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Co-Dx

Instructions for Use for Vector Smart™ North American Mosquito East

REF **NAMe-R-001**

Vector Smart™ North American Mosquito East RUO
CO-DIAGNOSTICS, INC.

RUO

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

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RUO

1 INTENDED USE

The Vector Smart™ North American Mosquito East is a research use only multiplex test, based on real-time PCR (qPCR) technology, for the simultaneous qualitative detection of the West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Eastern equine encephalitis virus (EEEV) specific RNA in mosquitoes.

For research use only (RUO). Not for use in diagnostics procedures.

2 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-e Master Mix	MM	NAMe-MM- 001	Proprietary blend of CoPrimers™ and PCR reagents	1x500 µL (100 reactions)
Red	Vector Smart™ NAM-e Positive Control	PC	NAMe-PC- 001	Proprietary blend of target templates	1x500 µL (100 reactions)
Clear	No Template Control	NC	NAMe-NC-001	DNase/RNase-free water	1x500 µL (100 reactions)
Orange	Extraction Control	EC	NAMe-EC-001	Proprietary blend of target	1x500 µL (100 reactions)

The product code is NAMe-R-001. Contact Sales at (801) 438-1036 ext. 01 or at www.co-dx.com/contact/ to order.

3 VECTOR SMART™ NORTH AMERICAN MOSQUITO EAST (NAM-E) STORAGE, HANDLING, AND DISPOSAL

- The **Vector Smart™ North American Mosquito East (NAM-e)** 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.
- Upon receipt of RUO, laboratory should follow internal procedures for quality control.
- 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-e** test RUO remains frozen at a temperature between -40°C and -16°C.
- Protect **Master Mix** from light.
- Expired products should not be used, as the integrity of the components cannot be guaranteed.
- 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.

4 WARNINGS AND PRECAUTIONS

WARNING!

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

Users should pay attention to the following:

- Use of this product should be limited to personnel instructed and trained in the techniques of real-time PCR.
- Samples should always be treated 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 PPE between areas.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents. Dedicate supplies and equipment to separate working areas and do not move them from one area to another.
- Consult appropriate Safety data Sheets (SDS) for safety. The SDS for the **Vector Smart™ NAM-e** test RUO is provided with the shipment. If not provided with shipment the SDS can be retrieved from Co-Diagnostics website at the 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.

5 BACKGROUND INFORMATION

5.1 West Nile Virus (WNV)

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

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5.2 St. Louis Encephalitis Virus (SLEV)

- **About:** Saint Louis encephalitis virus (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 (Saint Louis Encephalitis, n.d.)
- **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*.

5.3 Eastern Equine Encephalitis Virus (EEEV)

- **About:** Eastern equine encephalitis (EEEV) is an arbovirus that is associated with both human and equine encephalitis. The EEEV is a summertime infection found in the east of the US,usually around freshwater hardwood swamps in the Atlantic, Gulf coast areas, and Great Lakes region. It is more common in rural areas (Eastern Equine Encephalitis, n.d.). Approximately 30% of people with Eastern Equine Encephalitis die and many survivors have ongoing neurologic problems (Eastern Equine Encephalitis, n.d.).
- **The virus:** is an enveloped, single-stranded (+) RNA virus part of the *Alphavirus* genus of the family *Togaviridae*.
- **Transmission:** EEEV is maintained in a cycle between *Culiseta melanura* mosquitoes and avian hosts in freshwater hardwood swamps. *Cs. melanura* is not considered to be an important vector of EEEV to humans because it feeds almost exclusively on birds. Transmission to humans requires a bite from infected mosquito species, after biting an infected bird. Mosquito species known to “bridge” transmission from infected avian hosts to uninfected animal hosts includes *Aedes, Coquillettidia,* and *Culex* species (Eastern Equine Encephalitis, n.d.).

5.4 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 here to help laboratories develop better procedures for the analysis of results and troubleshooting other problems.

For more information visit the CDC website in the following addresses:

- CDC, West Nile virus: https://www.cdc.gov/westnile/index.html
- CDC, Saint Louis Encephalitis: https://www.cdc.gov/sle/index.html
- CDC, Eastern Equine Encephalitis Virus Disease: https://www.cdc.gov/easternequineencephalitis/index.html

6 PRODUCT DESCRIPTION

The **Vector Smart™ NAM-e** RUO is a research use only multiplex test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of ribonucleic acid (RNA) of the West Nile, St. Louis encephalitis, and Eastern equine encephalitis viruses. Specifically, in *Culex spp.* and *Aedes spp.* mosquitos. This test is designed for mosquito surveillance purposes which are especially important for public health officials working towards mosquito abatement.

The **Vector Smart™ NAM-e** test includes an internal control to identify possible qPCR inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The **Vector Smart™ NAM-e** test also includes a positive control which includes three synthetic RNA molecules carrying sequences that are homologous to West Nile (WNV), St. Louis encephalitis (SLEV), and Eastern equine encephalitis (EEEV) 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.

Vector Smart™ NAM-e test CoPrimers™ include the following:

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

7 MATERIALS AND DEVICES (REQUIRED BUT NOT PROVIDED)

- Appropriate 4-channel real-time PCR instrument, compatible with the fluorophores used in this test.
- Appropriate nucleic acid extraction system or kit
- 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 BSL-2 facility
- Copper coated premium BB’s (for extraction) or another sample homogenizer

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WARNING!

All instruments should be properly installed, calibrated, and maintained according to the manufacturer's instructions and recommendations. Do **not** use instruments with outdated calibration.

8 PROCEDURE

8.1 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 being tested for.

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.

8.2 Mosquito Preparation

The quality of the extraction of the RNA from the samples is essential for the performance of **Vector Smart™ NAM-e**. The extraction protocol to be followed should be performed following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with **Vector Smart™ NAM-e** must be validated by the user.

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.

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, into every sample pool being extracted. Due to the variability of mosquito populations, this will ensure that there is consistent amplification of the Mosquito Internal Positive Control (IPC).

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. Products like the RAMP grinding buffer is known as a PCR inhibitor and should not be used (Burkhalter, Horiuchi, Biggerstaff, Savage, & Nasci, 2014).

For additional information and technical support regarding preparation please contact Technical Support 1-(801) 438-1036 ext. 02.

8.3 Set Up the Reagent

Perform the steps below to set up the reagent.

8.3.1 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.

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

8.3.3 Vortex all **Vector Smart™ North American Mosquito East** DS MM, PC, nuclease-free water (used as a no template control or NC), and all sample tubes for 3 seconds.

8.3.4 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.

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8.4 Set Up the Reaction

Perform the steps below to set up the reaction.

8.4.1 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.

8.4.2 Pipet 5 µL of MM into each well collected.

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

8.4.4 Pipet 5 µL of PC into the appropriate well.

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

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

8.5 PCR Instrument Setup

8.5.1 For basic information regarding setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For programming instructions questions regarding the use of other real-time PCR instruments please contact the Laboratory (801) 438-1036 ext. 03 or at www.co-dx.com/contact/.

8.5.2 If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory (801) 438-1036 ext. 03 or at www.co-dx.com/contact/ 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.

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

8.5.3 Define the following settings in Table 2.

Table 2

Recommended Instrument Settings

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

8.5.4 Program PCR instrument with the cycling conditions in Table 3.

Table 3

PCR Instrument with 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

8.5.1 Ensure that PCR instrument being used is compatible with 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 or at: www.co-dx.com/contact/.

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

Table 4

Fluorescence Detector Definitions

WNV specific RNA	WNV	FAM™	BHQ® - 1
SLEV specific RNA	SLEV	Quasar® 670	BHQ® - 1
EEEV specific RNA	EEEV	CAL Flour® Orange 560	BHQ® - 2
Mosquito Internal Positive Control	IPC	CAL Flour® Red 610	BHQ® - 2

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

9 DATA ANALYSIS

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

9.1 Validity of Test Runs

9.1.1 Valid Test Run

9.1.1.1 Ensure that both the positive and no template control passed. The control conditions in Table 5 must be met.

Table 5

Required Control Conditions

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

9.1.1.2 If controls pass, interpret the sample results.

9.1.2 Invalid Test Run

9.1.2.1 If any of the controls fail, the run is invalid. Document the run and initiate troubleshooting.

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9.2 Interpretation of Results

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

- Positive
- Negative
- Inconclusive

A **Positive** result will show an amplification curve or cycle threshold value for WNV, SLEV, or EEEV. The cut off value should be determined by in house validation testing. However, internal studies have shown rare primer-dimer formation or other non-specific amplification at 45 cycles. This fact can be attributed to the nature of the CoPrimers™ (Satterfield, 2014) (Poritz & Ririe, 2014). The amplification of the RNaseP (IPC) shows that the extraction was successful.

A **Negative** result will show no amplification for WNV, SLEV, or EEEV; The absence of a curve for NAMe indicates a negative result ONLY when the RNaseP (IPC) marker 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

Marker	WNV	SLEV	EEEV	Mosquito Internal Positive Control (NAM.18S)	Vector Smart™ Positive Control	No Template Control (NC) Vector Smart™ Master Mix + Nuclease-Free Water	Result
Instrument Reading	+	+	+	Pass			**NAM-e +**
	-	-	-				NAM-e -
	+	-	-				**WNV +**
							SLEV-
							EEEV-
	-	+	-				WNV-
							SLEV+
							EEEV -
	-	-	+				WNV-
							SLEV-
							EEEV+
	+	+	-				**WNV+**
							SLEV+
							EEEV-
	-	+	+				WNV-
							SLEV+
							EEEV+
	+	-	+				**WNV+**
							SLEV-
							EEEV+
	Any Result			Fail	Pass		**Inconclusive: See Troubleshooting**
				Pass	Fail	Pass	
					Pass	Fail	

The cut off value will determine what results are to be considered positive or negative. It should be determined by the assay development.

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10 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and we would like to be informed of any issues with the **Vector Smart™ NAM-e RUO,** even if the recommended steps for troubleshooting solve the issue. To give feedback please fill out the Customer Feedback Form by visiting www.co-dx.com/contact/feedback/

10.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Presently, the expiration date of this product has been established as 12 months. It is not recommended to use expired RUO reagents, doing so may lead to inaccurate results.

Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

10.2 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.

90 minutes of online training 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

10.3 Invalid Results/Inconclusive Results

10.3.1 Vector Smart™ NAM-e Positive Control not amplifying

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

- 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,
- **Vector Smart™ NAM-e Master Mix** or **Vector Smart™ NAM-e 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 happens a third time after re-extraction and re-amplification, open a new **Vector Smart™ NAM-e Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 04 visiting www.co-dx.com/contact/.

10.3.2 NAM.18S (Mosquito Internal Positive Control (IPC)) not amplifying in samples

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

- 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 a crucial element for extraction.

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 a third time 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 or by visiting www.co-dx.com/contact/.

10.3.3 **No Template Control** showing amplification

- Amplification of NAM-e 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 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 or by visiting www.co-dx.com/contact/.

11 LIMITATIONS

- This product is intended for research use only. Not intended for use in clinical diagnostics for its performance for diagnostic applications has not been established.
- Strict compliance with this document is required for optimal results. Please, always use the most recent version of this document. This can be downloaded for free at: www.co-dx.com/resources/instructions-for-use/ or by visiting www.co-dx.com/contact/.
- Use of this product is to be limited to trained and instructed personnel in real-time PCR techniques.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents a test run be performed to check the integrity, and performance of the reagents prior to testing on samples.
- Appropriate collection, transport, storage, and processing procedures of samples are required for optimal results.
- Do not use the **Vector Smart™ NAM-e** RUO components directly on the specimens collected. Perform an appropriate nucleic acid extraction prior to using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the WNV, SLEV, and EEEV genome covered by this test RUO may result in failure to detect the presence of the pathogens.

12 QUALITY CONTROL

In accordance with the Co-Diagnostics Inc.'s ISO 13485-certified Quality Management System, each lot of **Vector Smart™ NAM-e** RUO is tested against predetermined specifications to ensure consistent product quality.

13 TECHNICAL ASSISTANCE

For technical assistance, please contact our Technical Support:

- Website: http://co-dx.com/contact/
- Email: support@co-dx.com
- Phone: (801) 438-1036 ext. 02

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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
	Catalog number
	Batch code
	Cap color
	Component
	Content/volume
	Number
	Use-by-date
	Contains sufficient for n tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Manufacturer
	Research Use Only