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## Comparison of the serum neutralization test and a competitive enzyme-linked immunosorbent assay for the detection of antibodies to vesicular stomatitis virus New Jersey and vesicular stomatitis virus Indiana

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**Abstract.** A competitive enzyme-linked immunosorbent assay (C-ELISA) for the detection of antibodies against vesicular stomatitis virus New Jersey (VSV-NJ) and vesicular stomatitis virus Indiana (VSV-IN) was compared with the serum neutralization test (SNT) using 1,106 serum samples obtained from dairy cattle on sentinel study farms in the Poás region of Costa Rica. Kappa coefficients between the C-ELISA and the SNT were 0.8871 (95% confidence interval [CI]: 0.8587–0.9155) and 0.6912 (95% CI: 0.6246–0.7577) for the VSV-NJ and VSV-IN tests, respectively. These results indicate good to excellent agreement between the 2 tests under these conditions.

Vesicular stomatitis (VS), a viral disease which primarily affects cattle, horses, and swine, occurs in endemic and epidemic forms in the tropical and subtropical areas of the Americas.<sup>1</sup> In temperate regions of Mexico and the United States, VS causes epidemics at variable intervals, whereas in southern Mexico and throughout Central and South America the disease is considered endemic.<sup>9</sup>

VS is mainly caused by 2 major serotypes of vesicular

stomatitis virus, New Jersey (VSV-NJ) and Indiana (VSV-IN), of the genus *Vesiculovirus* in the family Rhabdoviridae and order Mononegavirales.<sup>1</sup> Both viruses have a negative sense single-stranded RNA genome and 5 distinct proteins (L, G, N, P, and M). The serum of infected cattle contains high titers of virus-neutralizing antibodies induced by the surface glycoprotein (G). The nucleocapsid (N) protein is highly antigenic, induces a nonneutralizing antibody, and plays a central role in the regulation of transcription and replication of viral RNA.<sup>2</sup>

Both serotypes cause identical lesions on the epithelia of the mouth and tongue and on the coronary bands of cattle, pigs, and horses. In cattle, the 2 serotypes also affect the teats, making milking difficult. The importance of the disease resides in its clinical resemblance to foot-and-mouth disease (FMD) and the productivity losses it produces through anorexia, lameness, reduced milk yield, and mastitis. In addition, VS viruses infect humans, causing clinical

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symptoms ranging from a self-limited flu-like illness to severe encephalitis.<sup>4,8</sup>

The early diagnosis of VS is important to differentiate it from other more serious vesicular diseases such as FMD. Numerous serologic assays for detection of antibodies to VS viruses have been developed. The most widely used are the complement fixation test (CFT), the serum neutralization test (SNT), and more recently a competitive enzyme-linked immunosorbent assay (ELISA). However, the CFT and SNT have several disadvantages. The CFT has a low sensitivity, is complex to perform, and cannot be used with anticomplementary and hemolyzed serum samples. The SNT requires maintenance and use of live virus and cell cultures, must be performed under aseptic conditions, and requires up to 3 days for results. Recently, 2 serotype-specific competitive ELISAs for detection of antibodies to VSV-NJ and VSV-IN in animal serum samples were developed. These assays are rapid and inexpensive and show high sensitivity and specificity when compared with the SNT.<sup>1,3</sup>

The purpose of this study was to compare the competitive ELISA for the detection of antibodies to VSV-NJ and VSV-IN in cattle naturally infected with VS viruses, using the SNT as the reference test.

Serum samples from 88 cattle were collected monthly between April 1997 and July 1998 from 3 dairy farms in the Poás region of Costa Rica. The Poás region is premontane wet forest, with an average annual rainfall of >2,000 mm and temperatures ranging from 15 to 30 C throughout the year. The region is considered endemic for VS. Animals were between 16 and 38 months of age at the initial sampling. A total of 21 animals were repeatedly sampled between 1 and 15 times ( $\bar{x}$  = 12.1 times) on farm 1, a total of 22 animals were repeatedly sampled between 1 and 15 times ( $\bar{x}$  = 12.5 times) on farm 2, and a total of 45 animals were repeatedly sampled between 6 and 16 times ( $\bar{x}$  = 12.8 times) on farm 3. A total of 1,106 serum samples were obtained.

A microtiter neutralization test was used for the detection of neutralizing antibodies to VSV-NJ and VSV-IN.<sup>9</sup> Serum samples were heat inactivated (56 C, 30 minutes) and diluted from 1:100 to 1:6,400 (4-fold dilutions) with Dulbecco minimum essential medium in duplicate wells of tissue culture plates,<sup>a</sup> and 100–200 TCID<sub>50</sub> of VSV-NJ (Greentree strain) and VSV-IN (Chimayo strain) were added to each well. After incubation of the plates at 37 C in 5% CO<sub>2</sub> for 1 hour, Vero E6-cells were added as indicator cells (10,000 cells/well). Cell control, virus control, positive control, and negative control serum samples were included in each neutralization assay. All plates were read in 48 hours, and samples were considered positive if either well (New Jersey or Indiana) had a confluent monolayer of Vero cells.

The C-ELISA protocol developed by the National Veterinary Services Laboratories (NVSL, Ames, IA) was used throughout all experiments with some modifications.<sup>1</sup> Microtiter plates<sup>b</sup> were coated with 75  $\mu$ l recombinant antigen (protein N) of VSV-NJ and VSV-IN, diluted 1:2,500 in 0.01 M phosphate-buffered saline (PBS), pH 7.4 (wells G11 and G12 were used as blanks), and incubated for 16 hours at 4 C. The coating solutions were decanted, and 100  $\mu$ l/well of blocking solution (0.01 M PBS, 5% nonfat dry milk [NFDM]) was added to the plates and incubated for 1 hour at 37 C with constant agitation.

**Table 1.** Comparison of serum neutralization test (SNT) and competitive ELISA for detection of antibodies to VSV-NJ. Kappa coefficient = 0.8871 (95% confidence interval: 0.8587–0.9155).

SNT	ELISA		Total
	Positive	Negative	
Positive	360	50	410
Negative	7	689	696
Total	367	739	1,106

The plates were then washed 3 times with washing buffer (0.002 M PBS, 0.05% Tween-20), and bovine control and test serum samples diluted 1:8 in PBS (containing 1 M NaCl, 0.05% Tween-20, 1% NFDM) were added in duplicate wells (50  $\mu$ l/well) and incubated 30 minutes at 37 C with constant agitation. In wells A1/A2, only diluent was added as a control, and in wells G11/G12, no serum samples were added. Positive control serum samples were added to 2 wells. Polyclonal antibodies to VSV-NJ and VSV-IN were diluted 1:2,500 in PBS (containing 1 M NaCl, 1% NFDM), and 50  $\mu$ l of this solution was added to each well. The plates were incubated 30 minutes at 37 C with constant agitation. After washing the plates, the conjugate goat antimouse IgG (H+L) peroxidase<sup>e</sup> was diluted in 0.01 M PBS (containing 1% NFDM, 12.5% goat serum), added to each well (50  $\mu$ l/well), and incubated for 1 hour at 37 C with constant agitation. After washing, 50  $\mu$ l/well of peroxidase substrate (1 mg of tetramethylbenzidine-2HCl, diluted in 10 ml of 0.05 M phosphate citrate buffer, pH 5, containing 20  $\mu$ l of 3% H<sub>2</sub>O<sub>2</sub>) was added. The reaction was stopped with 2 M H<sub>2</sub>SO<sub>4</sub>, and the optical density (OD) was recorded at 450 nm.<sup>d</sup> The results were interpreted as a reduction percentage (RP) of the OD. The mean OD of the diluent control was considered 0 RP. The following formula was used to determine the RP of the test serum samples:  $RP = 100 - [(mean\ OD\ of\ the\ test\ serum / mean\ OD\ of\ the\ diluent\ control) \times 100]$ .

Cutoff points for the C-ELISA were calculated using 92 and 98 negative samples to VSV-NJ and VSV-IN, respectively. All of these samples had SNT titers of <1:10. The cutoff was defined as the mean of the RP of the negative serum samples plus 3 SD. The C-ELISA cutoff point was 16.89 RP for VSV-NJ and 17.48 RP for VSV-IN. The kappa coefficient was calculated to determine the magnitude of the statistical agreement between the 2 tests. Statistical analysis was conducted using commercially available software.<sup>e</sup>

Of the 1,106 samples tested for antibodies to VSV-NJ by the SNT, 410 (37.1%) samples were positive and 696 (62.9%) were negative (Table 1). Of the 1,106 serum samples analyzed by the C-ELISA, 367 (33.2%) samples were positive and 739 (66.8%) were negative. A kappa coefficient of 0.8871 (95% confidence limits of 0.8587–0.9155) was calculated between the SNT and C-ELISA for VSV-NJ. There were 57 discrepant results, with most (87%) of the SNT-positive results considered negative by the C-ELISA.

Of the 1,106 serum samples tested for antibodies to VSV-IN by the SNT, 151 (13.7%) samples were positive and 955 (86.3%) were negative (Table 2). Of the 1,106 samples analyzed by the C-ELISA, 113 (10.2%) were positive and 993 (89.8%) were negative. A kappa coefficient of 0.6912 (95% confidence limits of 0.6246–0.7577) was calculated between

**Table 2.** Comparison of serum neutralization test (SNT) and competitive ELISA for detection of antibodies to VSV-IN. Kappa coefficient = 0.6912 (95% confidence interval: 0.6246–0.7577).

SNT	ELISA		Total
	Positive	Negative	
Positive	96	55	151
Negative	17	938	955
Total	113	993	1,106

the SNT and C-ELISA for VSV-IN. There were 72 discrepant results, with most (76%) of the SNT-positive results considered negative by the C-ELISA.

Rapid and accurate diagnosis of VS virus infections is a key component to control of this disease and is imperative for the differentiation of VS from other more serious and clinically indistinguishable diseases such as FMD. The Office of International Epizootics lists the CFT, ELISA, and SNT as prescribed tests, i.e., those considered optimal for determining the health status of animals before shipment.<sup>7</sup> The disadvantages of the CFT and SNT have been discussed previously. The NVSL uses the C-ELISA as the initial procedure for serodiagnostic screening purposes. The NVSL reported that the C-ELISA has a specificity of 99% and a sensitivity of 88% relative to the SNT.<sup>3</sup> A kappa coefficient similar to that determined in the present study for VSV-NJ testing was calculated from the data published by the NVSL (0.8871 vs 0.8823).

The lowest SNT dilutions in this study were 1:100, and sera positive at this dilution and higher were considered positive. For diagnostic purposes, considering an SNT titer of <1:100 as negative increases the specificity of the test, reducing the number of false positives. However, for trade and other diagnostic purposes in nonendemic areas, SNT titers of  $\geq 1:32$  are generally considered positive. The SNT cutoff point of 1:100 in this study resulted in a number of sera considered negative by SNT being considered positive by C-ELISA (7 for VSV-NJ and 17 for VSV-IN). Discrepant results may also be due to detection of different antibodies in the SNT and C-ELISA. The SNT detects neutralizing antibodies or those directed against the glycoprotein of VS viruses.<sup>10</sup> The ELISA incorporates the nucleoprotein as antigen and thus detects antibodies made against the nucleoprotein.<sup>5</sup>

The kappa coefficient indicates how much the observed agreement exceeds that expected by chance alone. A kappa coefficient of  $>0.75$  represents excellent agreement, and kappa coefficients between 0.40 and 0.75 represent intermediate to good agreement.<sup>6</sup> The kappa coefficient calculated for the C-ELISA and SNT for VSV-NJ indicated excellent agreement between the 2 tests, and that calculated for VSV-IN indicated good agreement between the 2 tests.

The C-ELISA performed well when compared with the

SNT. The C-ELISA should be used as a screening test in VS outbreaks and can be used as a diagnostic test in nonoutbreak periods. The C-ELISA provides a more rapid and economical detection of antibodies to VSV-NJ and VSV-IN than does the SNT and should be incorporated into vesicular disease surveillance systems.

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