Rapid Field Immunoassay for Detecting Antibody to Sin Nombre Virus in Deer Mice

Tony Schountz,* Charles H. Calisher,† Tiffany R. Richens,‡ Audrey A. Rich,* Jeffrey B. Doty,† Mark T. Hughes,† and Barry J. Beaty†

We developed a 1-hour field enzyme immunoassay (EIA) for detecting antibody to Sin Nombre virus in deer mice (Peromyscus maniculatus). The assay specificity and sensitivity were comparable to those of a standard EIA. This test will permit identification of rodents with antibody to this and perhaps other hantaviruses.

Hantaviruses (family Bunyaviridae, genus Hantavirus) are rodentborne or insectivoreborne viruses; some are recognized causes of human hemorrhagic fever with renal syndrome or hantavirus pulmonary (or cardiopulmonary) syndrome (HPS) (1). The normal transmission cycle is rodent to rodent, without arthropod intermediate hosts. Each hantavirus has a single principal reservoir host, which suggests a coevolutionary relationship (2). In North America, the principal cause of HPS is Sin Nombre virus (SNV) because of the geographically widespread nature of its rodent host, the deer mouse (Peromyscus maniculatus), the most common mammal in North America.

As with other rodent reservoirs that harbor unique hantaviruses, most, if not all, deer mice become persistently infected without discernible pathologic consequences (3,4), which makes distinguishing infected from uninfected deer mice by simple observation impossible. Development of a field-relevant technique for detection of antibody to SNV would be of value; the technique could be exploited for further investigations of the virus–reservoir host interactions and characteristics and to determine whether experimental infections of deer mice with SNV accurately parallel natural infections (3,4).

Commonly used serologic tests for deer mice require a minimum of 3–5 hours to complete (2,5,6) and thus are impractical to use in the field in a single day without putting the rodents at risk for death from heat, cold, dehydration, trap injuries, and other hazards while tests are being conducted. We modified a previously described protein-A/G horseradish peroxidase enzyme-linked immunosorbent assay (PAGEIA) to detect antibodies to SNV in deer mice (7). The test can be completed in ≈1 hour under relatively primitive field conditions. The assay has advantages over more laborious assays used for similar purposes and, because it is mammal-specific rather than species-specific, we expect this assay will be applicable to serologic tests of mammals of many other species.

The Study

A fragment of the S segment (nt 43–394) encoding part of the nucleocapsid was cloned into pET21b with a C-terminal His tag to produce a 15-kDa truncated antigen (8) for use in the assay. Deer mice were trapped near Fort Lewis, Colorado, and blood was collected as previously described (9); whole blood was diluted in (1:100) 1 mL of phosphate-buffered saline (PBS) in 96 deep-well plates (P-DW-11-C, Axygen, Union City, CA, USA) at time of collection to expedite sample loading. The remainder of the blood was frozen on dry ice and returned to the laboratory for additional testing.

Wells of 96-well polystyrene microtiter plates (Falcon 353912, BD Biosciences, San Jose, CA, USA) were coated with 100 μL of 2 μg/mL recombinant nucleocapsid in PBS and blocked (0.25% gelatin in PBS) a week in advance. Wells were washed in the field 3× with 200 μL of PBS (pH 7.0) by using an 8-channel pipettor, and blood in PBS was added from the deep well plate; positive and negative (1:100) controls (diluted in PBS) were included. Plates then were incubated at ambient temperature (range ≈23°C–29°C) for 30 min. After 3 more washes with PBS/0.5% Tween-20, 100 μL of pretitrated staphylococcal protein-A/streptococcal protein-G horseradish peroxidase conjugate (Pierce Biotechnology, Inc., Rockford, IL, USA) diluted 1:1,000 in PBS was added for 30 min. Plates again were washed 3× with PBS-Tween-20, and 100 μL of activated ABTS substrate was added to each well. After 15 min of incubation at ambient temperature, wells were scored by using a 0–4+ system, with 0 indicating no reaction (i.e., clear, no color) and 4+ representing the strongest signal (i.e., dark green color). Samples deemed 1+, 2+, 3+, or 4+ were considered positive (very weak, weak, strong, very strong, respectively). Samples were retested under laboratory conditions with PAGEIA and standard Centers for Disease Control and Prevention (CDC) enzyme immunoassay (EIA) (5).

Blood samples from 222 deer mice were collected during 3 trapping sessions in the summer of 2006, and 39 samples were scored as positive in the field by PAGEIA; 183 were negative by the field PAGEIA, repeat laboratory PAGEIA, and the standard EIA in the laboratory. One sample (HA-2564) was scored negative by field and laboratory PAGEIA, but (low) positive (optical density [OD] of 0.327) by conventional EIA (Table).
Of the 39 samples that were scored positive in the field, 5 discrepancies between these and laboratory tests were found (Table). One sample (TS-0830–7) scored as 1+ in the field was determined to be negative on subsequent laboratory testing by both PAGEIA and conventional EIA. The other 4 samples (HB-2628, HA-2609, HA-2616, HB-2710) were scored as positive by field and laboratory PAGEIA but negative by conventional EIA. In the field, each of these samples was scored as 1+ or 2+ and had ODs of 0.331–0.664 by laboratory PAGEIA. However, ODs ranged from 0.076 to 0.228 by conventional EIA.
The PAGEIA results were similar to results of conventional EIA, with a specificity of 82.9% (184 negatives/222 total rodents) versus 84.7% (188/222) for conventional EIA. The sensitivity of the PAGEIA was 97.1% (34 positive by PAGEIA/35 positive by conventional EIA).

Conclusions

We have modified an existing serologic assay so that it is suitable for use in the field. The assay relies on a staphylococcal protein-A and streptococcal protein-G horseradish peroxidase conjugate (10). Each protein has the capacity to bind to the Fc portions of antibodies, including immunoglobulin M (IgM) and IgA for protein A (11,12), but has highest affinity for IgG subclasses of many mammalian species.

All samples scored 3+ or 4+ were also positive in laboratory tests when results were read by using a spectrophotometer. Thus, we are confident that such samples in the field will indicate seropositive animals. Because we are suggesting that this assay be used for identifying seropositive rodents and not for determining seroprevalence (although it appears to be adequate for those studies as well) and to be conservative, we considered only samples that appeared dark green (3+ and 4+) in the field assay to be positive with relative certainty. To minimize the complexity of the PAGEIA under field conditions, we did not use a negative control antigen to assess nonspecific reactivities of serum samples. Use of this test will allow deer mice with antibody to SNV to be identified. Deer mice are the population most likely to be naturally infected with that virus, and those rodents can be retained for further testing and for studies of tissues, live cells, and body fluids to be used for subsequent laboratory investigations, such as for determining cellular immunologic responses, viremia levels, viruria levels, and virus shedding in excreta and secretions.

Additional limitations of the PAGEIA are similar to those of other serologic tests. PAGEIA can detect only seropositivity, which is not necessarily indicative of current infection or of current shedding of virus. It also binds only with high affinity to IgG; thus, it is not useful for discriminating other immunoglobulin classes, such as IgM, the presence of which usually indicates recent infection.

Because of the broad mammalian species specificities of a protein-A and protein-G conjugate, the rapid PAGEIA likely can be used to test for antibodies to other antigens in other mammals. Lee et al. (7) characterized the reactivities of protein A and protein G with IgG from rodents of several species. They found that serum specimens from both sigmodontine rodent species (deer mice and hsdipoid cotton rats, Sigmodon hispidus) they tested were recognized by protein-A and/or protein-G conjugates. Similar laboratory-based PAGEIAs have also been used to detect antibody to antigens of agents causing other infectious diseases, including severe acute respiratory syndrome coronavirus-like viruses and Nipah virus in bats (13–15).

Acknowledgments

We thank Brian Hjelle for helpful discussions on the manuscript.

Funding was provided by NIH contract AI25489 (to T.S., C.H.C., and B.J.B.) and grant AI054461 (to T.S.). C.H.C. was also partially funded by CDC, contract US3/CCU 813420-09. Beta Betta Biological Honor Society and the University of Northern Colorado also provided support (to A.A.R. and T.S., respectively).

Dr Schountz is an assistant professor of microbiology in the School of Biological Sciences at the University of Northern Colorado. His research interest is the immunologic basis of persistence of zoonotic agents.

References


Address for correspondence: Tony Schountz, School of Biological Sciences, Box 92, University of Northern Colorado, Greeley, CO 80639, USA; email: tony.schountz@unco.edu