mice, as detected by use of the MPV IFA or HAI test, was affected by strain and viral dose, and none of these animals was seropositive by rNS1 ELISA. However, all ICR mice inoculated at four and eight weeks of age were seropositive by MPV HAI and IFA tests, and most were seropositive by rNS1 ELISA.

We describe an ELISA that utilizes bacterial-expressed MPV1a VP2 protein as antigen. It detects anti-viral antibodies in experimentally and naturally infected mice with high sensitivity and specificity. In addition, its ease of use and serogroup specificity make it an effective new tool in the armament of laboratory animal diagnostics.

Materials and Methods

Cloning of MPV-1a VP2 in the pET bacterial expression system. The MPV-1a DNA was isolated, using the Hirt procedure to select for low molecular weight DNA in infected cells (11), and it served as a template for polymerase chain reaction (PCR) amplification of the VP2 gene. The PCR primers were designed, using the published MPV-1a sequence (Genbank accession No. U12469). The 5'-end PCR primer linked vector coding sequence at the *Bam*HI site in-frame with the second through fifth amino acids of VP2, and the 3'-end primer linked the last five amino acids of VP2 in-frame with vector coding sequence at the *Hind*III site. The specific primers were: 5'primer, 5'CGCGGATCCGAGTGATGGCGCCGAG3' and 3'primer, 5'CCGTCAAGCTTGTAAGTATTTCTAGC3'. The PCR product was cloned through an intermediate vector, pCR2.1 (Invitrogen, Carlsbad, Calif.), which was transformed into electrocompetent *Escherichia coli* strain JM109. Resulting colonies were screened for the presence of the VP2 gene by restriction digest analysis, and plasmids containing the VP2 gene were purified (Qiagen, Valencia, Calif.). The VP2 gene was released from the vector by digestion with *Bam*HI and *Hind*III, isolated by electrophoresis on a 1% low melting point agarose gel, and purified by digestion of the agarose band, using β-agarase (New England Biolabs, Beverly, Mass.).

The pET20b+ plasmid (Novagen, Madison, Wis.), a bacterial expression vector containing a T7 RNA polymerase-inducible promoter, was digested with *Bam*HI and *Hind*III, treated with shrimp alkaline phosphatase (Amersham Pharmacia Biotech, Piscataway, N.J.), and gel purified as described previously. Purified plasmid and VP2 gene were ligated at 16°C overnight and transformed into JM109 cells, then resulting colonies were screened by plasmid size and restriction enzyme analysis. Plasmids containing the VP2 gene were purified and transformed into the expressor *E. coli* strain BL21(DE3) that contains a chromosomal copy of the T7 RNA polymerase gene under *lacUV5* control. As a result, transcription and translation of the MVP-1a VP2 gene does not occur until the cellular T7 RNA polymerase is expressed and translated. The expressed protein contains 31 vector amino acids on the N-terminus, all of VP2 except the starting methionine, and 13 vector amino acids (including six terminal histidines) on the carboxy terminus of VP2. Bacterial cultures were frozen and stored at -80°C.

Bacterial expression of MPV1a VP2. Frozen bacteria were used to inoculate tryptone yeast phosphate (TYP) broth containing 75 μg of ampicillin/ml, incubated at 37°C for six hours, and streaked for isolated colonies on L-ampicillin-containing agar plates. Three colonies were inoculated into TYP broth containing 50 μg of carbenicillin/ml and incubated in an orbital shaker at 37°C/225 rpm. When the cultures had reached approximate OD_{600} of 0.8, pre-induction samples were harvested. Isopropyl β-D-thiogalactopyranoside (IPTG) was added to the remaining culture at a final concentration of 0.7 m*M*, and incubation continued for an additional three hours. Cultures were then placed on ice for five minutes, postinduction samples were taken, and the remaining volume was centrifuged at 5,000 ×*g* for 5 min. Medium was decanted, and the pellet was washed with 50 m*M* Tris, pH 8.0. Cells were re-centrifuged at 5,000 ×*g* for 5 min, liquid was decanted, and the pellet was stored at -80°C until purification.

Pre- and postinduction samples were screened for expression of VP2. Sample pellets were re-suspended in 10 m*M* Tris-HCl (pH 8.0)/1 m*M* EDTA (1×TE), an equivalent volume of 2× loading buffer (0.125*M* Tris-HCl, 4% sodium dodecyl sulfate [SDS], 20% glycerol, 1.42*M* 2- mercaptoethanol, 0.02% bromophenol blue [pH 6.8]) was added, and samples were subjected to SDS-10% polyacrylamide gel electrophoresis (10% SDS-PAGE). Gels were stained with Coomassie blue, and protein bands present after induction were compared with those before induction for the presence of the 64-kDa MPV-1a VP2 protein.

Purification of MPV-1a VP2. Bacterial pellets were resuspended in buffer containing 5 m*M* imidazole, 500 m*M* NaCl, 40 m*M* Tris-HCl, pH 7.9 (1× binding buffer [BB]), sonicated on ice at four- 10-second pulses, and centrifuged in a Beckman SW28.1 rotor at 39,000 ×*g* for 25 min. The soluble fraction was discarded, and the insoluble fraction was resuspended in 1× BB containing 8*M* urea and was sonicated. After storage at 4°C overnight, the lysate was centrifuged as described previously. A column of His-bind resin (Novagen; Madison, Wis.) was equilibrated with three volumes of sterile deionized, double-distilled water, five volumes of 50 m*M* $NiSO_4$, and three volumes of 8*M* urea-1× BB. The supernatant from the second centrifugation was loaded onto the resin, the column was washed with 10 vol-

umes of 8*M* urea-1× binding buffer, and washed with six volumes of wash buffer (45 m*M* imidazole, 500 m*M* NaCl, 8*M* urea, and 20 m*M* Tris-HCl, pH 7.9). Protein was eluted, using 200 m*M* imidazole, 500 m*M* NaCl, 8*M* urea, 80 m*M* Tris-HCl, pH 7.9. To determine quality of the purification, samples from various steps in the purification were electrophoresed by use of 10% SDS-PAGE, and the gel was stained with Coomassie blue. Typically, only MPV-1a VP2 was evident in eluted fractions 1 and 2. Protein concentration of an antigen pool, typically 400 to 800 μg/ml, was determined, using the BioRad (Bradford) protein assay (BioRad, Hercules, Calif.), and was verified by SDS-PAGE comparison with known concentrations of bovine serum albumin. The identity of the protein as MPV-1a VP2 was confirmed by immunoblot (western blot) analysis, using rabbit anti-VP2 sera as described (3).

Cloning and purification of bacteria-expressed MVM VP2. The VP2 gene from the lymphotropic strain of MVM, MVMi, was cloned, using a procedure similar to that described previously. The specific PCR primers for MVMi VP2 were: 5' primer, 5'GAATTCGGATCCAAGTGATGGCACCAGCCAAC3' and 3' primer, 5'GAATTCGAAAGCTTGTAAGTATTTCTAGCAACAGG3'. However, after cleavage of the PCR product with *Bam*HI and *Hind*III, the PCR product was cloned directly into pET 20b+. Test inductions, large scale inductions, and denaturing purification were performed as described for MPV-1a VP2.

Enzyme-linked immunosorbent assay conditions. Microtitration plates (96-well Nunc Immulon or MaxiSorp plates, Naperville, Ill.) were coated, using 100 μl of phosphate-buffered saline (PBS; Dulbecco's phosphate-buffered saline without calcium chloride, Gibco BRL, Rockville, Md. [negative control]), MPV-1a VP2 or MVM VP2 diluted in PBS (test wells), or β-galactosidase diluted in PBS (negative control). The amount of antigen used to coat the plate was optimized by testing various concentrations of antigen against the same sera in the ELISA. Use of the lowest concentration, 0.75 μg/ml, yielded a reduced signal, but use of 1.5, 3.0, and 5.0 μg/ml yielded similar $OD_{450\ nm}$. Thus, a concentration of 1.5 μg/ml was used in all subsequent ELISAs. Plates were incubated at room temperature (RT) for two hours, then were kept overnight at 4°C. Antigen was removed, and wells were washed three times with 0.5% Tween 20 in PBS. To block non-specific binding of mouse serum, 200 μl of 3% gelatin/well was added, and plates were incubated for one hour at 37°C. After washing as before, 100 μl of diluted sera (1:50 or 1:100)/well was added and plates were incubated for one hour at 37°C. Each plate included positive- and negative-control sera. Plates were washed as before, then 100 μl of a 1:10,000 dilution of horse-radish peroxidase-linked goat anti-mouse immunoglobulin antibody was added, and plates were incubated for one hour at 37°C. After washing, tetramethylbenzidine substrate (TMB) was added to each well and plates were left at RT for five minutes. The substrate reaction was stopped by addition of 1*N* HCl, and reactivities were read at 450 nm on an ELISA reader. The threshold for a positive result in the ELISA, $OD_{450\ nm}$ of 0.3, was determined by calculating the mean $OD_{450\ nm}$ reading plus two standard deviations for 100 known negative sera.

Indirect fluorescent antibody test. The L3 cells (4) were infected with MPV-1a and were incubated at 37°C until approximately 25% of the cells in the culture were infected. Cells were spotted onto 12-well spot slides and were fixed in acetone. Serum was diluted 1:10 in PBS, and slides were incubated for 30 min at RT. Slides were rinsed three times in PBS, affinity-purified fluorescein-labeled goat anti-mouse IgG was added, and slides were incubated as before. Slides were rinsed three times in PBS, air-dried, cover-slipped, and examined by use of transmitting-fluorescence microscopy. Sera were judged positive if a pattern of green fluorescence of infected cells was seen similar to that for positive-control sera.

Sources of sera for testing. Sera were obtained from mice experimentally infected with MPV-1a or from field cases (naturally infected mice) submitted to the virology diagnostic laboratory in the Section of Comparative Medicine at Yale University School of Medicine. Husbandry conditions and experimental infections have been described (6), included humane care and use of animals with approval from the university's institutional animal care and use committee, and involved oronasal inoculation with three-hundred 50% tissue culture infective doses of MPV-1a in four- to six-week-old mice. Sera were tested at various postinfection (PI) times, from PI day 10 through approximately 9 weeks. Sera obtained at PI day 21 was used to titrate MPV antibodies. Sera were first screened by MPV IFA test, and results were used to classify them as MPV positive or negative. Positive-control serum was obtained from a seropositive, experimentally infected mouse, and negative-control serum was obtained from an uninfected mouse.

The ELISA conditions at Charles River Laboratories. The MPV-1a VP2 ELISA was licensed to Charles River Laboratories (CRL) by the Office of Cooperative Research at Yale University. Although CRL used the same MPV-1a VP2 as that used at Yale University, they used a slightly different procedure for the test. Antigen was diluted in 0.1*M* carbonate-bicarbonate buffer (pH 9.6) containing ovalbumin (5 mg/L) and bound to microtitration plates overnight at 2 to 8°C. Buffer alone was put into wells as a negative control. Plates from each batch were evaluated, using a panel of standard mouse and rat control sera and an indirect ELISA. Briefly, 50 μl of standard serum, diluted no lower than 1:50 in PBS with 5% bovine serum or in BLOTTO*(5% non-fat dry milk in PBS, [12]) with 20% bovine serum, was added to appropriate antigen wells and negative-control wells. After incubation for 40 min at 37°C, the plate was washed several times with 0.9% saline containing 0.05% Tween 20. Next, 50 μl of horseradish peroxidase-conjugated, affinity-purified goat anti-mouse IgG was added to each well, followed by incubation at 37°C for 40 min. Plates were washed as described previously, and 100 μl of 0.4 m*M* ABTS-2.0 m*M* H_20_2 chromogenic substrate was added to each well, then the plate was incubated at RT for 40 min. To stop the reaction, 25 μl of 1% SDS in water/well was added. Reaction intensity at 405 nm was determined by use of an ELISA reader. The net absorbance values (antigen-negative control) were converted to scores by dividing by 0.13. Net score ≥ 3 was considered a positive result. Serum was routinely tested by use of ELISA with antigens: MVM purified virions, MPV VP2 (from Yale), and their purified, baculovirus-expressed MPV-1a NS1. Additional tests were performed if a serum had positive results, and these could include IFA testing with MVM, KRV, or MPV-infected cells, or MVM HAI testing. In most instances, serum that only tested positive by MPV ELISA was re-assayed, using MPV IFA testing to confirm the result.

Data analysis. Data were analyzed, using Microsoft Excel.

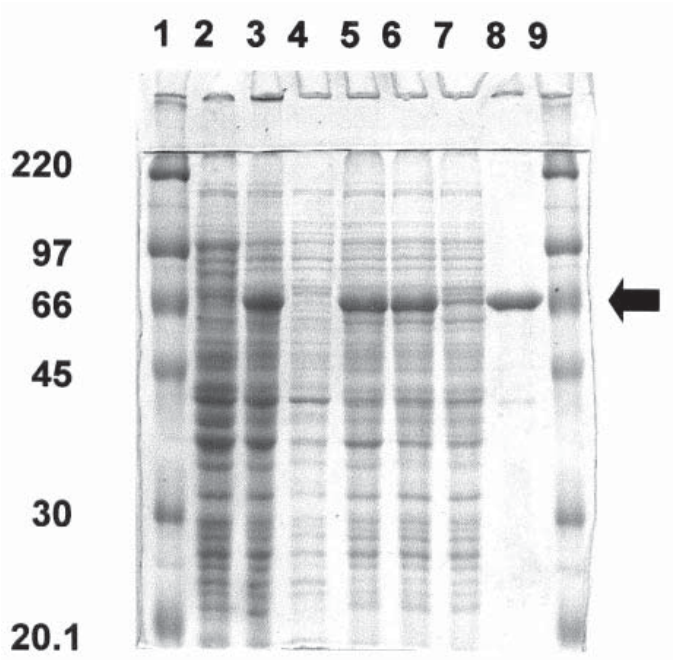

Figure 1. Analysis of bacterial lysates and purified proteins by use of 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Lanes 1 and 9 are high molecular weight protein markers, with the sizes (in kDa) indicated. Lanes 2 and 3 are bacterial lysate before and after induction, respectively. Lane 4 is total soluble fraction, lane 5 is total insoluble fraction, and lanes 6 and 7 are insoluble fraction after clarifying but before (6) and after (7) loading the column. Lane 8 shows eluted 64-kDa mouse parvovirus (MPV) VP2 protein. Arrow indicates the location of MPV VP2.

When comparing groups of equal size, Student's *t* test assuming equal variance was used, and when groups were not of the same size, Student's *t* test assuming unequal variance was used.

Results

Optimization of expression and purification of bacterial-expressed MPV VP2. Because MPV-1a is difficult to grow in vitro, we used a bacterial expression system to synthesize large amounts of capsid protein, VP2. In the pET expression system (Novagen), the VP2 gene is under control of the T7 bacteriophage promoter, and the expressor *E. coli* strain (BL21(DE3)) contains a genomic copy of T7 RNA polymerase expressed only when IPTG is added to the culture. Eight BL21(DE3) transformants were tested for VP2 expression after induction. Three cultures expressed Coomassie blue-detectable amounts of MPV1a VP2, and most VP2 was localized to the insoluble fraction. To maximize solubilization of VP2, buffers used to resuspend the insoluble fraction and to purify the protein contained 8*M* urea. Figure 1 illustrates a typical induction and purification of MPV1a VP2 using metal-chelation chromatography. Pre- (lane 2) and postinduction (lane 3) samples indicate that IPTG induces a large protein band at approximately 64 kDa. Comparison of soluble (lane 4) and insoluble (lane 5) fractions shows that most of the VP2 protein is present in the insoluble fraction, and comparison of pre-column and flow-through samples (lanes 6 and 7) shows that most of the protein binds to the resin during purification. As shown in lane 8, VP2 is the only protein detected in the eluted fraction. Typically, a 150-ml culture yields 1.2 mg of purified VP2.

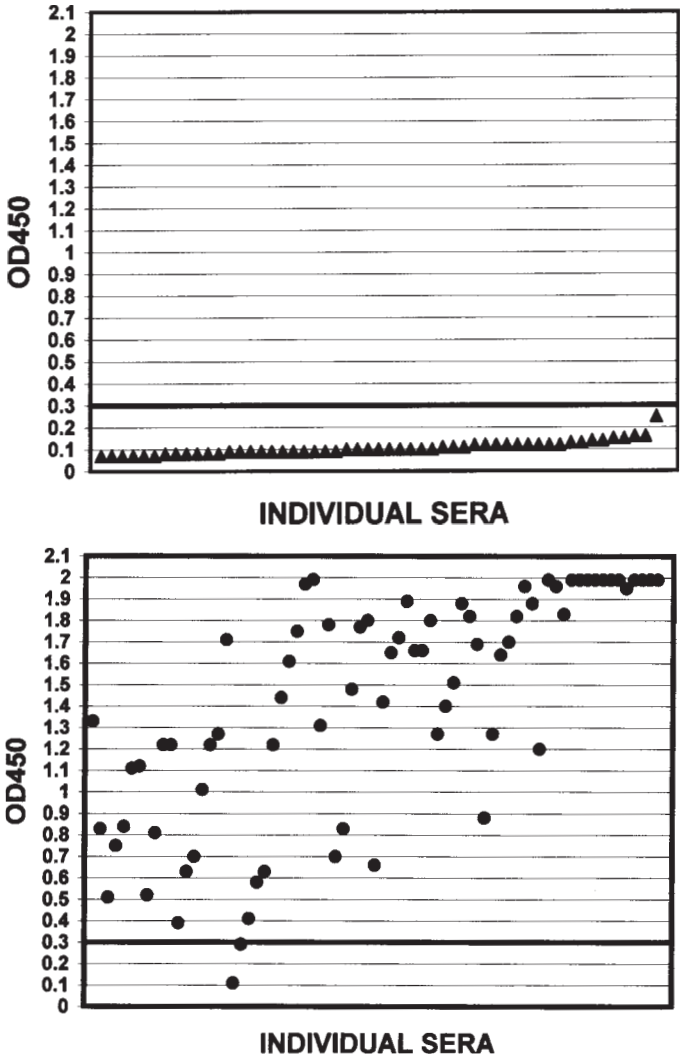

Figure 2. The MPV ELISA results for individual sera from experimentally infected mice. Mouse parvovirus indirect fluorescent antibody (IFA) test-negative (a; ▲) and positive (b; ●) sera were tested by use of MPV ELISA. Results are given for individual sera. The bold line at 0.3 $OD_{450\ nm}$ indicates the cut-off for test-positive samples.

Detection of MPV antibodies in experimentally infected mice. Initial validation of the ELISA was performed using sera obtained from 53 negative-control mice and 73 mice experimentally infected with MPV-1a. Sera were screened by MPV IFA test, then were re-assayed by MPV-1a VP2 ELISA (MPV ELISA) and MVM VP2 ELISA (MVM ELISA). All sera with negative results by IFA test had negative results by MPV ELISA (Fig. 2a), and 71 of 73 sera that had positive results by IFA test had positive results by MPV ELISA (Fig. 2b). Mean MPV ELISA $OD_{450\ nm}$ results were significantly ($P < 0.001$) different for MPV IFA-positive (Fig. 3; 1.41 ± 0.54) and -negative sera (Fig. 3; 0.106 ± 0.03). The sera with positive MPV IFA test results also were tested against MVM antigen, and the mean $OD_{450\ nm}$ value (Fig. 3; 0.56 ± 0.43) was significantly ($P < 0.001$) lower than that for the MPV antigen. Thirty-five of these sera were test negative against MVM antigen, and 38 had a lower $OD_{450\ nm}$ value than that for the MPV antigen. Thus, MPV infection can be differentiated from MVM infection by ELISA when serum reactivity is tested using

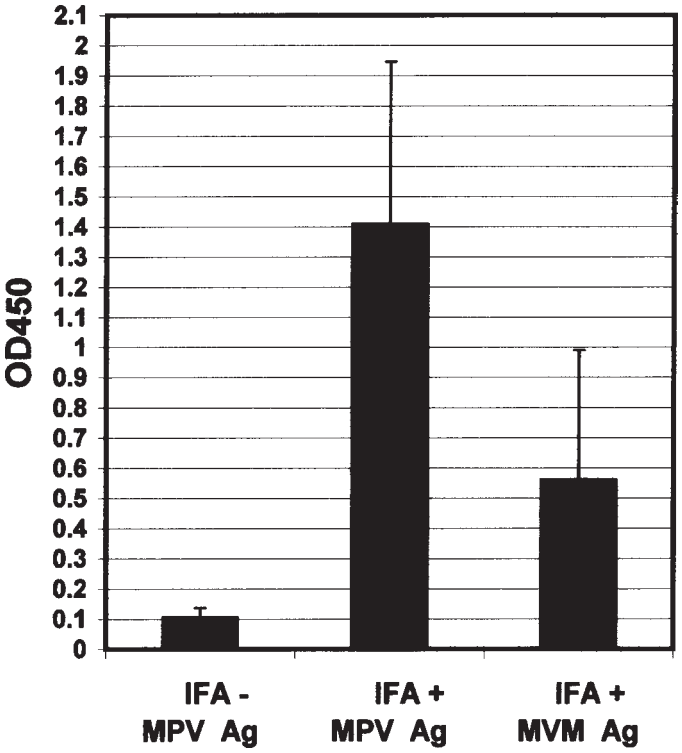

Figure 3. Summarized results for sera from experimentally infected mice. Mean (+ SD) is shown for the following groups: MPV ELISA results for MPV IFA test-negative sera, MPV ELISA results for MPV IFA test-positive sera, and minute virus of mice (MVM) ELISA results for MPV IFA test-positive sera.

both MVM and MPV VP2 antigens.

Ten MVM-positive sera from experimentally infected mice were tested by ELISA against MVM and MPV antigens, and all sera reacted positive to MVM antigen and negative to MPV antigen. Mean ± SD $OD_{450\,nm}$ against MVM antigen was 1.118 ± 0.51, whereas against MPV antigen, it was 0.18 ± 0.05. Thus, MVM infection also can be differentiated from MPV infection by use of the ELISA.

Detection of MPV antibodies in naturally infected mice. Sera from 457 mice were screened by use of MPV IFA testing; 387 were MPV negative, and 70 were MPV positive. All but one of the IFA test-negative sera were test negative by MPV ELISA (Fig. 4a), and 61 of 70 IFA test-positive sera were test positive by MPV ELISA (Fig. 4b). For IFA test-negative sera, mean $OD_{450\,nm}$ against MPV antigen (Fig. 5) was 0.105 ± 0.068, and for IFA test-positive sera, it was 0.927 ± 0.497. These values were significantly ($P < 0.001$) different, allowing easy diagnosis of positive sera. Only 20 of 70 IFA test-positive sera tested positive against MVM antigen. For IFA test-positive sera, mean $OD_{450\,nm}$ of 0.374 ± 0.270 for MVM antigen was significantly ($P < 0.001$) lower than the mean value for MPV antigen.

Results comparing the MPV VP2 ELISA with the MPV-infected cell IFA test are summarized in Table 1. Sensitivity, as determined by dividing the number of ELISA positive results by the number of ELISA positive plus false-negative results (samples positive by IFA testing, but negative by ELISA), was 92.3%. Test specificity, as determined by dividing the number of ELISA negative results by the number of ELISA false-positive

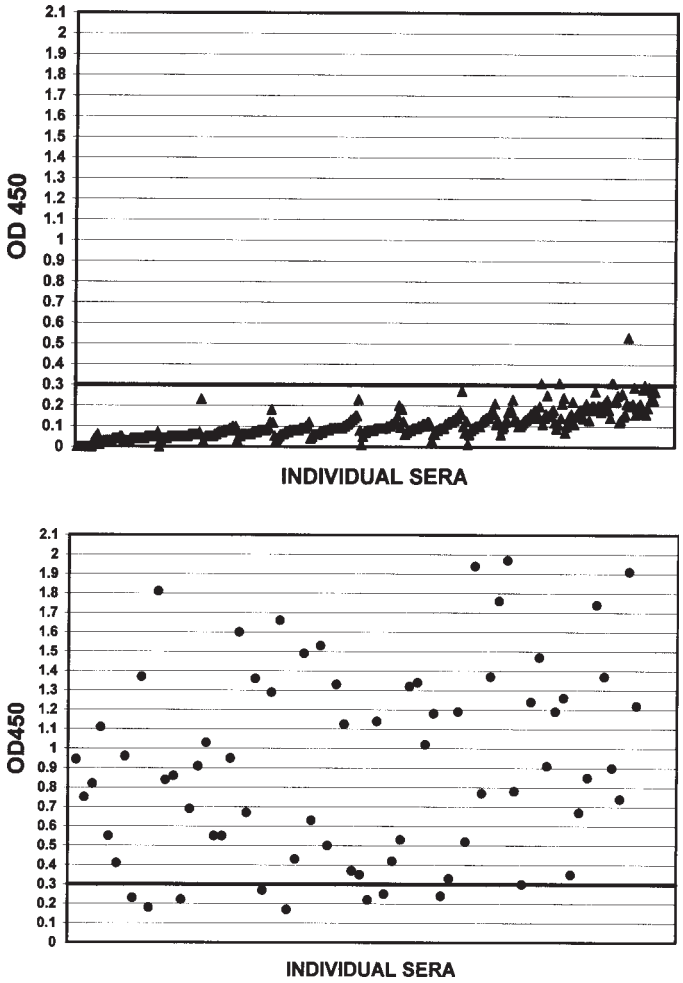

Figure 4. The MPV ELISA results for individual, diagnostic sera. Mouse parvovirus IFA-test- negative (a; ▲) and test-positive (b; ●) sera were tested by MPV VP2 ELISA. Results are given for individual sera. The bold line at 0.3 $OD_{450\,nm}$ indicates the cut-off for test-positive samples.

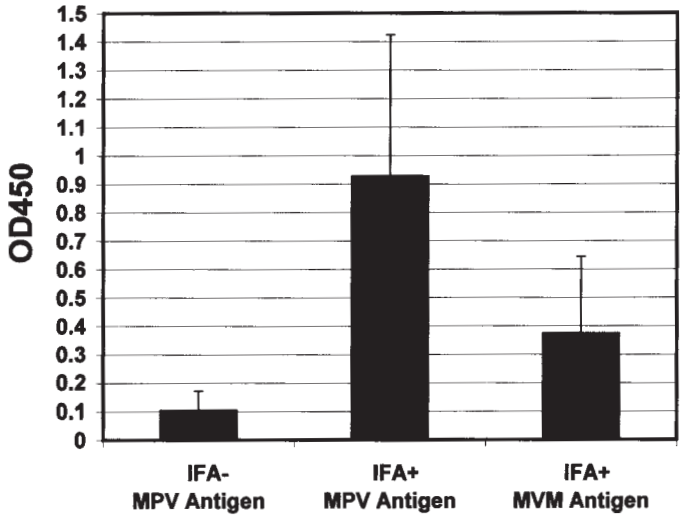

Figure 5. Summarized results for diagnostic sera. Mean (+ SD) is shown for the following groups: MPV ELISA results for MPV IFA test-negative sera, MPV ELISA results for MPV IFA test-positive sera, and MVM ELISA results for MPV IFA test-positive sera.

Table 1. Comparison of mouse parvovirus (MPV) indirect fluorescent antibody (IFA) test and MPV ELISA results

Result	IFA positive	IFA negative
ELISA positive	132	1
ELISA negative	11	439
Sensitivity (132/[132+11])	92.3%	
Specificity (439/[439+1])	99.8%	

Table 2. Comparison of serum titers for mice with experimentally induced infection

Sera	IFA[a]	ELISA[a]
1	500	1,000
2	500	15,000
3	500	2,500
4	500	2,500
5	500	5,000
6	500	2,500
7	500	7,500
8	500	2,500

[a]Number corresponds to the last dilution of sample with positive results.

results (samples positive by ELISA, but negative by IFA testing) plus ELISA-negative results, was 99.8%. Thus, the MPV VP2 ELISA is a sensitive and specific test for detection of MPV infection.

We also compared the anti-MPV titers for sera from 8 experimentally infected mice, using the IFA test and MPV ELISA. The endpoint dilution at which a positive reaction was detected was at least fivefold higher by ELISA than by IFA testing in seven of the mice (Table 2).

Comparison of diagnostic results obtained using MPV-1a VP2 and NS ELISAs. During two, one-week periods, CRL tested a total of 3,728 mouse sera by use of the NS1 ELISA (rNS1 ELISA), MPV-1a VP2 ELISA (MPV ELISA), and MVM virion ELISA (MVM ELISA) (Table 3), and the results of all positive sera were confirmed by IFA or HAI test. Ten parvovirus-positive sera were negative by MPV ELISA (90.3% sensitivity), whereas 36 parvovirus-positive sera were negative by NS ELISA (65% sensitivity). Although most of the negative results were confirmed by both ELISAs, the MPV ELISA had a higher number of false-positives results. Sixteen of these were not confirmed as false-positive results by the MPV IFA test, and 26 were at the threshold used to define a positive reaction (values of three to four, where three is the threshold for positive results). Overall, the specificity of the MPV ELISA was 98.3% and of the NS1 ELISA was 99.6%.

Discussion

Serologic testing for rodent viruses must be sensitive and specific to detect and discriminate between infections caused by related agents that differ pathogenetically and epidemiologically. Accurate diagnosis is essential before an appropriate strategy can be implemented to control and eliminate infection. Considering current trends toward high-density housing of genetically unique animals, tests with high sensitivity are necessary to detect infection at the earliest possible time. Additional factors required of screening tests are ease of use, cost-effectiveness, and adaptability to high-throughput as volume and frequency of testing increase.

The results show that the MPV ELISA meets the aforementioned criteria and is an effective diagnostic method for detection of MPV infection. Comparison with the IFA test indicated

Table 3. Comparison of MPV VP2 and rNS1 ELISAs

Result	All tests[a]	MPV ELISA	rNS1 ELISA
True positive (TP)	103	93	67
True negative (TN)	3625	3565	3611
False positive (FP)		60[b]	14
False negative (FN)		10	36
Sensitivity (TP/[TP+FN])		90.3%	65%
Specificity (TN/[FP+TN])		98.3%	99.6%

[a]Includes ELISAs and confirmation by results of MVM, KRV, or MPV IFA testing.
[b]16 of these sera were not tested by use of MPV IFA testing.
MVM = minute virus of mice; KRV = Kilham rat virus.

that the MPV ELISA has high sensitivity and specificity. In the ELISA, most of the IFA positive sera were confirmed and the results were easily distinguished from those of negative sera. In addition, serogroup of the infecting agent was distinguished by direct comparison of reactivity to MPV and MVM antigens. Many MPV-positive sera were test negative against MVM antigen; however, when sera contained MVM cross-reactive antibodies, OD readings were at least twofold higher against MPV antigen, allowing serogroup identification. Definition of serogroup by IFA testing requires extensive experience because this test detects antibodies to viral NS and VP proteins, and distinctions are made by qualitative differences in fluorescent signal or localization patterns. In addition, the MPV ELISA proved more sensitive when determining antiviral antibody titers in sera from animals with experimentally induced infection. A range of endpoints at higher dilutions was attained by use of ELISA, whereas a single endpoint was attained at a low dilution by use of IFA testing. Thus, the MPV ELISA may be more sensitive than the IFA test in detecting VP2 antibody.

During MPV ELISA testing at Yale University, there were low numbers of sera that were IFA test positive and ELISA negative. One possibility for the discrepancy is incorrect classification of sera by IFA testing because all diagnostic tests have some degree of false-positive and false-negative results. If this were the case, test sensitivity and specificity would be affected because those calculations were based on IFA test results to simplify data analysis. Therefore, actual sensitivity and specificity of the MPV ELISA may be higher than that calculated during the validation process. A second possibility for the discrepancies could be loss of low-titer MPV antibodies during storage of sera. All sera were screened by IFA testing, then were stored at -80°C prior to testing by ELISA.

Non-immune mouse sera did not react with VP2 antigen in the ELISA. The $OD_{450\ nm}$ results were comparable in PBS-, β-galactosidase-, and VP2-coated wells. In addition, there was only one serum from experimentally or naturally infected mice at the university that had a false-positive result. Although the diagnostic screening of 3,728 sera resulted in 60 sera with false-positive results at CRL, the total number of negative results confirmed by MPV ELISA was 3,565. As a result, specificity of the test is excellent at 98.3%.

In direct comparison, sensitivity of the MPV ELISA was higher than that of the rNS1 ELISA (90.3% versus65%, respectively). Although the rNS1 ELISA may detect new, highly divergent strains of parvovirus because of conserved NS1 homology, it is unable to distinguish serogroup of the infecting agent. In addition, data presented here and previously published results (10) indicate a high rate of false-negative results by rNS1 ELISA. In a screening test, false-negative results are problematic be-

cause an infected colony will go undetected. Thus, application of the MPV ELISA will increase specificity and sensitivity of diagnostic detection of MPV infection of laboratory mice.

Future research should address two issues regarding the diagnosis of MPV infection. Sera from experimentally infected animals should be tested at multiple times after inoculation of virus to define the kinetics of seroconversion to individual viral proteins, time of peak anti-viral titer, and duration of humoral immunity. Additional potential variables, such as mouse strain and age, also should be examined in such experiments. The effect of dual or staggered parvovirus infection on seroconversion should be defined as infection with one agent may affect the pathogenesis of and seroconversion to the second agent. Parvoviruses continue to be troubling infectious diseases of laboratory rodents, and current evidence suggests there may be more unrecognized agents in animal colonies (8, 13). As these agents are isolated and characterized, specific diagnostic tests need to be developed to detect their presence in colonies of laboratory rodents.

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