Multiple Evaluation of the RAMP West Nile Virus Test

A Reliable, Sensitive and Rapid System for Detecting West Nile Virus in Mosquitoes and Corvids

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Results from five independent external studies confirm in-house data and demonstrate that Response Biomedical Corp.'s RAMP West Nile Virus (WNV) Test is able to rapidly and reliably detect West Nile Virus in both mosquitoes and corvids with sensitivity and specificity approaching both PCR and ELISA.

- **>75% Sensitivity in comparison to PCR**
- **>95% correlation to ELISA**
- **At least 10 times more sensitive than Vectest**
- **Independently validated by the US CDC and Canada's NML**

RAMP is a platform technology that can be adapted to quantify virtually any immunologically active substance. The RAMP System consists of two components: a disposable Test Cartridge that houses an analyte-specific immunochromatographic strip, and a portable fluorescence Reader that is used to quantify the analyte. The assay uses latex particles that are fluorescently labeled and tagged with antibodies. Immunoassays based on fluorescence have substantially greater sensitivity and dynamic range than those based on earlier-generation detection techniques such as reflectance, or colored particles detected by eye. A proprietary Internal Control runs concurrently in every assay, allowing the RAMP System to compensate for test-to-test variability.

The RAMP Reader has received clearance from the U.S. FDA as a Class II medical device and a Medical Device License from the Canadian Therapeutic Products Directorate.

The RAMP System is well suited to perform environmental tests for detection of infectious agents, such as West Nile Virus. A sample is collected and then added to the Sample Buffer and mixed. An aliquot of the sample in the buffer is placed in the Test Cartridge that is then inserted into the RAMP Reader. In approximately 1 minute the Reader measures the amount of fluorescence emitted from the Test Cartridge, and the test result is displayed as a quantitative value.* This result can be stored, printed or uploaded to a computer system as needed.

Performance of the RAMP WNV Test has been evaluated at five external sites: the US CDC; the Canadian National Microbiology Laboratory; Pennsylvania Department of Environmental Research; Clarke Mosquito Control/Walter Reed Army Hospital and Harris County Mosquito Control District, Texas. Data from these five evaluations support the claims of a high level of sensitivity and specificity for the RAMP System.

US CDC – Comparison to Vectest

Sensitivities of both the RAMP WNV Test and Vectest were evaluated by testing serial dilutions of WNV seed virus and mosquito pools. Prepared samples were recovered into RAMP or Vectest Buffer and assays were performed according to the respective package instructions. Results from all samples were confirmed by

* After the test has dried

PCR. Results for both stock WNV and mosquito pools were tabulated.

The results show that RAMP is at least ten times more sensitive than Vectest (Table 1 and Table 2.)

Table 1. Stock WNV Dilution Results From US CDC

Sample #	Stock WNV Dilution	RAMP Rep # 1	RAMP Rep # 2	Vectest Rep #1 (0,1- 3+)	Vectest Rep #2 (0,1- 3+)
1	- 3 Log	> 640	> 640	1, 1, 1	1, 1, 1
2	- 4 Log	208.9	191.7	1, 0, 1	0, 0, 0
3	- 5 Log	33.7	24.1	0, 0, 0	0, 0, 0
4	negative	0.0	0.0	0, 0, 0	0, 0, 0

Sample #	Mosquito Pool Dilution	RAMP Rep # 1	RAMP Rep #2	Vectest Rep #1 (0,1- 3+)	Vectest Rep #2 (0,1- 3+)
1	- 1 Log	> 640	> 640	2, 2, 2	2, 2, 2
2	- 2 Log	> 640	>640	1, 1, 1	1, 1, 1
3	- 3 Log	48.4	74.9	0, 0, 0	0, 0, 0
4	negative	0.0	0.0	0, 0, 0	0, 0, 0

Canadian National Microbiology Laboratory – Comparison to Vectest (Corvids)

A second evaluation was conducted at the Canadian National Microbiology Laboratory in Winnipeg, Manitoba. Both the RAMP WNV test and Vectest were evaluated using either throat or cloacal swabs from corvids that had been submitted to the laboratory from sites throughout Manitoba. Swabs containing sample were recovered into BA1 culture media and assays were performed according to the respective package instructions. Results from all samples were then compared to PCR to assess the sensitivity.

The results show RAMP to have 100% sensitivity. Vectest did not detect 2 of the 4 PCR positive samples, showing 50% sensitivity (Table 3).

Table 3. Corvid Throat (Th) and Cloacal (Cl) Swab Results From National Microbiology Laboratory

Sample #	Crow Samples	Ramp Result	Taqman (CT)	1-step RT-PCR	VecTest
1	Crow (Cl)	Negative	40	ND	Negative
2	Crow (Th)	Negative	40	ND	Negative
3	Crow (Th)	Positive	29	Positive	Negative
4	Crow (Cl)	Positive	19	Positive	Positive
5	Crow (Cl)	Positive	18	Positive	Positive
6	Crow (Th)	Positive	25	Positive	Negative

Department of Environmental Research, Pennsylvania – Comparison to PCR

The third evaluation was conducted at the Pennsylvania State Laboratory Department of Environmental Research. Sensitivity of the RAMP WNV test was compared to PCR in 90 unknown mosquito pools collected from throughout Pennsylvania. Samples containing up to 50 mosquitoes were recovered into RAMP Buffer and assayed according to the package insert instructions. The same samples were then tested on RT-PCR to compare the sensitivity. Results were tabulated.

The results showed RAMP to have 76% sensitivity compared to PCR, with no false positives (Table 4).

Table 4. Unknown Mosquito Pool Results From Pennsylvania State Laboratory

RT-PCR Count (CT)	Number of PCR+ ve samples	Number of RAMP +ve samples
20-25	13	13
26-30	10	6
31-39	6	3
40	0	0

Clarke Environmental Mosquito Control/Walter Reed Army Hospital, Virginia

The fourth evaluation was conducted by Clarke Environmental and Walter Reed Army Hospital. Sensitivity and specificity of the RAMP WNV test was compared to PCR in 100 unknown mosquito pool samples collected from Virginia. Samples containing up to 50 mosquitoes were collected into RAMP buffer and assayed according to the Package insert instructions by laboratory staff at Clarke Environmental. Following this, the samples were sent to Walter Reed for confirmatory RT-PCR.

The results show that RAMP correctly identified the single PCR positive mosquito pool, and showed no false positives (Table 5).

Table 5. Unknown Mosquito Pool Results From Clarke Environmental/Walter Reed Army Hospital.

		PCR	
		Positive	Negative
RAMP	Positive	1	0
	Negative	0	99
		Sensitivity	100%
		Specificity	100%

Harris County Mosquito Control District – Comparison to ELISA (WNV-EIA) and Inhibition Assay (WNV-IA)

A fifth evaluation was conducted by Dr. Ray Parsons and his department at Harris County MCD in Texas. Sensitivity of the RAMP WNV Test was compared to both ELISA and Inhibition Assay in 100 unknown mosquito pool samples. Up to 50 mosquitoes were recovered into RAMP buffer and assayed according to the package insert instructions. The same samples were than tested on ELISA and Inhibition Assay.

The results show that RAMP had >95% correlation to ELISA and >93% correlation to inhibition assay. (Table 6).

Table 6. Unknown mosquito pool results from Harris County, Texas.

	# of SAMPLES TESTED	RAMP TEST (MATCH)	% Correlation
ELISA (WNV-EIA)	97	94	96.9
Inhibition Assay (WNV-IA)	87	81	93.10

Conclusion

Results from five external independent studies confirm in-house data and demonstrate that the RAMP West Nile Virus Test has >76% sensitivity and 100% specificity in comparison to PCR, and >95% correlation to ELISA.