Frequently Asked Questions
Response Environmental

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1. PRODUCT INFORMATION

RAMP® Platform

a) What is the RAMP® Platform and how does it work?
The RAMP® Platform is an immunofluorescence-based technology, which consists of a Reader (RAMP® Reader or RAMP® 200) and disposable test cartridges. Mosquitoes are collected, species sorted, gender sorted and mixed with RAMP® sample buffer using the supplied test tip that contains fluorescently-bound WNV antibodies. The fluorescent antibodies bind to WNV, if present in the sample.

An aliquot of mixed sample is taken and added to the RAMP® WNV test cartridge. The cartridge is left to dry for 90 minutes or longer. As the sample migrates along the test strip, WNV-bound particles are immobilized at the Detection Zone and excess of un-bound antibodies are immobilized at the Control Zone.

After the development period, the test cartridge is inserted into the Reader. The Reader then measures the amount of fluorescence emitted by the particles bound at each zone. Using a ratio between the two values, a test result is determined and displayed on the Reader. The process is the same for corvids; however a throat swab is the required sample type.

b) What other equipment is required to run the RAMP® WNV test?
In addition to the Reader (RAMP® Reader or RAMP® 200) and a box of RAMP® WNV tests, pipettes measuring 1mL and 120uL volumes, a benchtop micro-centrifuge and a vortex mixer are required. This additional lab equipment is available for purchase from ADAPCO Inc.

RAMP® Reader and RAMP® 200

a) How many test results can be stored in the RAMP® Reader memory?

RAMP® Reader	Up to 500 test results can be stored
RAMP® 200	Up to 300 test results can be stored

Once the maximum amount of results have been stored, the Reader will proceed to overwrite the oldest result.

b) How many tests can the RAMP® Reader and RAMP® 200 process at one time?

RAMP® Reader — One test cartridge can be run at a time. It takes approximately 1 minute for the cartridge to be scanned.

RAMP® 200 — Between 2 to 6 test cartridges can be run simultaneously, depending on how many RAMP® 200 Test Modules are being used. Based on feedback from our WNV customers, a maximum of 2 Test Modules (4 test ports) is usually sufficient. It takes approximately 1 minute for each cartridge to be read.

Data Management

a) Can I print test results?

RAMP® Reader — Yes, results can be printed. The compatible dot matrix printer is manufactured by Citizen (Citizen CBM-910 Type II). The printer is connected to the RAMP® Reader through the RS-232 serial port on the back panel of the Reader, using a Reader-to-printer cable. Both serial printer and Reader to printer cable are available for purchase through Response Biomedical Corp. The Reader can be set up to print individual results as required or automatically as each test is performed. Instructions are available in the Operator's Manual.

RAMP® 200 — Yes, results can be printed. The compatible printer is manufactured by Zebra Technologies (TLP2824™ and TLP2824 Plus™). The printer is connected to the RAMP® 200 Control Module through

the USB port at the back of the Reader. Instructions are available in the Operator's Manual. Alternatively, results can be transferred to excel and printed using the File Converter Utility (refer to b).

b) Can I download test results to a computer?

RAMP® Reader The Reader can be connected to a computer/laptop via the RS-232 cable and/or USB to a RS-232 adapter. The results can be downloaded using HyperTerminal and opened in Excel. Instructions are available at the end of the Operator's Manual.

RAMP® 200 Results can be transferred from the RAMP® 200 to USB, then uploaded to a computer/laptop and converted to excel via the File Converter Utility program. Alternately, files can be transferred through an Ethernet connection to a network. Instructions on transferring results are available in the Operator's Manual.

RAMP® Platform Disposables

a) How many tests come in a RAMP® WNV test kit?

Each kit contains 100 test cartridges.

b) What is included in the RAMP® WNV test kits?

Each kit contains 100 test pouches (each containing a RAMP® test cartridge and RAMP® test tip), 120mL RAMP® sample buffer vial, 100 2.0mL dilution vials, 100 0.5mL sample vials, 70µL transfer device, a container of copper coated ball bearings, 1 lot card and 1 Instructions for Use.

c) Why are the RAMP® test tips important?

It is important to use the test tips provided as they contain the fluorescently labeled antibody, which will bind to the sample of interest, if present. The binding of the antibody to the sample of interest begins outside of the test cartridge during mixing with the supplied sample buffer. Without the fluorescent tag, the RAMP® Reader would not be able to detect any sample flow and would generate a "Sample Error #2" message.

d) Why is the RAMP® sample buffer important?

RAMP® sample buffer is specifically formulated for optimal sensitivity and performance of the test. For this reason, the RAMP® sample buffer cannot be substituted or omitted; nor can buffers be interchanged between different tests or different kit lots of the same test.

2. SAMPLE PREPARATION

a) What sample types can be tested on the RAMP® WNV test?

Mosquitoes and corvids are the recommended sample types for use with the RAMP® WNV test. It is recommended that the use of mosquitoes with blood-engorged abdomens and decomposing corvids is avoided (refer to d).

b) How long can I wait before testing the trapped mosquitoes?

Mosquitoes should be analyzed immediately once collected. Depending on the environmental conditions (i.e. extreme temperature, wet weather, etc.) mosquitoes can degrade quickly. If a time delay is anticipated before testing, mosquitoes should be frozen as collected, not frozen in buffer solution.

c) How many mosquitoes can be tested at one time?

Up to 50 mosquitoes are recommended as per the RAMP® WNV test Instructions for Use. Exceeding the recommended number of mosquitoes may lead to hindrance of the sample flow and/or error messages. As some mosquito species are larger in size than others, it is recommended that a lower number of mosquitoes per pool are used for larger species.

d) Can I test blood or tissue samples in the RAMP® WNV test?
Blood or tissue homogenates must not be used with the RAMP® WNV test due to a strong risk of interference. Mosquitoes should be examined for blood-engorged abdomens and any blood-engorged mosquitoes should be removed from the sample prior to preparation to avoid the potential for erroneous results and/or error messages. For corvids, avoid using decomposing samples and/or swabs contaminated with blood.

e) Can the RAMP® Platform be used to test sentinel chickens?
When chickens are infected with WNV, their viral load is not particularly high. In addition, the viraemic window is narrow (approx. 2 days). In order to detect the virus, RAMP® WNV tests would have to be run every day in order to have a chance at detection. Response Biomedical Corp. has not performed any evaluations on sentinel chickens.

f) Can the mosquitoes be prepared in RAMP® buffer and frozen until able to test?
It is recommended that testing of mosquitoes be carried out immediately once collected. If a time delay is anticipated, freeze the mosquitoes without RAMP® sample buffer.

g) After homogenization of mosquito samples, can the extracted supernatant be stored for use at a later date?
Supernatant samples should be run as soon as possible after preparation; however they can be stored in refrigerated conditions (2 to 8°C) for up to 24 hours prior to testing. Failure to test the supernatant samples within this time frame may cause erroneous results.

h) Can corvid swabs be stored for use at a later date?
No. If there will be a delay in testing, the carcasses should be frozen for testing at a later date to ensure the stability of RNA in the sample.

i) Why does the test cartridge take 90 minutes to dry? Can the process be accelerated?
The RAMP® WNV test is optimized for reading dry test cartridges. The 90 minute drying time ensures that the sample is completely dry in the cartridges. Do not do anything to speed up the drying process (i.e. hairdryer, etc.). Place the cartridge on a clean, dry surface and allow it to dry at room temperature.

j) Can the test cartridge sit overnight with added sample before reading?
The test cartridge can be left for longer than 90 minutes and read the next day. The cartridge must not be exposed to direct sunlight or extreme temperatures as this may affect test results.

k) When re-testing a sample, should more RAMP® buffer be added to the original mosquito pool?
When re-testing a sample, additional sample buffer is not required. The sample may need to be re-centrifuged if the original pellet has mixed with the buffer. Take 120µL of the supernatant and transfer to a separate tube. Mix the 120µL with the supplied mini-pet and a new pipette tip (from a fresh test cartridge pouch). Transfer 70µL into the new test cartridge.

l) Can RAMP® sample buffer be mixed with reagents from another kit?
No, the RAMP® sample buffer is specifically formulated for optimal sensitivity and performance of the test, and for this reason it cannot be mixed or interchanged between the different tests or different kit lots of the same test.

3. SENSITIVITY AND ACCURACY

a) How does RAMP® Platform compare to Vectest?
The RAMP® WNV test limit of detection can be approximated at 3 Log plaque-forming units (pfu)/mL (1000 plaque-forming units) of West Nile virus and above; however the VecTest will detect WNV at approximately 5 Log pfu/mL (100,000 plaque forming units).[1,2]

4. RESULT INTERPRETATION

a) What is the reportable range of the RAMP® WNV test?
The reportable range of the RAMP® WNV test is 10.0-640.0 units.

Note: Results above the reportable range will be output as ">" the upper limit of the reportable range; results below the reportable range will be output as "<=" the lower limit of the reportable range.

b) What do the numerical results of the RAMP® WNV test mean?
Numerical results above the recommended cutoff are reflective of the viral concentration present in the sample. Numeric values below the recommended cutoff, are reflective of the inherent variability of the test. Each Reader and RAMP® WNV test has been specifically calibrated to reduce the risk of variability; however this cannot be completely eliminated. Some factors contributing to the variability of the RAMP® WNV test are from the type of sample tested (i.e. species, size, location) and sample preparation technique.

c) Does a higher result indicate higher antigen concentration?
Yes, the higher the RAMP® WNV test result, the greater the amount of West Nile Virus present in the sample.

d) As per the Instructions for Use, the recommended cut off values for mosquitoes and corvids are 30 and 50 RAMP® units, respectively. What if the RAMP® Reader result falls around the cut off?
Occasionally, results fall near the cut off value causing uncertainty. In general, this is related to the diversity of the sample types tested, the local variation in mosquito, bird and virus populations, potential differences in sample preparation techniques and the inherent variability of the RAMP® WNV test. These factors make setting discrete cut off values extremely challenging. If a result falls close to the cut off, we recommend that the sample either be re-run using leftover sample, or be sent for further confirmation by RT-PCR. Each center is encouraged to set local cut-off values that more closely reflect the local populations being tested.

e) What cut off is recommended by the Centers for Disease Control and Prevention (CDC) for the RAMP® WNV test?
According to a study conducted by the Kristen Burkhalter *et al.* (to be published), the CDC will be releasing the following recommendations:

- Use 50 RAMP® units as a cutoff for positives; no RT-PCR confirmation is required above that value
- For a more conservative cut off, use 100 RAMP® units as a cutoff for positives; RT-PCR confirmation required for samples of 50-100 RAMP® units.

If you have any questions regarding the study, please obtain Kristen Burkhalter's contact information from our Technical Support team at techsupport@responsebio.com.

f) What is the purpose of a "grey zone"? Is there a recommended grey zone for the RAMP® WNV test?
Occasionally, RAMP® WNV test results fall near the suggested positive/negative cut off value causing uncertainty. In general, this is related to the diversity of the sample types tested and the local variation in mosquito, bird and virus populations. All of these factors make setting a discrete cut off value extremely challenging. One possible solution implemented by many customers is the introduction of a 'grey zone' as a way to improve the decision-making process with RAMP® WNV test results.

Many RAMP® WNV test users determine their own grey zone to improve result interpretation based on the actual mosquito/bird population being tested and previous experience with the RAMP® WNV test. Based on data supplied by customers (approx. 750 samples), the most commonly used grey zone is between 30-80 RAMP® units. This was the area where most disagreement between RAMP® and RT-PCR results occurred. It is recommended that results falling within this area should be re-run using leftover sample and/or sent for further confirmation by RT-PCR. Each center is encouraged to set local grey zone values that more closely reflect the local populations being tested. Further information on the grey zone is available from our Technical Support team at techsupport@responsebio.com.

5. SAFETY

a) Does the RAMP® WNV test need to be operated in a BL3 lab?
The RAMP® WNV test does not need to be contained in a BL3 laboratory; however each testing facility should adhere to their local guidelines on the handling and disposal of potentially biohazardous material including deceased corvids. Universal laboratory precautions should be followed including lab coat, goggles, gloves, etc.

6. STORAGE

a) How should the RAMP® WNV test kits be stored?
RAMP® WNV test kits should be stored at room temperature (18-25°C or 64-77°F) as per the storage instructions included in the Instructions for Use. Do not expose any test components to extreme temperatures.

b) What are the storage requirements for the RAMP® Reader and RAMP® 200?
The recommended storage temperature range for the RAMP® Reader is -10-50°C (14-122°F) and the recommended storage temperature for the RAMP® 200 is -10-50°C (14-122°F).

c) What is the shelf life of the RAMP® WNV test kits?
Each RAMP® WNV test kit is shipped with a minimum of 12 months until expiration. The RAMP® Reader and RAMP® 200 will not process a test cartridge after the last day of the month of expiration.

7. MAINTENANCE AND CALIBRATION

a) Does the RAMP® Reader and/or RAMP® 200 require maintenance or calibration?
The RAMP® Reader and/or RAMP® 200 do not require maintenance or calibration. When the Reader is first switched on, it performs a Power On Self Test (POST) to ensure the system is operating within certain parameters. The POST includes calibration verification, optical system verification, a real-time clock test and a transport mechanism test. In the event of a failure, the Reader will display a message along with an audible alarm.

8. RESULT CONFIRMATION

a) Can RT-PCR be run on a sample that is in RAMP® sample buffer?
Yes, many customers carry this out routinely. If RT-PCR testing is to be performed, the remaining supernatant should be removed immediately and stored at < -70°C as quickly as possible, to reduce the risk of RNA degradation within the sample. Please note that additional RNA wash cycles may be required during RNA isolation.

If mosquito or corvid samples are to undergo off-site RT-PCR confirmation, they should be shipped on sufficient dry ice to ensure that the samples arrive at the RT-PCR facility in a frozen state.

9. TECHNICAL SUPPORT

a) Does Response Biomedical Corp. have a Technical Support Department?

Yes, Response has a Technical Support department, available 24/7.

North America (PST)	8am - 4:30pm	1-888-591-5577
	4:30pm - 8am	1-866-525-7267
International	8am - 4:30pm	604-456-6010
	4:30pm - 8am	604-219-6119
E-mail	techsupport@responsebio.com	

10. REFERENCES

[1] Burkhalter, K.L., Lindsay, R., Anderson, R., Dibernardo, A., Fong, W. & Nasci, R. (2006). Evaluation of Commercial Assays for Detecting West Nile Virus Antigen. *J Am Mosquito Contr,* 22(1):64-69. Referenced from: http://responsebio.com/support/publications#section-4

[2] Stone, W.B., Therrien, J.E., Benson, R., Kramer, L., Kauffman, E.B., Eidson, M. & Campbell, S. (2005). Assays to Detect West Nile Virus in Dead Birds. *Emerging Infectious Diseases (CDC)*. 11(11):1770-1773. Referenced from: http://responsebio.com/support/publications#section-4