MVCAC 75th Annual Conference, February 4-7, 2007

The Assessment of RAMP® and Real-Time PCR for West Nile Virus Testing

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Introduction

Since 2003, West Nile virus has been detected in mosquito populations in California and is now endemic throughout the state. It is therefore vital to mosquito abatement and surveillance programs to have a reliable, fast and efficient method to detect WNV in mosquito samples.

Receiving positive results has an important impact on mosquito control operations. A quick response time:

- can prevent virus transmission to humans, equines, birds, squirrels, etc by decreasing vectors and suppressing the mosquito life cycle.
- can reduce pesticide use (especially adulticides) and be more cost-effective by treating smaller and more specific areas.
- can streamline the public notification process (hence it is less likely to be controversial with anti-pesticide advocates).

Specific Aims of the Study

RAMP® is an antigen/antibody based assay which is quick (< 2 hours for results) and easy to perform. However, it is a less sensitive test compared to using Real-Time PCR. Additionally, RAMP® assays do not detect other arboviruses such as SLE and WEE that are endemic to California. With decreased sensitivity, it is possible that the RAMP® may miss lower levels of viremia in the mosquitoes, especially early in the season and possibly late in the season.

The purpose of our study was to compare RAMP® units with Ct values using Real-Time PCR (RT-PCR) on mosquito samples collected from San Joaquin County. Our Specific Aims were:

Specific Aim 1: To determine how RAMP® units compare with Ct values.

Specific Aim 2: To determine the seasonality of positive mosquito pools in San Joaquin County.

Materials and Methods

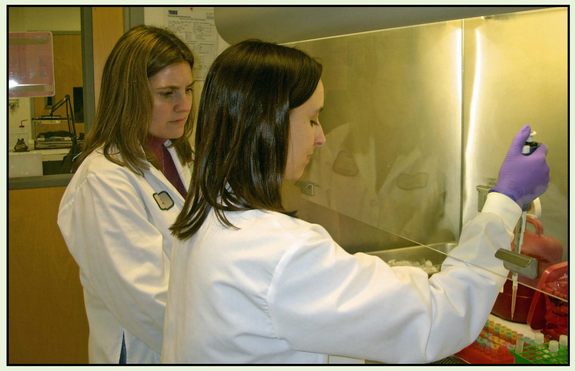
Sample Preparation: Mosquito samples were collected from May to Nov. 2006 from 8 sites in San Joaquin County. Pools of 1-50 *Culex spp*. per pool were ground in an appropriate diluent or RAMP® buffer. Aliquots were removed for both RAMP® and RT-PCR testing*. RAMP® testing was conducted according to manufacturer's protocol, with modification where non-RAMP® buffer diluents were used. RNA was extracted according to the manufacturer's protocol for QIAamp® Viral RNA Mini Kit.

Procedure for Splitting Mosquito Pools

Mosquitoes were ground in diluent for RAMP® and RT-PCR testing

60 ul of sample + 60 ul of RAMP® buffer (1:2 dilution) → 70 ul used in RAMP® assay

140 ul of sample + 560 ul of RNA lysis buffer (1:5 dilution) → RNA extraction → Reverse-transcription and Real-Time PCR assay



Dr. Stacy Bearden (left) and Kimberly Heilig (right) performing extraction of RNA.

Real-Time PCR Analysis: Samples were run in duplicate wells in a 96-well plate using the ABI PRISM 7000 Sequence Detection System/Applied Biosystems 7500 Real Time PCR System and TaqMan® One-Step RT-PCR Master Mix reagents kit (ABI catalog #4309169). Positive and negative controls and a no template control were run for each assay.

20.0 uL WNV Master Mix + 5.0 uL RNA (RNA not quantified) = 25 ul total reaction volume

Reaction Cycle Times and Temperatures:

Stage: 1 Reps: 1, 48 C for 30 minutes

Stage: 2 Reps: 1, 95 C for 10 minutes

Stage: 3 Reps: 45, 95 C for 15 seconds, 60 C for 1 minute

Kristen Holt loading samples into the Real-Time PCR system.

Loading sample cartridge into RAMP® reader.

Results

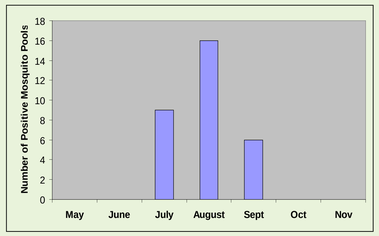


Figure 1. The positive pools were found in July (9), August (16) and September (6) of 2006.

Date Collected	RAMP units	Ct (ENV)	Ct (SP)
9/8/2006	640	30.635	26.675
8/29/2006	640	26.16	27.705
8/15/2006	640	21.32	29.395
8/29/2006	640	21.68	28.685
8/9/2006	631.7	29.925	26.465
8/4/2006	483	25.995	27.685
7/27/2006	425.3	23.84	27.095
8/29/2006	392.7	25.145	28.29
7/18/2006	212.3	26.85	28.41
7/25/2006	59.2	23.035	26.19
7/18/2006	56.4	27.575	29.29
8/2/2006	56.1	29.72	31.355
7/25/2006	53.5	26.575	29.57
8/8/2006	51.5	21.135	29.36
8/22/2006	44.7	28.765	35.805
7/25/2006	42.3	23.455	29.205
8/2/2006	40.7	31.54	32.9
9/20/2006	40.1	28.125	34.42
8/9/2006	38.1	26.18	28.955
9/27/2006	34.5	undet./43.06	32.21
8/29/2006	25	28.51	35.995
8/15/2006	20.2	27.57	36.235
8/23/2006	16.9	undet./undet.	undet./undet.
8/23/2006	15.9	undet./undet.	undet./undet.
8/23/2006	15.4	undet./undet.	undet./undet.
9/7/2006	15.2	26.19	33.31
9/7/2006	10.2	32.475	41.865
9/8/2006	5.1	undet./undet.	
9/8/2006	2.5	undet./undet.	
8/29/2006	2.1	undet./undet.	
8/29/2006	1.8	undet./undet.	
9/7/2006	1.7	undet./undet.	
9/8/2006	1.4	undet./undet.	
7/12/2006	1.4	26.19	32.64
9/8/2006	1.3	undet./undet.	
8/15/2006	1.3	undet./undet.	
8/15/2006	1.3	35.77	undet./undet.
9/26/2006	1.2	undet./undet.	
8/29/2006	1.2	undet./undet.	
9/6/2006	1.1	undet./undet.	
8/22/2006	1.1	undet./undet.	
9/14/2006	1	undet./undet.	
9/14/2006	0.8	undet./undet.	
8/29/2006	0.8	undet./undet.	
8/22/2006	0.8	undet./undet.	
9/7/2006	0.8	undet./undet.	
9/9/2006	0.7	undet./undet.	
8/29/2006	0.7	undet./undet.	
8/29/2006	0.7	undet./undet.	
8/29/2006	0.7	undet./undet.	
8/29/2006	0.7	undet./undet.	
9/8/2006	0.6	undet./undet.	
9/8/2006	0.5	undet./undet.	
9/26/2006	0.4	undet./undet.	
9/7/2006	0.4	undet./undet.	
9/8/2006	0.3	undet./undet.	
9/6/2006	0.3	undet./undet.	
8/29/2006	0.3	undet./undet.	
10/20/2006	0.2	undet./undet.	
9/26/2006	0.2	undet./undet.	
9/12/2006	0.1	undet./undet.	
8/15/2006	0.1	undet./undet.	
8/25/2006	0.1	undet./undet.	
8/29/2006	0.1	undet./undet.	
9/7/2006	0.1	undet./undet.	
9/26/2006	0.1	undet./undet.	
7/14/2006	0	33.92	34.695
5/19/2006	0	38.58/undet.	undet./undet.
7/25/2006	0	37.495	undet./undet.
8/15/2006	0	42.05/undet.	undet./undet.
8/15/2006	0	38.15/undet.	undet./undet.
8/22/2006	0	31.905	38.115
8/29/2006	0	38.165	undet./39.80
8/29/2006	0	38.21/undet.	undet./undet.
9/20/2006	0	37.485	undet./41.02

* In addition to the above samples, there were 466 samples that were negative by both RAMP® and RT-PCR.

Table 2. Comparison of RAMP® values with Ct values using primers to the envelope sequence (ENV) and single-plex primers (SP). Samples were considered positive (in RED) if Ct values were less than 42.0 for either set of primers.

Discussion

A total of 541 mosquito samples from 8 locations in San Joaquin County were tested using both RAMP® and Real-Time PCR. Results using RT-PCR indicate that positive pools were detected during months of July (9), August (16) and September (6), with the peak occurring during August, 2006 (Figure 1).

Table 2 shows the RAMP® units (in descending order) compared to the Ct values obtained using RT-PCR. The RED color indicates those samples which were considered positive by RT-PCR, i.e. Ct values of <42 for either ENV (envelope) or SP (single-plex) primers. Samples considered negative by RT-PCR are in **BLACK**.

Comparison of the two values indicates strong agreement with RAMP® units >20 and Ct values (avg. Ct value for ENV = 27; avg. Ct value for SP = 30). 519 samples had RAMP® units of 0 – 19. Of these, 9 (1.7%) were positive by RT-PCR.

This suggests that as RAMP® units decrease, there is less agreement between the two methods. In addition, of the samples that had 0 RAMP® units, 5/475 (1.1%) were positive by RT-PCR. Therefore, although few in number, some samples assayed using RAMP® may be false-negatives.

In conclusion, this study suggests that the results of RAMP® testing correlate well with results obtained by RT-PCR when RAMP® units are above 20 units. Below 20 units, the test is less reliable when compared to RT-PCR. Because mosquito samples with low RAMP® units may be false-negatives, confirmation by the more sensitive method of RT-PCR should be considered.

Acknowledgements

We would like to thank the staff at San Joaquin County MVCD for processing the mosquito pools as well as John Stroh and the San Joaquin County MVCD Board of Trustees for financial support. From the Marin/Sonoma MVCD, we would like to thank Sarah Klobas for database management, Chris Canterbury for poster development, Jim Wanderscheid, and the Board of Trustees of the Marin/Sonoma MVCD for financial support.