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EVALUATING THE USE OF COMMERCIAL WEST NILE VIRUS ANTIGENS AS POSITIVE CONTROLS IN THE RAPID ANALYTE MEASUREMENT PLATFORM WEST NILE VIRUS ASSAY

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Abstract

We evaluated the utility of two types of commercially available antigens as positive controls in the Rapid Analyte Measurement Platform (RAMP®, Response Biomedical Corp., Burnaby, British Columbia, Canada) West Nile virus (WNV) assay. Purified recombinant WNV envelope antigens and whole killed virus antigens produced positive RAMP results and either type would be useful as positive controls. Killed virus antigens provide operational and economic advantages and we recommend their use over purified recombinant antigens. We also offer practical applications for RAMP positive controls and recommendations for preparing them.

Keywords

RAMP® test; commercial antigen; WNV; positive control

The Rapid Analyte Measurement Platform (RAMP®, Response Biomedical Corp., Burnaby, British Columbia, Canada) West Nile Virus (WNV) assay is an antigen detection lateral flow assay used by mosquito abatement districts (MADs) to detect WNV in field-collected mosquito pools. In brief, pools of 1–50 mosquitoes are ground in the proprietary RAMP buffer included in the kit and centrifuged. An aliquot of the resulting supernatant is mixed with a conjugate-antibody complex and applied to an immunochromatographic strip housed in a cartridge. After a 90-minute incubation period, the cartridge is inserted into the RAMP reader, which reads the strip and produces the results in RAMP units. According to the manufacturer a RAMP score of 30 or higher is considered positive for WNV, while the Centers for Disease Control (CDC) recommends a cutoff of ≥ 50 (Burkhalter et al. 2014). The cut-off used is at the discretion of the agency based on the goals of their arbovirus surveillance program.

The RAMP system employs several internal quality control measures that ensure reproducibility of RAMP results between cartridges, antibody-complexes, and grinding buffer manufactured in the same lot. However there are no WNV positive controls provided in the kit. RAMP operators who would like to verify the validity of RAMP results from their

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field-collected samples must seek other means to do so. Typically, positive controls are generated from verified tissue culture isolates or other samples that have been determined to contain the target antigen. These positive controls are then processed and tested according to the protocols applied to unknown samples to determine the efficacy and accuracy of the test assay. However, most MADs do not have access to live WNV from which to create positive controls, the proper permits required to accept shipped virus, nor sufficient biosafety containment facilities in which to work with known infectious material. Relying on previously-tested RAMP positive field-collected pools for use as controls has several disadvantages. If a MAD does not typically encounter positive pools in their routine surveillance, limited quantities of positive material will be available. Field-collected pools can vary in viral titer so positive controls from field-collected samples would not be standardized. Finally, the MAD would be relying on the validity of positive controls determined to be positive by the assay they're testing (RAMP) rather than an independent source.

Commercially-prepared purified WNV antigens can be purchased from several sources, and are most often used in serological diagnostic assays, such as enzyme immunoassays (EIA). The RAMP assay works similarly to an antigen-capture EIA; that is, antigens of interest present in a sample are captured by monoclonal antibodies specific to the target antigen and detected visually by a color change produced by an enzyme-substrate reaction or by fluorescent tags attached to the antibodies (Porstmann and Kiessig 1992). The RAMP assay utilizes the latter detection method in a "dry" format (i.e., on a strip). We purchased two types of commercially-available WNV antigens that are normally sold for use in EIAs and tested them with the RAMP assay to determine their utility as positive controls.

The first type of antigen tested was purified recombinant WNV. Recombinant viral antigens contain only the region of the protein recognized by antibodies, no other virus particles or nucleic acid, and are not infectious. Because the antibodies used in the RAMP assay are proprietary and unknown to us, we chose three antigens representing two WNV surface glycoproteins, the envelope (ENV) and precursor membrane (Pre-M), for this investigation (Table 1). All antigens were available as 100 µl stock solutions with concentrations of 1 mg/mL. Dilutions were made from the stock solutions in RAMP buffer to produce samples with a range of antigen concentrations (2 – 40 µg/mL) and mixed well. A 120 µl aliquot of each sample was removed and mixed with the antibody-conjugate provided in the RAMP kit; 70 µl of this mixture was applied to the cartridge according to the kit insert. After a 90-minute incubation period the cartridges were read by the RAMP reader.

The second type of antigen tested was WN killed virus antigen (Table 2). Unlike recombinant antigens, killed virus antigens contain whole virions, including surface protein antigens, nucleic acids, and other virus particles, but have been rendered inactive by one of several available methods (World Health Organization 2004). The 2 units of WNV formalin-killed antigens we purchased were prepared from a 2005 equine WNV isolate, and shipped on dry ice as 1 mL aliquots with concentrations of 9.7 $\log_{10}$ tissue culture infectious dose $(TCID)_{50}$/ml and 8.5 $\log_{10}$ $TCID_{50}$/ml according to the accompanying certificates of analyses. Six panels consisting of 10-fold serial dilutions of the antigens were made in RAMP buffer and mixed well. Sets of 1:2 dilutions were made between the 10^{-2} and 10^{-3}

dilutions to produce an intermediate range of RAMP results. A 120 μl aliquot was removed from each sample and tested with the RAMP assay as described above. In order to report the concentration of virus as $\log_{10}$ plaque forming units (PFU)/mL, the estimated titer of the panel samples was determined by quantitative real-time RT-PCR. Briefly, serial dilutions of each lot of killed virus antigens were made in cell culture medium Bovine Albumin (BA)-1 and tested by real-time RT-PCR against a WNV RNA standard curve of known concentrations.

The RAMP results of the recombinant antigens are presented in Table 1. The recombinant ENV antigens produced positive results according to the CDC recommendation that ≥ 50 RAMP units are considered positive (Burkhalter et. al. 2014); however the Pre-M antigen did not (suggesting that the antibodies used in the RAMP assay do not target this antigen). The two ENV recombinant antigens purchased from different vendors produced positive results for samples containing ≥ 10 μg/mL.

The RAMP results produced by the killed virus antigens are presented in Table 2. Because the concentration of virus was given in $\log_{10}$ $TCID_{50}$/ml, the dilutions of each lot were tested by real-time RT-PCR against a standard curve of a known concentration of WNV RNA to determine an estimated concentration of each sample in $\log_{10}$ PFU/ml. Samples with estimated titers ≥ 3.5 $\log_{10}$ PFU/ml produced positive RAMP results (≥ 50 RAMP units), and samples with estimated titers ≤ 3 $\log_{10}$ PFU/ml produced negative results (< 50 RAMP units). The sensitivity limit and range of RAMP scores for positive results generated by these samples were consistent with previously published ranges (Burkhalter et al. 2014, Burkhalter et al. 2006).

Both the purified recombinant WNV envelope antigens or killed virus antigens could be used as positive control for the RAMP assay; however, several characteristics support the use of killed virus antigens over recombinant antigens. Because the killed virus antigen contains all parts of the virion, it better represents real-world samples than the purified recombinant antigen. Also, while the purchase price of both antigen types is comparable, enough volume is available (1 ml) to make several panels of 1:10 dilutions of killed virus antigen (~9 mL of each 1:10 dilution can be produced from one 1 ml tube of killed virus antigen), while the volume of recombinant antigen (100 μl) is only sufficient to generate a handful of samples.

The handling of recombinant or killed virus antigens does not need to be performed under biosafety containment; however, the use of personal protective equipment (PPE) such as gloves, safety googles, and lab coats is still advised. Operators should be mindful that they are handling a highly concentrated WNV sample and care should be taken when preparing the controls so as not to contaminate their work area or other samples. While positive controls can be freshly prepared every time they are needed, we recommend choosing a dedicated time and place to prepare many controls at once where and when no other RAMP work is being performed (such as field testing). After the controls are serially diluted in RAMP buffer, small volumes of each dilution can be dispensed into individual tubes and stored in the freezer. Controls that are prepared and aliquoted in advance can simply be thawed when needed, tested, and discarded. Maintaining an inventory of pre-made controls is not only convenient; it also reduces the frequency of RAMP operators handling highly

concentrated stock antigen in their work area. Please note that antigen samples prepared and stored in RAMP buffer that had been through several freeze-thaw cycles produced inconsistent RAMP results in our laboratory (data not shown); however one freeze-thaw did not interfere with expected RAMP results. The killed virus antigen certificate of analysis also discourages repeated freeze-thaws of the antigen. Therefore, we recommend that each frozen aliquot be only thawed and tested once.

One minor complication of both types of antigens is the antigen concentrations reported by the companies (μg/mL or $\log_{10}$ $TCID_{50}$/ml) differ from the viral concentrations ($\log_{10}$ PFU/ml) that have been correlated to RAMP scores previously (Burkhalter et al. 2014, Burkhalter et al. 2006). Comparing RAMP results of purchased antigens to previously published RAMP scores may be difficult. In our lab, we were able to determine the estimated concentration of the killed virus antigen in $\log_{10}$ PFU/ml using real-time RT-PCR but that capability is unavailable to most agencies that use the RAMP assay. We present the results of two serially diluted lots of the killed virus antigen with different $\log_{10}$ $TCID_{50}$/ml concentrations to provide RAMP operators with an estimated RAMP result range if the lots they purchase contain similar concentrations, or if they produce samples containing similar concentrations by diluting the stock antigen.

The use of positive controls in the RAMP assay can serve different purposes depending on the needs of the agency. Having a set of samples that give an expected range of RAMP scores can demonstrate consistency (or lack thereof) in the assay from year to year, or more frequent intervals. Positive controls can be used to create blind-coded panels when training new RAMP users or for training in the off-season when mosquitoes are not available. They can be used to confirm the validity of the RAMP assay for agencies that have not yet detected WNV-positive mosquito pools from the field and wish to distinguish between the lack of positive mosquitoes in their area and possible problems with the RAMP assay or their processing protocols. In all of these examples, using RAMP-positive field-collected mosquito pools as positive controls would be problematic, for reasons discussed earlier. We show in this evaluation two types of commercial antigens intended for EIA that can be used as positive controls in the RAMP assay and provide recommendations for practical applications.

The purchase of antigens from the vendors listed does not imply endorsement by the CDC.

References Cited

Burkhalter KL, Lindsay R, Anderson R, Dibernardo A, Fong W, Nasci RS. 2006 Evaluation of commercial assays for detecting West Nile Virus antigen. J Am Mosq Contr Assoc 22: 64–69.

Burkhalter KL, Horiuchi K, Biggerstaff BJ, Savage HM, Nasci RS. 2014 Evaluation of a rapid analyte measurement platform and real-time reverse-transcriptase polymerase chain reaction assay West Nile virus detection system in mosquito pools. J Am Mosq Contr Assoc 30:21–30.

Porstmann T and Keissig ST. 1992 Enzyme Immunoassay techniques: an overview. J Immunol Methods 150:5–21. [PubMed: 1613258]

World Health Organization. 2004 Guidelines on viral inactivation and removal procedures intended to assure the viral safety of human blood plasma products. Technical Report, Series No. 924. http://www.who.int/bloodproducts/publications/WHO_TRS_924_A4.pdf

Table 1.

RAMP assay results of commercially-available recombinant West Nile virus (WNV) antigen diluted in RAMP buffer. RAMP results are considered positive if ≥ 50 RAMP Units.

Manufacturer	Product Name	Catalogue Number	Lot Number	Concentration of test sample (μg/mL)	RAMP result (RAMP Units)
ProSpec[1]	Recombinant WNV Envelope	wnv-001	1011PWNVE24	40	359.4
				20	137.1
				10	90.5
				5	45
				2	<10
ProSpec	Recombinant WNV Pre-M	wnv-002	112PWNVPM31	40	<10
				20	<10
				10	<10
				5	<10
				2	<10
Feldan[2]	Recombinant WNV Envelope *rWNVE*	7G-25–001	13H8936	40	396.6
				20	162.8
				10	72.5
				5	32.7
				2	13.6

[1]ProSpec-Tany Technogene Ltd.,East Brunswick, NJ

[2]Feldan Proteins and Reagents, Quebec, QC, Canada

Table 2.

RAMP results of two units of WN Killed Virus Antigen (catalogue number K100–1) purchased from Hennessey Research Associates, LLC (Shawnee, KS). Six panels of serial dilutions made in RAMP buffer were prepared from each unit. RAMP results are considered positive if ≥ 50 RAMP Units.

Lot Number	Pre-inactivation titer of stock antigen[1] (log_{10} $TCID_{50}$/mL)	Dilution of stock antigen	Estimated titer of test sample[2] (log_{10} PFU/mL)	RAMP results (RAMP Units)					
E09002	9.7			Panel 1	Panel 2	Panel 3	Panel 4	Panel 5	Panel 6
		10^{-2}	4.7	>640	>640	>640	>640	>640	>640
		$10^{-2.5}$	4.2	>640	>640	>640	>640	>640	>640
		$10^{-2.75}$	4.0	336.2	302.8	371.8	443.8	364	355.3
		10^{-3}	3.7	117.5	127.7	117	144.4	191.3	131.2
		10^{-4}	2.6	13.3	10.5	10.5	14.7	19.8	15
		10^{-5}	1.8	<10	<10	<10	<10	<10	<10
WN089F	8.5								
		10^{-1}	5.3	>640	>640	>640	>640	>640	>640
		10^{-2}	4.3	>640	565.6	>640	>640	>640	>640
		$10^{-2.5}$	4.1	398.4	307.7	329.9	449.7	378.7	319.3
		$10^{-2.75}$	3.8	172.8	144.4	150.5	193.7	165.1	177.7
		10^{-3}	3.5	61.2	56.5	66.7	68	56.4	51
		10^{-4}	2.4	<10	<10	<10	<10	<10	<10

[1] According to manufacturer's accompanying certificate of analysis

[2] Estimated titer in log_{10} pfu/mL determined by real-time RT-PCR of serial dilutions of killed virus compared against known concentrations of WNV RNA