

SCIENTIFIC NOTE

EFFECTIVENESS OF A COMMERCIAL COMPETITIVE ELISA FOR THE DETECTION OF WEST NILE VIRUS ANTIBODIES IN SENTINEL CHICKENS

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ABSTRACT

The use of sentinel chickens is a powerful tool to aid in arboviral surveillance efforts. Results from this type of surveillance is used to help guide abatement efforts for vector species. Many mosquito control programs use outsourced laboratories for testing sentinel chicken samples. This study evaluated the use of a commercially available competitive enzyme-linked immunosorbent assay (cELISA) for in-house testing for West Nile virus antibodies from chicken serum. The commercial cELISA detected more positive sentinel chickens compared to the outsourced laboratory results. Results obtained from the in-house testing of chicken serum were reported sooner compared to the outsourced laboratory results, enhancing mosquito control programs timeliness for response to mosquito-borne disease.

Key Words: Antibody; chicken serum; ELISA; sentinel; West Nile virus

Vector-borne diseases remain a major human, veterinary, and wildlife health threat globally, and monitoring transmission is a critical process in the management of such diseases (Langevin et al. 2001). Mosquito control programs are tasked with the control and prevention of arboviral activity within their communities. In the United States, West Nile virus (WNV) is the most prevalent mosquito-borne disease and leads to human morbidity and mortality annually (Danforth et al 2021). As treatment and prevention options are limited for WNV, control of the pathogen vector is most effective in the reducing disease. Screening sentinel chickens for viral activity provides the relevant authorities the knowledge needed to guide their vector abatement decisions (Olson et al. 1991, Johnson et al 2003, Van Den Hurk et al. 2012, Day et al. 2015). Studies have shown that sentinel chicken surveillance can be one of the most sensitive indicators of virus activity in an area (Day and Lewis 1992, Reisen et al. 1994, Ramirez et al. 2018) and the use of enzyme-linked immunosorbent assays (ELISA) to test sentinel chicken serum is an effective tool (Johnson et al 2003).

As arbovirus infected female mosquitoes feed on sentinel chickens, the arbovirus is passed directly into

the chicken blood from the mosquito saliva (Komar 2000). After an intrinsic incubation, antibodies develop in the sentinel chicken and can then be detected through serological assays. Though serological surveillance has an inherent delay (the need to wait for antibody development in the sentinel animal) and is less precise compared to other assays such as PCR, serology provides the benefit of providing location and time of transmission events. (Langevin et al. 2001). On the other hand, surveillance of arboviruses using mosquito pools can be difficult since mosquito infection rates are often extremely low in nature and does not represent transmission events (Day et al. 2015, Tang et al. 2020).

Some state health departments provide local mosquito control programs free or low-cost testing of sentinel samples. However, testing through large, centralized laboratories has its advantages and limitations. Advantages of the centralized laboratories include the comparability of results and the quality assurance of samples. Limitations of using centralized laboratories often include the delay in receiving results due to the large number of samples to be tested and the time required for shipping and receiving samples between programs (Day and Lewis 1992, Reisen

et al. 1994, Sutkowska et al. 2021). The ability of mosquito control programs to receive their results in a timely manner helps maintain the relevance of those results for management and abatement decisions (Calisher et al. 1986, Johnson et al 2003). Outsourced laboratories often use a two-fold testing regimen which includes the initial “presumptive” result followed by confirmatory testing on presumptive positive samples. Though presumptive positive samples from outsourced laboratories may be provided within a few days, confirmatory results often take much longer. As districts often use confirmatory results to guide abatement decisions, a delay in confirmatory results being reported may negate the purpose of using sentinel samples as arboviruses would have been actively circulating before treatment measures have been deployed.

This study evaluated the effectiveness of a commercial competitive enzyme-linked immunosorbent assay kit (ID Screen West Nile Competition Multi Species; Innovative Diagnostics, Grabels, France – hereafter referred to as ‘ID Screen’) for in-house testing of sentinel chicken serum for WNV antibodies compared to the results from the traditionally utilized outsourced laboratory. As such, aliquoted subsamples from this study were also shipped to the outsourced laboratory for WNV antibody testing and confirmation. Sentinel chicken serum samples from this study were collected from the Anastasia Mosquito Control District (AMCD) and Lee County Mosquito Control District (LCMCD).

Sentinel chickens were bled weekly via a brachial or jugular venipuncture using a 3-mL syringe and a 25-gauge needle (Johnson et al 2003, FDOH 2021). Blood was transferred into 3.5-mL serum separator tubes (SST) and allowed to clot for at least 30-min and then centrifuged at >2,000 rpm for at least 10-min (Grasedieck et al. 2012). Serum was then removed from SSTs for testing and aliquots were shipped to the outsourced laboratory.

Sentinel chicken serum was tested in-house at both AMCD and LCMCD for WNV antibodies using the ID Screen kit according to manufacturer’s instructions. In short, controls and samples were diluted with buffer and transferred into the kit provided pre-coated microwells of the ELISA plate. After a 90-min incubation at room temperature, the plates were washed three times (Model 1575 Immunowash Microplate Washer, Bio Rad, Hercules, CA) with approximately 300 μ L of IX wash solution. Diluted conjugate was then added to all microwells, and the plates were incubated for another 30-min at room temperature. After another round of washing, substrate solution was added to all microwells, and a final 15-min incubation was conducted in the dark at room temperature. After the final incubation, stop solution was

added to all microwells, and the plates were read at 450 nm (iMark Microplate Absorbance Reader, Bio Rad, Hercules, CA). Optical density (OD) values were then converted into a signal-to-noise ratio (S/N%) using the following equation: $((OD_{\text{sample}} / OD_{\text{average negative control}}) * 100)$. Final results were reported as follows: S/N% of >50 were considered negative, >40 but \leq 50 were considered doubtful, and \leq 40 were considered positive (ID.Vet 2020).

Subsamples that were sent to the outsourced laboratory were initially screened (presumptive testing) using a hemagglutination inhibition assay (HAI). With the high volume of samples that come through the outsourced laboratory, this HAI assay was initially used to identify samples as being flavivirus antibody positive or not. If samples tested positive via the HAI assay, they were later confirmed positive for WNV (and/or SLEV) antibodies using an IgM ELISA assay and if needed a plaque reduction neutralization test (PRNT) (FDOH 2021). Assay protocols utilized by the outsourced laboratory are proprietary as they were provided to them by the Centers for Disease Control and Prevention.

During 2020 and 2021, a total of 311 sentinel chickens tested positive for antibodies (Table 1). Despite all 311 positive samples being tested by both the in-house and outsourced assays, only 300 (96.5%) were reported positive through in-house testing (WNV, ID Screen). The outsourced laboratory (WNV/SLEV, HAI) reported 212 (68.2%) positive samples (Table 1). Of the overall 311 antibody positive sentinel samples, 201 (64.3%) were positive via both in-house and outsourced testing, though not necessarily from the same week of testing.

Both the in-house ID Screen assay and the outsourced laboratory assay detected positive samples during the same sample week 182 times (90.6%, 182/201), however, the outsourced laboratory results took an average of 3.7 days longer to be reported in those instances (Table 1). The outsourced laboratory assay detected a positive sample prior to the week the in-house ID Screen assay was positive 17 times (8.5%, 17/201), averaging 9.7 days prior to the in-house ID Screen assay (Table 1). When taking into account the delay in results being reported from the outsourced laboratory, the results from these 17 instances were reported on average 6.0 days prior to the in-house assay results. The in-house ID Screen assay detected a positive prior to the week the outsourced laboratory assay was positive two times (1.0%, 2/201), averaging 46.0 days prior to the outsourced laboratory. When accounting for the delay, results from the in-house ID Screen assay were reported on average 49.5 days prior to the outsourced laboratory in these instances.

Table 1. Positive sentinel chickens from the Anastasia Mosquito Control District (AMCD) and Lee County Mosquito Control District (LCMCD) during 2020 and 2021 using the in-house commercial competitive ELISA kit and the hemagglutination inhibition assay results provided by the outsourced laboratory testing.

	2020		2021		Total
	AMCD	LCMCD	AMCD	LCMCD	
Number of positive birds	1	208	12	90	311
Positive birds from in-house testing	1	208	12	79	300
Positive birds from outsourced testing	1	110	12	89	212
Number of confirmed outsourced birds	1	71	4	41	117
Average days to receive initial outsourced results (range)	4 (4)	3.6 (3 to 4)	5.2 (4 to 11)	3.5 (3 to 4)	3.7
Average days to receive confirmed outsourced results (range)	60 (60)	10.3 (3 to 23)	8.5 (4 to 15)	13.0 (3 to 50)	11.6

Of the 212 presumptive antibody positive sentinel samples from the outsourced laboratory, 117 (55.2%) were later confirmed WNV positive via the PRNT. These confirmatory results were reported, on average, 11.6 days after samples were collected (Table 1).

In this study, the commercial ID Screen assay detected more of the positive samples compared to the outsourced laboratories presumptive HAI testing. A majority of the time, both testing methods detected a positive sample from the same sampling period; however, the outsourced laboratory had an inherent delay in reporting results, which is an important outcome of this study. The use of ELISAs for the detection of IgM antibodies has proven to have quicker turnaround times compared to HAI and PRNT assays from the outsourced laboratory (Calisher et al. 1986). The sooner these results can be reported to the control programs the sooner abatement efforts can be enacted, which directly relates to the overall effective control of potential WNV exposure. On that same note, confirmatory results from the outsourced laboratory took over three times as long to be reported when compared to the presumptive positive results. This delay in receiving confirmatory results from the outsourced laboratory may not be an issue if control programs base their abatement decisions on the presumptive results (WNV/SLEV).

The outsourced laboratory presumptive testing did detect positive samples from a sampling period prior to the in-house ID Screen assay at a higher rate (17 times verses 2 times). However, the magnitude between those differences was drastically greater when the in-house ID Screen assay detected a positive from a sampling period prior to the outsourced laboratory testing (average of 46.0 days prior compared to 9.7 days prior). This is something that must be considered by a program when deciding

whether or not to use in-house testing methods for their sentinel program. An additional point of consideration is the associated cost of in-house testing. Unlike the free service often provided by outsourced public health laboratories, in-house testing is not free. There is an initial investment required to purchase the microplate washer and microplate reader as well as pipettes. Then there is the yearly cost of purchasing the commercial ELISA kits and consumables (pipette tips, etc.). For some control programs, this cost might be too great and thus the free service might outweigh the benefits provided through in-house testing.

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