

Rapid Detection of West Nile Virus in Fox Squirrels (*Sciurus niger*) Using RAMP® Test

Steve Schutz, Eric Ghilarducci, Damien Clauson and Mike McCoy

Contra Costa Mosquito & Vector Control District, 155 Mason Circle, Concord, CA 94520

ABSTRACT: A trial was conducted to determine whether the RAMP® rapid antigen test could detect West Nile virus in oral swabs from dead fox squirrels. Although only two squirrels out of 15 tested were RAMP® positive using the standard cutoff value of $R = 50$, six out of seven squirrels with R values greater than zero were confirmed to be PCR positive while all squirrels with $R = 0$ were PCR negative. Test results on corvids were species-specific; results were more accurate for American crows than for western scrub-jays. Adjusting the test conditions or lowering the cutoff value for positive results might improve the utility of RAMP® for squirrel and scrub-jay testing.

INTRODUCTION

During 2005, the Contra Costa Mosquito and Vector Control District utilized the RAMP® rapid antigen test for early detection of West Nile virus (WNV) in corvid oral swab samples. While we considered our use of these tests to be supplemental to PCR testing by the University of California Davis Center for Vectorborne Diseases (CVEC), the California Department of Health Services (CDHS) accepted RAMP® test results on dead crows as accurate enough to be considered 'official' for reporting purposes.

While corvids and certain other bird species are known to be susceptible to WNV, data collected by CDHS in 2004 suggested that tree squirrels may also be susceptible. The fox squirrel *Sciurus niger*, an introduced species, is very common in densely populated residential areas of the San Francisco Bay area (Byrne 1979), has a limited home range (Flyger and Gates 1982), and therefore could prove to be a useful sentinel for local virus transmission in urban areas. The California Department of Health Services, in collaboration with the Lindsay Wildlife Museum, a large wildlife rehabilitation center located in central Contra Costa County, had already initiated a study of WNV in squirrels submitted to the wildlife hospital (Padgett et al. 2005). Suspect squirrels reported to the CDHS statewide WNV hotline by the Museum were picked up by District personnel for shipment to the California Animal Health and Food Safety Laboratory (CAHFS) for necropsy and CVEC for PCR testing, a process which took up to 14 days. Since rapid testing is capable of providing same-day results and a much quicker operational response, we decided to investigate whether RAMP® could detect WNV in squirrel oral swabs.

MATERIALS AND METHODS

We used the standard RAMP® procedures recommended by the manufacturer, Response Biomedical, for testing oral swabs from corvids and squirrels and the standard cutoff value of $R \geq 50$ (determined automatically by the RAMP® reader from the ratio of a test band to a control) to decide whether a test result was positive (Response Biomedical Corp., 2005). All squirrels tested by RAMP® were subsequently submitted to CVEC for PCR testing. Western scrub-jays (*Aphelocoma californica*) testing positive ($R \geq 50$) were reported to DHS but not submitted for further testing while those

testing negative were submitted for PCR testing. Since RAMP® results on crows (*Corvus brachyrhynchos*) were considered definitive by CDHS, we did not submit the majority of crows we tested for PCR confirmation.

RESULTS

A total of 15 fox squirrels were RAMP® tested. Of these, only two had R values above the standard cutoff value of 50 recommended for corvids. These were both confirmed by CVEC to be PCR positive, as were five additional squirrels with R values less than 50 (Table 1). Therefore, using the standard cutoff value, 87% of our squirrel test results were false negatives. However, further examination of the data revealed that all seven of the PCR positive squirrels had RAMP® R values greater than zero, and all but one of eight PCR negative squirrels had $R = 0$. R values for PCR positive squirrels ranged from 8.6 to > 640 (mean = 134.6, standard error = 101.6) (Table 2).

Table 1. Fox squirrel RAMP test results. Samples with $R \geq 50$ were considered WNV positive.

Tested	RAMP +	RAMP -	False +	False -
15	2 (13%)	13 (87%)	0 (0%)	13 (87%)

Table 2. RAMP test results for individual fox squirrels with $R > 0$.

Date	DHS ID Number	City	RAMP	R	PCR
9/26	Q5-196	Concord	negative	8.6	positive
9/26	Q5-195	Walnut Creek	negative	33.4	positive
9/28	Q5-201	Pleasant Hill	positive	81.3	positive
10/4	Q5-213	Martinez	negative	19.0	positive
10/11	Q5-225	Martinez	positive	> 640	positive
10/18	Q5-232	Concord	negative	19.9	negative
10/21	Q5-233	Concord	negative	24.7	positive