RESPONSE BIOMEDICAL

INSTRUCTIONS FOR USE

RAMP® West Nile Virus

E1101-1.3

INTENDED USE

The RAMP® West Nile Virus (WNV) test is intended for the screening of environmental samples for the presence of West Nile virus, the causative agent of West Nile viral encephalitis. This test is intended for trained users only. It is not for use by the general public or individuals untrained in the handling of hazardous materials. This test is not to be used for human medical or veterinary diagnostic purposes.

WARNING!

Failure to follow RAMP® test procedures may result in invalid and/or erroneous results. Read the entire Instructions For Use prior to performing test.

WWW.RESPONSEBIO.COM | SPEED. PRECISION. ACCURACY.

24-HOUR TECHNICAL SUPPORT
Tel: 1-866-525-7267 (toll free) Tel: 1-604-219-6119 (int'l)
Email: techsupport@responsebio.com

RESPONSE CORPORATE OFFICE
Tel: 1-888-591-5577 (toll free) Tel: 1-604-456-6010 (int'l)
Email: customersupport@responsebio.com

Running a test

Add 1 mL of buffer into unused dilution vial; secure lid. Swab inside of bird's throat, ensuring no blood or tissue gets on swab.

Submerge swab into filled dilution vial and rotate to transfer specimen into buffer. Pipette 120 µL of mixed sample to unused 0.5 mL vial.

Add ≤ 50 female mosquitoes, 2 ball bearings, and 1 mL of buffer into unused dilution vial; secure lid.

Vortex sample for 1-5 min. Centrifuge for 3-5 minutes. Pipette 120 µL of mixed sample to unused 0.5 mL vial, avoiding mosquito particles.

Open foil pouch and firmly attach test tip to the transfer device.

Insert filled test tip into buffer and slowly depress plunger 10 times to fully mix. Transfer 70 µL of mixed sample into test cartridge well.

Insert cartridge into RAMP® instrument port. When test is finished, read result.

Discard all used components.

TEST PRINCIPLE

The RAMP® West Nile Virus test is a quantitative immunochromatographic test for the detection of West Nile virus (WNV). The suspect material is sampled by either homogenizing mosquitoes or swabbing a dead corvid, mixed with buffer and antibody-coated, labeled particles, and applied into the sample well of the test cartridge. The mixed sample migrates along the test strip. Fluorescent-dyed particles coated with anti-WNV antibodies bind to WNV, if present in the sample. As the sample migrates along the strip, WNV-bound particles are captured at the detection zone and excess fluorescent-dyed particles are captured at the control zone.

The RAMP® instrument then measures the amount of fluorescence emitted by the complexes bound at the detection zone and at the control zone. Using a ratio between the two fluorescence values, a quantitative reading is calculated. For further information on the use of the instrument, refer to the RAMP® User Manual.

REAGENTS

- The RAMP® test kit contains all the reagents necessary for the detection of West Nile virus.
- The sample buffer contains phosphate buffer, animal protein, surfactant, and ProClin® 300 / ProClin® 900 as preservatives.

WARNINGS AND PRECAUTIONS

- For environmental testing only; not for *in vitro* diagnostic use.
- Read the entire instructions for use (IFU) prior to use. Directions should be read and followed carefully, or invalid or erroneous results may occur.
- Do not interchange or mix components of different RAMP® tests, RAMP® lots or components from other manufacturers.
- Do not use the kit, or kit components, beyond the stated expiry date.
- Do not use any visibly damaged components.
- Do not insert a cartridge on which test sample or any other fluid is spilled into the instrument.
- Disposal of all waste materials should be in accordance with local guidelines.
- Exercise standard precautions required for collection, handling, storage and disposal of raw or diluted samples and used kit contents.
- The device contains material of animal origin and should be handled as a potential biohazard.
- Avoid generating aerosols.
- The sample buffer provided contains ProClin®, a potential skin sensitizer. Avoid spilling or splashing reagents containing ProClin® on skin or clothing. In case of contact, thoroughly flush with water.
- The sample buffer is intended to facilitate the immunoreaction of the assay and is NOT intended to inactivate the virus. Used buffer should be considered a potential biohazard and disposed of accordingly.

STORAGE AND STABILITY

Store at 2 to 25°C (36 to 77°F) up to stated expiry. Do not freeze.

SAMPLE COLLECTION & PREPARATION

Testing should be completed as soon as possible after sample collection.

- Appropriate training in specimen collection is highly recommended to ensure specimen quality.
- All samples must be mixed thoroughly before testing, regardless of consistency, to ensure a representative sample prior to testing.

The following specimen collection methods are recommended.

MOSQUITO METHOD

1. Obtain and sort mosquitoes (do NOT use mosquitoes with blood-engorged abdomen).
2. Utilizing an empty 2.0 mL dilution vial; fill with up to fifty (50) female mosquitoes (smaller numbers are required with larger mosquito species) and insert 2 copper-coated ball bearings.
3. By inversion, mix one RAMP® buffer bottle and, via set-volume pipette, transfer 1 mL (1000 µL) of buffer to mosquito-filled dilution vial.
4. Vortex filled dilution vial for 1 to 5 minutes. The more mosquitoes used; the longer the vortex time required. If any whole mosquito parts are noticed, re-vortex until sample is fully homogenized.
5. Once complete, transfer the dilution vial to micro-centrifuge and spin down the solids for 3 to 5 minutes. If any particulates are observed in the supernatant, re-centrifuge until liquid appears clear.
6. Transfer 120 µL of supernatant into an empty 0.5 mL buffer vial.

If samples are to be sent for PCR confirmation following RAMP® testing, remove the supernatant from the mosquito sample(s) and immediately freeze and aliquot for PCR testing.

CORVID METHOD

Use a provided swab for liquid sampling.

1. By inversion, mix one buffer bottle and, via set-volume pipette, transfer 1 mL (1000 µL) of buffer to an empty 2.0 mL dilution vial.
2. Place thumb and forefinger around the dead corvid's jaw and carefully open beak. Swab inside of the throat, using absorbent end of a dry swab, and slowly move swab in an up-and-down motion to gather sample of mucus. NOTE: You should be able to feel the swab moving between your fingers.
3. Remove swab from corvid's throat and immediately submerge swab into buffer-filled dilution vial; stir/rotate for 10 seconds to transfer specimen. Avoid foaming.
4. Remove swab from buffer, rotating against inside of vial to remove excess liquid. Discard used sampling swab.
5. Transfer 120 µL of mixed sample into an empty 0.5 mL RAMP® buffer vial.

MATERIALS PROVIDED

- 100 pouches, each containing 1 RAMP® test cartridge and 1 test tip
- 2 bottles RAMP® buffer, each 60 mL (120mL total)
- 100 buffer vials, each 0.5 mL (empty)
- 100 dilution vials, each 2.0 mL (empty)
- 210 copper-coated ball bearings
- 1 transfer device for 70 µL
- 1 lot card
- 1 instructions for use (IFU)

MATERIALS REQUIRED (BUT NOT PROVIDED)

- REF: E1100 RAMP® Reader instrument; or
- REF: C2100 RAMP® 200 instrument control module, and
  REF: C3100 RAMP® 200 instrument test module
- Optional accessories such as RAMP® printer and/or barcode scanner
- Micro-centrifuge
- Vortex mixer
- Set –volume pipette (1 mL sample)
- Set-volume pipette (120 µL sample)
- Swabs, for corvid sampling (optional)
- Marking pen (optional)

Use only the listed RAMP® instruments with this test.

LOT CARD CALIBRATION

Each RAMP® test kit includes a lot card that is individually packaged in an anti-static pouch. The lot card provides information specific to the kit test cartridge lot, including lot number, expiration date, and standard curve information. For further details on loading lot-specific information, see the RAMP® instrument User Manual. No additional calibration beyond insertion of the lot card is necessary. This operation is required only once per test kit lot.

For each new lot, remove the lot card from its pouch and insert it into the lot card slot on the instrument. Once the lot card has been uploaded, return to its pouch and do not discard. Avoid touching the contacts at the end of the lot card.

PROCEDURE

If stored refrigerated, prior to sample preparation, allow all components to come to ambient temperature for at least 15 minutes.

- Keep the test cartridge and test tip in the sealed foil pouch until ready for use. Once opened, test cartridges and test tips must be used or discarded within 60 minutes.
- The test cartridge, test tip, and buffer vial should be discarded after a single-use. Do not reuse.

1. Prepare RAMP® instrument for test cartridge. Refer to the RAMP® User Manual for detailed instructions on Starting a Test.
2. Uncap the buffer vial containing the mixed buffer/ suspected agent and place upright on a clean, dry level surface; or in a holder.
3. Open a test pouch and remove the test cartridge and tip. Place the test cartridge on a clean, level surface and, if desired, write a Sample ID on test cartridge with a marking pen. Firmly attach the test tip to the supplied transfer device.
4. Fully depress the transfer device plunger and submerge the test tip into the buffer vial close to, but not touching, the bottom.
5. Mix sample slowly by fully pressing and releasing the plunger 10 times; keep the tip submerged in the buffer for optimal mixing and to minimize air bubbles.
6. Once mixing is complete, draw 70 µL of sample into the test tip by releasing the plunger one final time and immediately dispense liquid into the sample well of the test cartridge. Small droplets may remain in the tip; this is expected.
7. Once in the test cartridge, allow sample to dry at room temperature for a minimum of 90 minutes before continuing.
8. After the minimum sample drying time has lapsed, insert the test cartridge fully into the instrument and press until firm resistance is felt.
9. The instrument will draw the cartridge in and test development will begin.
10. The instrument will analyze the cartridge and report the result in approximately 2 minutes.
11. Record the result, if required. For additional information on printing and/or uploading results, please refer to the RAMP® User Manual.
12. Remove the used test cartridge and discard all used test components according to local biohazard procedures. DO NOT reuse.

For additional information on the general operation and troubleshooting of the instrument, please refer to the RAMP® User Manual.

QUALITY CONTROL

Refer to the RAMP® User Manual for full details on quality control operation and troubleshooting.

SYSTEM QUALITY CONTROL

The RAMP® instrument has error checking and self-diagnostic functions (Internal Quality Control (IQC)) that assure system integrity. These include algorithms and measurements used to confirm acceptable operator technique, sample handling, and test performance. Frequency of IQC may be programmed at desired intervals.

Valid results are displayed only after all performance requirements have been met.

PROCEDURAL CONTROLS

- Each RAMP® test has built-in controls. Test cartridges have a control zone that is scanned as part of the test protocol to ensure proper sample flow.
- Control limits for each lot of test cartridges are established during the manufacturing process and are incorporated in the test-specific lot parameters. If a control result does not meet specifications, the sample result is not reported and a message is displayed.

TEST RUN MESSAGES

When the RAMP® instrument is unable to continue a specific task it will emit an audio alarm and display a message. Refer to the RAMP® User Manual 'Troubleshooting Guide' section for a full description of all messages. If repeated tests give unexpected results, contact Response Biomedical Technical Support for assistance.

LIMITATIONS

- Where required by local regulations, results obtained from the use of this product should be used only as an adjunct to other, conformational, procedures.
- Adulterant such as bleach in the specimen may produce an erroneous result. If adulteration is suspected, the test should be repeated with a new or diluted sample.
- Do not use samples containing blood or tissue as this may give erroneous results.

TEST CUT-OFF AND EXPECTED VALUES

RAMP® West Nile Virus test results are reported in Units. 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, corvid and virus populations, potential differences in sample preparation techniques and the inherent variability of the test. All of these factors make setting a discrete cut-off value extremely challenging. One possible solution is the introduction of a 'grey zone' as a way to improve the decision making process with RAMP® West Nile Virus results. Customers can determine their own RAMP® West Nile Virus grey zone to improve result interpretation and efficiency based on the actual mosquito / corvid population being tested and previous experience with the RAMP® West Nile Virus test.

- For mosquitoes: ≤ 10.0 to 30.0 Units (Negative); greater than or equal to 30.0 Units (Positive)
- For corvids: ≤ 10.0 to 50.0 Units (Negative); greater than or equal to 50.0 Units (Positive)

For assistance with result interpretation, contact Response Biomedical Technical Support.

PERFORMANCE CHARACTERISTICS

MEASUREMENT RANGE

10.0 to 640 Units

WNV levels in excess of 640 Units are reported as greater than > 640 ng/mL, values less than 10.0 Units should be reported as ≤ 10.0 Units.

SENSITIVITY

Consistent with studies conducted in collaboration with Health Canada's National Microbiology Lab and the US Centers for Disease Control and Prevention, the RAMP® West Nile Virus test limit of detection (LoD) can be approximated at 3 x 10^3 plaque-forming units (pfu)/mL WNV. However, due to the variable nature of WNV populations and preparations, actual test sensitivity can vary.

GLOSSARY OF SYMBOLS

LOT **Batch Code**	REF **Catalogue Number**	**Caution**
Consult Instructions for Use	Σ **Contains Sufficient for <n>Tests**	**Do Not Reuse**
Harmful, Irritant	**Manufacturer**	**Temperature Limit**
Use-by Date		

PRODUCT SUPPORT / ASSISTANCE

If you have any questions regarding the use of this product please contact Response Biomedical Corp. Technical Support:

- Within US or Canada (+1.866.525.7267)
- Outside US or Canada (+1.604.219.6119)
- By email at techsupport@responsebio.com

MANUFACTURER

RESPONSE BIOMEDICAL

Response Biomedical Corp.
1781 – 75th Avenue W
Vancouver, BC, Canada V6P 6P2
T. +1.604.456.6010
T. +1.888.591.5577 (Toll-free North America)
E. customersupport@responsebio.com
2014-04, V 1.3, English

REF E1101