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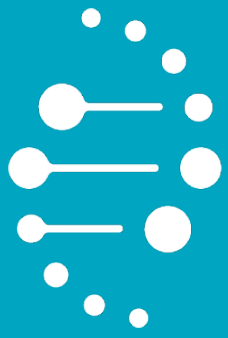

Co-Dx

Instructions for Use for Vector Smart™ North American Mosquito West

REF **NAMw-R-001**

Vector Smart™ North American Mosquito West (NAM-w) RUO
CO-DIAGNOSTICS, INC.

RUO

CO-DIAGNOSTICS, INC. | 2401 Foothill Dr., Ste D, Salt Lake City, UT 84109 USA

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1 MANUFACTURER

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RUO

2 INTENDED USE

The **Vector Smart™ North American Mosquito West (NAM-w)** Research Use only (RUO) product is a research use only multiplex test, based on real-time polymerase chain reaction (qPCR) technology, for the simultaneous qualitative detection of the West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Western equine encephalitis virus (WEEV) specific ribonucleic acid (RNA).

For research use only. Not for use in diagnostics procedures.

3 PRODUCT DESCRIPTION

The **Vector Smart™ NAM-w** RUO is a research use only multiplex test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of RNA of WNV, SLEV, and WEEV. Specifically, in *Culex spp.* and *Aedes spp.* mosquitos. This RUO is designed for mosquito surveillance purposes which are especially important for public health officials working towards mosquito abatement.

The **Vector Smart™ NAM-w** RUO includes a mosquito derived internal control to identify possible qPCR inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The **Vector Smart™ NAM-w** RUO also includes a positive control which includes three synthetic RNA molecules carrying sequences that are homologous to WNV, SLEV, and WEEV viruses and are targeted by this multiplex assay. Positive controls represent a source of cross-contamination. Precautions should be taken to prevent and minimize the risk.

CoPrimers™ included in the **Vector Smart™ NAM-w** RUO include the following:

- CoPrimers™ that are targeting WNV are labelled with the FAM™ fluorophore
- CoPrimers™ that are targeting WEEV are labelled with the CAL Fluor® Orange 560 fluorophore
- CoPrimers™ that are targeting SLEV are labelled with the Quasar® 670 fluorophore
- CoPrimers™ that are targeting the Mosquito Enhancer of the Internal Positive Control (IPC) DNA are labelled with CAL Fluor® Red 610 fluorophore

4 RUO COMPONENTS

See Table 1 for a list of RUO components.

Table 1

RUO Components

Lid Color	Component	Symbol	Catalog Number	Description	Amount
Brown	Vector Smart™ NAM-w Master Mix	MM	NAMw-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1x500 µL (100 reactions)
Red	Vector Smart™ NAM-w Positive Control	PC	NAMw-PC-001	Proprietary blend of target templates	1x500 µL (100 reactions)
Clear	No Template Control	NC	NAMw-NC-001	DNase/RNase-free water	1x500 µL (100 reactions)
Orange	Extraction Control	EC	NAMw-EC-001	Proprietary blend of target templates	1x500 µL (100 reactions)

The RUO Catalog Number is NAMw-R-001. Contact Sales at (801) 438-1036 ext. 01 to order.

5 STORAGE

Note the following during storage, handling, and disposal of this product:

- The **Vector Smart™ NAM-w** RUO is shipped on dry ice. The components of the RUO should arrive frozen. If one or more of the components are not frozen upon receipt, or are compromised during shipment, contact your distributor for assistance.
- All components should be stored below -16°C upon arrival to prevent degradation of reagents.
- Repeated thawing and freezing of components (more than four times) should be avoided, specifically the master mix, as this might affect the performance of the assay. The reagents should be frozen in multiple aliquots if they are to be used intermittently.
- Co-Diagnostics recommends, storage between +2°C and +8°C should not exceed a period of 4 hours.
- If you work in an area prone to power outages, it is recommended to have a back-up generator for your freezer as well as a temperature data log system to ensure that the **Vector Smart™ NAM-w** RUO remains frozen at a temperature between -40°C and -16°C.
- Expired products should not be used, as the integrity of the components cannot be guaranteed.

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- The product is not a biological waste. See Safety Data Sheets (SDS) for hazard classification. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

6 MATERIALS AND DEVICES (REQUIRED BUT NOT PROVIDED)

The following materials and devices are required but not provided with this product:

- Appropriate 4-channel qPCR instrument, compatible with the fluorophores used in this test.
- Appropriate nucleic acid extraction system or kit, with associated equipment according to extraction manufacturer protocol.
- Vortex mixer
- Centrifuge with a rotor for 2 mL reaction tubes
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Ice
- Biosafety cabinet, ideally a Biosafety Level 2 (BSL-2) facility
- Copper coated premium BBs (for extraction) or another sample homogenizer

7 BACKGROUND INFORMATION

7.1 West Nile Virus (WNV)

The following is information about WNV:

- **About:** WNV is the leading cause of mosquito-borne disease in the continental United States. The virus was introduced to the US in 1999 after the New York outbreak where there were 62 cases and 6 fatalities. The WNV had other outbreaks in the US from time to time.
- **The Virus:** is an enveloped, single-stranded (+) RNA virus part of the *Flaviviridae* family.
- **Transmission:** Most commonly spread to people by the bite of an infected *Culex spp.* mosquito, in special *Culex pipiens* in the northern half of the US, *Culex quinquefasciatus* in the southern states, and *Culex tarsalis* in the western states where it overlaps with *Cx pipiens* and *quinquefasciatus*. Cases of WNV occur during mosquito season, which starts in the summer and continues through fall. 94% of human cases are reported from July through September, however cases of WNV can happen year-round. The transmission can also happen through blood transfusion and organ donation. Since 2003, the US blood supply and organs are tested for WNV year-round. For more information consult: *West Nile Virus in the*

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United States: Guidelines for Surveillance, Prevention, and Control (Division of Vector-Borne Diseases, 2013).

- **Signs and Symptoms:** Fortunately, most people infected with WNV do not feel sick. About 1 in 5 people who are infected develop a fever and other symptoms. About 1 out of 150 infected people develop a serious, sometimes fatal, illness.
- **Detection:** Detection of WNV in mosquito pools for surveillance is an essential tool for directing spraying of pesticides in Vector Control programs throughout the United States.

7.2 St. Louis Encephalitis Virus (SLEV)

The following is information about the SLEV:

- **About:** SLEV is an arbovirus that is largely spread through the US, but periodic outbreaks and epidemics have primarily occurred in the Mississippi Valley and along the Gulf Coast. In temperate areas of the United States, SLEV disease cases occur primarily in the late summer or early fall. In southern states, cases can occur year-round.
- **The virus:** is an enveloped, single-stranded (+) RNA virus part of the *Flaviviridae* family.
- **Transmission:** SLEV is spread to people by the bite of Culex species mosquito. The most common vectors are *Culex pipiens*, *Culex quinquefasciatus*, *Culex tarsalis*, and *Culex nigripalpus*.
- **Signs and Symptoms:** Most people infected with SLEV have no apparent illness. Initial symptoms of those who become ill include fever, headache, nausea, vomiting, and tiredness. Severe neuroinvasive disease (often involving encephalitis, an inflammation of the brain) occurs more commonly in older adults. In rare cases, long-term disability or death can result. There are no vaccines to prevent nor medications to treat SLEV. Care is based on symptoms.
- **Detection:** Detection of SLEV in mosquito pools for surveillance is an essential tool for directing spraying of pesticides in Vector Control programs throughout the United States.

7.3 Western Equine Encephalitis Virus (WEEV)

The following is information about the WEEV:

- **About:** WEEV is an arbovirus that is associated with both human and equine encephalitis throughout the Americas. The WEEV is a summertime infection found in the west of the US. It is more common in rural areas.
- **The Virus:** is an enveloped, single-stranded (+) RNA virus part of the *Alphavirus* genus of the family *Togaviridae.*
- **Transmission:** The natural transmission cycle of WEEV involves a variety of mosquitoes and avian species. Most often it is transmitted from avian hosts to equines and humans, which are presumed to be dead-end hosts.

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- **Signs and Symptoms:** Most infections are subclinical but may present with a nonspecific viral syndrome consisting of fever, chills, malaise, and muscle aches. More serious symptoms are rare; however, complications vary from different levels of central nervous system (CNS) impairment to death.
- **Detection:** Detection of WEEV in mosquito pools for surveillance is an essential tool for directing spraying of pesticides in Vector Control programs throughout the United States.

7.4 Multiplex (WNV, SLEV, and WEEV)

Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any real-time RT-PCR-based test system that accumulation of mutations over time may lead to false negative results. Make sure to always use the most current version of the **Vector Smart™ NAM-w** RUO and avoid use of expired RUO components.

7.5 Mosquito Selection, Collection, Storage, and Handling Recommendations

The sample selection, collection, storage, and handling play an essential part on the performance of nucleic acid assays. Thus, valuable information is presented in this section to help laboratories develop better procedures for the analysis of results and troubleshooting other problems.

For more information, visit the following Centers for Disease Control and Prevention (CDC) website locations:

- CDC, West Nile virus: https://www.cdc.gov/westnile/index.html
- CDC, Saint Louis Encephalitis: https://www.cdc.gov/sle/index.html
- CDC, Western Equine Encephalitis Virus Disease: https://wwwn.cdc.gov/nndss/conditions/western-equine-encephalitis-virus-disease/

8 ACCESSORIES (NOT INCLUDED)

8.1 Thermocycler

Co-Diagnostics, Inc. can either directly or through reagent rental programs, provide the CoDx Box™ thermocycler machines (manufactured for Co-Diagnostics, Inc. by Bio Molecular Systems).

The CoDx Box thermocycler is recommended due to its ease of use, small size, durability, and fast report generation. The CoDx Box thermocycler software was developed by Bio Molecular Systems solely for Co-Diagnostics, Inc., and it has been verified for use with Co-Diagnostics, Inc. real-time PCR products, simplifying result interpretation.

The CoDx Box thermocycler reads fluorescence in real-time, generated from the PCR reagents loaded into CoDx Box PCR reaction tubes, amplifies the virus RNA by thermal cycling using magnetic induction, and displays output data through the integrated software. The CoDx Box thermocycler is available with 48 reaction wells and 4 channels. Other Co-

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Diagnostics, Inc. real-time PCR products also utilize this CoDx Box thermocycler. The Microsoft Surface™ Pro 4 System (MSPRO-4) is available for use with CoDx Box software in a windows-based operation system. The output device used with the CoDx Box thermocycler can be a printer or external computer. Alternately, the results can be manually recorded. The method of reporting is left to the discretion of the user.

9 WARNINGS AND PRECAUTIONS

WARNING!

Read the *Instructions for Use* carefully before using the product. Before first use check the components for integrity and frozenness upon arrival.

Users should do the following:

- Always treat mosquito samples as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling samples. Always wear gloves when handling RUO components.
- Always use DNase/RNase-free disposable pipette tips with filters.
- Use segregated working areas for sample preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional workflow. To prevent contamination, change gloves between areas.
- Consult appropriate SDS for safety. The SDS for the **Vector Smart™ NAM-w** RUO is provided with the shipment. If not provided with shipment the SDS can be retrieved from Co-Diagnostics website at the following link: Safety Data Sheets | Co-Diagnostics, Inc. (co-dx.com).
- Do not open the reaction tubes/plates post amplification.
- Do not autoclave reaction tubes/plates after the PCR, since this will not degrade the amplified nucleic acid and will pose a risk to the laboratory area to contamination.
- Do not use components of the RUO that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

10 SAMPLE INFORMATION

10.1 Sample Storage

Ensure the following when storing samples:

- Process all specimen types within 48 hours after collection, if storage is needed after 48 hours, store the samples frozen, preferably at -70°C (ECDC, 2020).

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- Avoid repeated freezing and thawing of any specimen. If you need to keep a specimen for retesting, aliquot the specimen in different tubes to avoid freezing and thawing cycles.
- Monitor the temperature in the storage areas and recorded temperatures regularly to identify potential fluctuations.
- Do not use domestic refrigerators/freezers with wide temperature fluctuations. Domestic refrigerators/freezers are not suitable for the storage of frozen specimens (CDC, 2020).

11 PROCEDURE

11.1 Mosquito Collection

Mosquitos are typically collected using commercially available mosquito traps, such as the CDC miniature light trap Model 512. The mosquitoes collected from a single collection site are often called a pool. The pool of mosquitoes is sexed and speciated based upon the specific target for which they are being tested.

After being sexed and speciated, the mosquitoes are either stored frozen or can go through the extraction process. After extraction, the mosquito extract can then be tested or stored frozen, preferably at -70°C for future testing.

11.2 Mosquito Preparation

The quality of the extraction of the RNA from the samples is essential for the performance of **Vector Smart™ NAM-w**. The extraction protocol to be followed should be performed following manufacturer's instructions or an internally validated protocol. Suggestions of extraction methods and system include:

- QIAamp® Viral RNA Mini RUO (QIAGEN)
- MagMAX™ Viral RNA Isolation RUO (Applied Biosystems)
- MagMAX™ Viral Pathogen Nucleic Acid Isolation RUO (Applied Biosystems)
- Sbeadex Livestock RUO (LGC)

To prepare the mosquitoes before the extraction, place a pool of 10-50 mosquitoes in a snap top 1.5 or 2.0 mL microcentrifuge tube, and add 10 µL per mosquito of (TE Buffer with 1% Triton X-100) to the tube, and 1 copper coated premium BB (for 19 or less mosquitoes) or 2 BB's (for 20 or more mosquitoes). Vortex the tube for 5 minutes, and centrifuge at 21,380 x g for 5 minutes. Remove the supernatant and continue with the extraction.

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WARNING!
An important step to ensure that the extraction process is working is to add 5 µL of **Extraction Control**, after the lysis step or when instructed by the extraction kit, into every sample pool being extracted. Due to the variability of mosquito populations, this will ensure that there is consistent amplification of the Mosquito IPC.

For additional preparation information or Technical Support, contact Technical Support at (801) 438-1036 ext. 02.

WARNING!

If your sample preparation system is using washing buffers containing ethanol, make sure to eliminate any traces of ethanol prior to elution of the nucleic acid. Ethanol is a strong inhibitor of real-time PCR.

The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.

Do not use buffer from other products besides the buffer in the sample extraction kit. Products like the RAMP grinding buffer is known as a PCR inhibitor and should not be used (Burkhalter, Horiuchi, Biggerstaff, Savage, & Nasci, 2014).

11.3 Vector Smart™ NAM-w Reagent Setup

11.3.1 Set Up the Reagent

Perform the steps below to set up the reagent.

11.3.1.1 Clean all working surfaces with a fresh 10% bleach solution followed by a molecular-grade alcohol or another equivalent method of cleaning that disinfects and degrades nucleic acids.

11.3.1.2 Thaw all reagents and samples on ice, or a cold block, before starting the setup.

11.3.1.3 Vortex all Vector Smart™ DS MM, PC, NC, and all sample tubes for 3 seconds.

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11.3.1.4 Briefly spin the MM, PC, NC down before using to ensure reagents are properly mixed and to ensure removal of any condensation or residue from the lids.

11.3.2 Set Up the Reaction

Perform the steps below to set up the reaction.

11.3.2.1 Collect enough reaction wells for each of the following:

- One for each NC,
- One for each sample you want to test, and
- One (or more) for each PC

Note: The example below shows the number of wells needed for 5 known samples.

PC	1
NC	1
Unknown samples	5
Total wells required	**7**

Important:

- Pipette on ice, if possible.
- Perform PC pipetting and sample setup in a separate area, or at a separate time from the MM and NC.
- Change pipette tips between samples and change pipette tips after pipetting each component.
- Pipet the PC last, if possible, to avoid contamination events.

11.3.2.2 Pipet 5 µL of MM into each well collected.

11.3.2.3 Pipet 5 µL of the sample or 5 µL of NC control to the appropriate wells (in addition to the 5 µL of MM already in the well).

Note: Ensure that at least one NC control is included in each run and that enough space remains for at least one PC.

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11.3.2.4 Pipet 5 µL of PC into the appropriate well.

11.3.2.5 Seal the reaction plate with an optical adhesive film or seal each reaction tube with its appropriate lid.

11.3.2.6 Place the plate or tubes into the real-time PCR instrument in the correct orientation and start the run.

11.4 PCR Instrument Setup

11.4.1 If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory (801) 438-1036 ext. 03 for the template file for download. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, or using another device, use the settings outlined below to program the PCR instrument.

11.4.1.1 To achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.

11.4.2 Define the settings as displayed in Table 2.

Table 2

PCR Instrument Settings

Item	Setting
Reaction Volume	10 µL
Ramp Rate	Default
Passive Reference	None

11.4.3 Program PCR instrument with the cycling conditions outlined in Table 3.

Table 3

Recommended Cycling Conditions

Item	Stage	Cycles	Temperature	Time
Reverse Transcription	Activation	1	45˚C	15 minutes
Initial Denaturation	Hold	1	95˚C	2 minutes
Amplification	Cycling	50	95˚C	3 seconds
			55˚C	32 seconds

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11.4.4 Ensure that PCR instrument being used is compatible with the fluorophores below. Some devices may not have options for the quencher. If needing help or have questions, contact Co-Diagnostics Inc. Technical Support at (801) 438-1036 ext. 02.

11.4.5 Define the fluorescence detectors (dyes) as displayed in Table 4.

Table 4

Fluorescence Detector Definitions

Target	Detector Name	Reporter	Quencher
WNV specific RNA	WNV	FAM™	BHQ® - 1
WEEV specific RNA	WEEV	CAL Fluor® Orange 560	BHQ® - 1
SLEV specific RNA	SLEV	Quasar® 670	BHQ® - 2
Mosquito Internal Positive Control	IPC	CAL Fluor® Red 610	BHQ® - 2

11.4.6 When the run is finished, ensure that the run file is saved.

12 DATA ANALYSIS

For basic information regarding data analysis on specific real-time PCR instruments please refer to the user manual of the respective instrument.

12.1 Positive and No Template Controls

12.1.1 Do the following to validate the test runs:

12.1.1.1 Ensure that both the positive and no template controls have passed.

12.1.1.2 Ensure the control conditions in Table 5 are met.

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Table 5

Control Conditions

Control Type	Control Name	Purpose of Control	WNV	SLEV	WEEV	Mosquito Internal Control (NAM.18s)
NAM Positive Control	WNV (FAM™)	Verifies the performance of the master mix	+	+	+	+
	WEEV (CF®560)					
	SLEV (Q®670)					
	IPC (CF®610)					
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-	-

12.1.1.3 If controls pass, interpret the sample results.

12.1.2 Invalid Test Run

12.1.2.1 If any of the controls fail, an investigation should be made to decide whether the run is valid or not. For investigation, document the run and initiate the troubleshooting procedures in section 0.

12.2 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on the following three possible outcomes:

- Positive
- Negative
- Inconclusive

A **Positive** result will show an amplification curve or cycle threshold value for WNV, SLEV, or WEEV at or below 45 cycles. Amplification curves greater than 45 cycles for NAM-w are in the uncertainty zone. The presence of a curve for positive sample in all or any of the WNV, SLEV, or WEEV indicates a positive result. The amplification of the NAM.18S shows that the extraction was successful.

A **Negative** result will show no amplification for WNV, SLEV, or WEEV; however, occasionally amplification greater than 45 cycles occurs due to the uncertainty zone (less

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than 95% confidence). The absence of a curve for NAM-w indicates a negative result ONLY when the Mosquito IPC marker (NAM.18S) is positive.

An **Inconclusive** result will result if any of the controls fail. See troubleshooting.

The interpretation of results can be translated to Table 6.

Table 6

Interpretation of Results

<table>
<tr><th>Marker</th><th>WNV</th><th>SLEV</th><th>WEEV</th><th>Mosquito Internal Positive Control (NAM.18S)</th><th>Logix Smart™ Positive Control</th><th>No Template Control (NC) Logix Smart™ Master Mix + Nuclease-Free Water</th><th>Result</th></tr>
<tr><td rowspan="20">Instrument Reading</td><td>+</td><td>+</td><td>+</td><td colspan="3" rowspan="18">Pass</td><td>NAMw +</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>NAMw -</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">-</td><td rowspan="3">-</td><td>WNV +</td></tr>
<tr><td>SLEV -</td></tr>
<tr><td>WEEV -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">+</td><td rowspan="3">-</td><td>WNV -</td></tr>
<tr><td>SLEV +</td></tr>
<tr><td>WEEV -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">-</td><td rowspan="3">+</td><td>WNV -</td></tr>
<tr><td>SLEV -</td></tr>
<tr><td>WEEV +</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">+</td><td rowspan="3">-</td><td>WNV +</td></tr>
<tr><td>SLEV +</td></tr>
<tr><td>WEEV -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">+</td><td rowspan="3">+</td><td>WNV -</td></tr>
<tr><td>SLEV +</td></tr>
<tr><td>WEEV +</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">-</td><td rowspan="3">+</td><td>WNV +</td></tr>
<tr><td>SLEV -</td></tr>
<tr><td>WEEV +</td></tr>
<tr><td colspan="3" rowspan="3">Any Result</td><td>Fail</td><td colspan="2">Pass</td><td rowspan="3">Inconclusive: See Troubleshooting</td></tr>
<tr><td rowspan="2">Pass</td><td>Fail</td><td>Pass</td></tr>
<tr><td>Pass</td><td>Fail</td></tr>
</table>

Anything before 45 cycles is considered a positive reading (+). Anything after 45 cycles is considered a negative or inconclusive due to confidence lower than 95%.

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13 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Vector Smart™ NAM-w,** even if the recommended steps for troubleshooting resolves the issue. To give feedback please fill out the Customer Feedback Form by visiting http://co-dx.com/contact/feedback/

13.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Presently, we establish the expiration date of this product to be 12 months. We do not recommend the use of expired RUO reagents because doing so may lead to inaccurate results.

Always use the most recent version of this document because we add more stability information when studies are completed.

13.2 User Errors

Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.

It is essential for the user to have some molecular biology experience and be familiar with proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be replaced after every pipetting. Gloves must be replaced often. Equipment, such as pipettes and real-time PCR instruments, should be calibrated when applicable.

A 90-minute online training course for Good Laboratory Practices for Molecular Genetics Testing (Centers for Disease Control and Prevention, 2017) is available at the CDC website at the following link https://www.cdc.gov/labtraining/training-courses/good-lab-practices-molecular-genetics-testing.html

13.3 Invalid Results/Inconclusive Results

13.3.1 Vector Smart™ NAM-w Positive Control not amplifying

No amplification from the positive control could be the result of one or multiple factors, such as:

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,

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- **Vector Smart™ NAM-w Master Mix** or **Vector Smart™ NAM-w Positive Control** degradation (result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, it is best to disregard the results from the samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the positive control, after re-extraction and re-amplification, happens a third time, open a new **Vector Smart™ NAM-w Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02.

13.3.2 NAM.18s (Mosquito IPC) not amplifying in samples

No amplification from the NAM.18s (IPC) channel could be the result of one or multiple factors, such as the following:

- Not enough nuclear material in the sample,
- PCR inhibitors such as: ethanol and heparin,
- the extraction was performed incorrectly,
- or the extraction RUO used is not compatible or has a step that eliminates the mosquito DNA (e.g., a DNase Digestion step).

Negative results cannot be trusted and re-testing by re-amplification should be performed. If the IPC fails again, then samples should be re-extracted and re-amplified. If it fails after that an investigation should be conducted to identify possible causes for error. If the cause for the error is clear, the test can either be signed out as **inconclusive** due to either PCR inhibitors being present or not enough nuclear material being present. If the cause for error is unclear contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02 for help.

13.3.3 **No Template Control** showing amplification

Amplification of NAM-w in a No Template Control indicates contamination in one or more of the reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors.

None of the results can be trusted and re-testing by re-amplification should be performed. If the NC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in

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the process. If failure of the NC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02.

14 REFERENCES

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15 LEGEND OF PACKAGE SYMBOLS

See Table 7 for a legend of package symbols.

Table 7

Legend of Package Symbols

Icon	Description
REF	Catalog number
LOT	Batch Code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
Σ x	Contains sufficient for x tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Manufacturer
RUO	Research Use Only

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