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Co-Dx

Instructions for Use for Co-Dx Logix Smart™ Mpox (2-Gene)

REF **MPX2-R-001**

Instructions for Use for Co-Dx Logix Smart™ Mpox (2-Gene)
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 **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** is for the simultaneous qualitative detection of Mpox (monkeypox) and other related Orthopoxviruses.

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

3 PRODUCT DESCRIPTION

The **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** is a research-use-only multiplex test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of deoxyribonucleic acid (DNA) of the Mpox virus and other related Orthopoxviruses.

The **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** includes an internal control to identify possible quantitative polymerase chain reaction (qPCR) inhibition, confirm the integrity of the reagents, and verify the quality of the sample extraction. The **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** also includes a positive control (PC) which includes synthetic DNA molecules carrying sequences that are homologous to Mpox viruses targeted by this multiplex assay. PCs represent a source of cross contamination. Take precautions to prevent and minimize the risk of cross contamination.

CoPrimers™ in the **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** include the following:

- CoPrimers™ that target Mpox (MPX) F8L are labelled with the Quasar® 670 fluorophore.
- CoPrimers™ that target Mpox (MPX) L6R are labelled with the CAL Fluor® Orange 560 fluorophore.
- CoPrimers™ that target the Human RNaseP DNA, which acts as the Internal Positive Control (IPC), are labelled with CAL Fluor® Red 610 fluorophore.

3.1 Cross Reactivity

The **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** is expected to cross-react with some Orthopoxviruses, based on In Silico analysis or wet testing. That are expected to be detected by In Silico analysis include the following:

- Mpox
- Variola
- Vaccinia*
- Cowpox
- Camelpox
- Akhmeta virus
- Buffalopox
- Alaskapox

* The vaccinia virus is present in some forms of the smallpox vaccine. There is a chance that individuals who were recently vaccinated for smallpox could show a positive result.

4 RUO COMPONENTS

See Table 1 for components included in the **Co-Dx Logix Smart™ Mpox (2-Gene) RUO**.

Table 1

RUO Component Information

Lid Color	Component	Symbol	Catalog Number	Description	Amount
Brown	Co-Dx Logix Smart™ Mpox Master Mix	MM	MPX2-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1×1000 µL (100 reactions)
Red	Co-Dx Logix Smart™ Mpox Positive Control	PC	MPX2-PC-001	Proprietary blend of target templates	1×1000 µL (100 reactions)
Clear	Co-Dx Logix Smart™ Mpox No Template Control	NC	MPX2-NC-001	DNase/RNase-free water	1×1000 µL (100 reactions)

The product code is MPX2-R-001. Contact Sales at (801) 438-1036 ext. 01 or go to www.co-dx.com/contact/ to order.

5 COLLECTION, HANDLING, STORAGE, AND SHIPPING

See the following information for reagent storage and handling:

- The **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** is shipped on dry ice and should arrive frozen. Contact your distributor if one or more of the components are not frozen upon arrival or are compromised during shipment.
- Store all components immediately upon arrival at a temperature between -40°C and -16°C to prevent degradation of reagents.
- Do not use expired products. Integrity of expired components cannot be guaranteed.
- Follow internal laboratory procedures for quality control when using this product.
- Protect the master mix (MM) from light.
- If you will be using reagents intermittently, freeze the reagents in multiple aliquots to ensure that less than 10 freeze/thaw cycles occur. Excessive thawing and freezing of components (specifically the MM) could affect the performance of the assay.
- Avoid storing components at temperatures between +2°C and +8°C for more than 4 hours.
- If you work in an area prone to power outages, keep a back-up generator for your freezer as well as a temperature data log system to ensure that the product remains frozen at a temperature between -40°C and -16°C.
- Dispose of the product in accordance with applicable regional, national, and local laws and regulations. This product is not biological waste. The Safety Data Sheet (SDS) for this product can be viewed from Co-Diagnostics website at Safety Data Sheets | Co-Diagnostics, Inc. (co-dx.com).
- Always follow the most recently recommended product stability data as it becomes available. This data can be found in our latest version of the Instructions for Use at Instructions For Use - Co-Diagnostics, Inc. (co-dx.com).

6 MATERIALS REQUIRED (NOT INCLUDED)

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

- 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
- Adjustable pipettes
- Disposable pipette tips with filters

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- Disposable powder-free gloves
- Ice
- Biosafety cabinet, ideally BSL-2 facility

WARNING!

Install, calibrate, and maintain all instruments properly according to the manufacturer's instructions and recommendations. Do not use instruments with outdated calibration.

7 BACKGROUND INFORMATION

Mpox is a viral zoonosis similar to smallpox, which is also part of the Orthopoxvirus genus. Mpox primarily occurs in central and west Africa, often in proximity to tropical rain forests, but also near urban areas.

The Mpox virus is an enveloped double-stranded DNA virus with a variety of mammalian hosts, including rope squirrels, Gambian pouched rats, dormice, non-human primates, among others. (Reference https://www.who.int/news-room/fact-sheets/detail/Mpox) Mpox virus is transmitted by close contact with lesions, body fluids, respiratory droplets, and contaminated materials such as bedding.

The incubation period of Mpox is usually from 6-13 days but can range from 5-21 days. The infection can be divided into two periods: (Reference https://www.who.int/news-room/fact-sheets/detail/Monkeypox.

The invasion period (lasts between 0-5 days) characterized by fever, intense headache, lymphadenopathy (swelling of the lymph nodes), back pain, myalgia (muscle aches) and intense asthenia (lack of energy).

Lymphadenopathy is a distinctive feature of Mpox compared to other diseases that may initially appear similar (chickenpox, measles, smallpox) (Reference https://www.who.int/news-room/fact-sheets/detail/monkeypox). The skin eruption usually begins within 1-3 days of appearance of fever. The rash tends to be more concentrated on the face and extremities rather than on the trunk. It affects the face (in 95% of cases), and palms of the hands and soles of the feet (in 75% of cases). Also affected are oral mucous membranes (in 70% of cases), genitalia (30%), and conjunctivae (20%), as well as the cornea. The rash evolves sequentially from macules (lesions with a flat base) to papules (slightly raised firm lesions), vesicles (lesions filled with clear fluid), pustules (lesions filled with yellowish fluid), and crusts which dry up and fall off. The number of lesions varies from a few to several thousand. In severe cases, lesions can coalesce until large sections of skin slough off (Reference https://www.who.int/news-room/fact-sheets/detail/Monkeypox).

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In May of 2022, cases of Mpox were reported to WHO from 12 member states across three WHO regions, where Mpox virus is not endemic. These areas include the United States, Canada, Australia, United Kingdom, Spain, and Portugal. (Reference https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON385) -This reference is different than the above links.

8 ACCESSSORIES (NOT INCLUDED)

8.1 Thermocycler

Thermocyclers validated but not included with the test are displayed in Table 2.

Table 2

Thermocyclers Validated but Not Included with the Test

Thermocycler Machine	Catalog Number	Manufacturer
CoDx Box	MIC-4	Co-Diagnostics, Inc.
Mic qPCR Cycler	MIC-4	BMS, Bio Molecular Systems

8.2 Extraction System

Extraction System required but not included with the test are displayed in Table 3.

Table 3

Extraction and Automation Systems Validated with the Test

Extraction Reagent		Automation Platform (If applicable)	Manufacturer	Sample Input Volume/Sample Elution Volume by Sample Type			
Name	Cat. Number			Protocol Used	Sample Type	Input	Output
QIAamp DNA Mini Kit (Qiagen)	51304 (50 extractions) 51306 (250 extractions)	N/A	Qiagen	Buccal Swab	dry lesion swab	400 µL	60 µL x 2

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9 WARNINGS AND PRECAUTIONS

WARNING!

Before performing any testing or running any sample, verify that all instruments have been properly installed, calibrated, and are well maintained. Do **not** use instruments with an outdated calibration.

Read this *Instructions for Use* document carefully before using the product. Before first use, check the components for the following:

- Integrity
- Frozenness upon arrival
- Users should ensure the following:
- Limit use of this product to personnel instructed and trained in the techniques of real-time PCR.
- Always treat samples as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling samples and 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 personal protective equipment (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 the appropriate SDS for safety. The SDS for the **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** is provided with the shipment. If not provided with the 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 that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

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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 (CDC, 2020).
- 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).

10.2 Sample Handling

Laboratory workers should wear appropriate PPE, which includes disposable gloves, laboratory coat/gown, and eye protection when handling potentially infectious specimens.

Conduct samples suspected to be or confirmed to be infected with Mpox under a certified Class II biosafety cabinet in a BSL-2 containment facility. More details are provided in the Biosafety in Microbiological and Biomedical Laboratories (BMBL) (CDC, 2009) or the WHO Laboratory Biosafety Manual (WHO, 2004).

For specific instructions on handling clinical specimens for the Mpox virus, see also the CDC's webpage for the Preparation and Collection of Specimens – Mpox (Guidelines for Collecting and Handling Specimens for Mpox Testing | Mpox | Poxvirus | CDC) (CDC, 2022) and *Interim Guidance for Collection Diagnostic Specimens from Persons with Suspect Monkeypox* (CDC, 2003).

The quality of the extraction of the DNA from the samples is essential for the performance of **Co-Dx Logix Smart™ Mpox (2-Gene) RUO**. Perform extraction by following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** must be validated by the user.

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WARNING!

If your sample preparation system uses 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.

11 PROCEDURE

11.1 Real Time RT-PCR Setup

11.1.1 Set Up the Reagents

Perform the steps below to set up the reagents.

11.1.1.1 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.

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

11.1.1.3 Vortex all **Co-Dx Logix Smart™ Mpox (2-Gene) RUO** MM, PC, nuclease-free water (used as an NC), and all sample tubes for 3 seconds before using.

11.1.1.4 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.

11.1.2 Set Up the Reaction

Perform the steps below to set up the reaction.

11.1.2.1 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

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Note: The example below displays the minimum number of wells needed for 5 samples.

PC	1
NC	1
Samples	5
Total wells required	**7**

11.1.2.2 Pipet 10 µL of MM into each well collected.

11.1.2.3 Pipet 10 µL of the NC into the appropriate wells (in addition to the 10 µL of MM already in the well).

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

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.

11.1.2.4 Pipet 10 µL of sample or PC into the appropriate well.

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

11.1.2.6 Place the plate or tubes into the RT-PCR instrument in the correct orientation and start the run.

11.2 PCR Instrument and Thermocycler Setup

11.2.1 For programming instructions questions regarding the use of other real-time PCR instruments, contact the Laboratory (801) 438-1036 ext. 03 or at www.co-dx.com/contact/.

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11.2.2 If using Co-Diagnostics Inc. CoDx Box, contact the Laboratory (see contact information in Section 7.4.2) 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.

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

11.2.3 Define the settings as displayed in Table 4.

Table 4

Recommended Instrument Settings

Item	Setting
Reaction Volume	20 µL
Ramp Rate	Default
Passive Reference	None

11.2.4 Program PCR instrument with the cycling conditions displayed in Table 5.

Table 5

Recommended Cycling Condition Settings

Item	Stage	Cycles	Temperature	Time
Initial Denaturation	Hold	1	95˚C	2 minutes
Amplification	Cycling	45	95˚C	3 seconds
			53˚C	32 seconds

11.2.5 Ensure that the PCR instrument being used is compatible with the fluorophores below. Some devices may not have options for the quencher. If you need help or have questions, contact Co-Diagnostics Inc. Technical Support at (801) 438-1036 ext. 02 or at: support@co-dx.com.

11.2.6 Define the fluorescence detectors (dyes) as displayed in Table 6.

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Table 6

Fluorescence Detector Definitions

Target	Detector Name	Reporter	Quencher
MPX-F8L (T4)	MPX-F8L (T4)	Quasar® 670	BHQ®-2
MPX-L6R (T3)	MPX-L6R (T3)	CAL Fluor® Orange 560	BHQ®-1
RNaseP (IPC)	RNaseP (IPC)	CAL Fluor® Red 610	BHQ®-2

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

12 DATA ANALYSIS

For basic information regarding data analysis on specific qPCR instruments, please refer to the user manual of the respective instrument.

12.1 Positive Controls

Validate the test run by checking to see that the PC has passed and that the control conditions displayed in Table 7 are met.

12.2 No-Template Controls

Validate the test run by checking to see that no-template control has passed and that the control conditions displayed in Table 7 are met.

Table 7

Required Control Conditions

Control Type	Control Name	Purpose of Control	MPX L6R Result	MPX F8L Result	RNaseP (IPC) Result
MPX Positive Control (PC)	MPX-L6R (CF®560)	Verifies the performance of the master mix	+	+	+
	MPX-F8L (Quasar® 670)				
	RNaseP (IPC) (CF®610)				
No Template Control (NC)	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-

12.2.1.1 If controls pass, interpret the sample results.

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12.2.2 Invalid Test Run

12.2.2.1 If any of the controls fail, the run is invalid.

12.2.2.2 Document the run and initiate troubleshooting.

12.3 Interpretation of Results

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

- Positive
- Negative
- Invalid

A Positive result will show an amplification curve or cycle threshold value for Mpox. 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 shows no amplification for Mpox; The absence of a curve for Mpox indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An Inconclusive result occurs if any of the controls fail. See the Troubleshooting section.

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See Table 8 for results translation.

Table 8

Results Translation

	Sample Result			Co-Dx Logix Smart™ Mpox Positive Control	No Template Control (NC) (Master Mix + Water)	Interpretation of Results
	MPX-L6R CF560 Channel	MPX-F8L Q670 Channel	Internal Positive Control (RNaseP) CF610 Channel			
Instrument Reading	+	+	+	+	-	*Mpox Virus DNA +
	+	-	+	+	-	*Mpox Virus DNA +
	-	+	+	+	-	*Mpox Virus DNA +
	-	-	+	+	-	Mpox Virus DNA -
	Any Result (+/-)		-	+	-	INVALID: See Troubleshooting
			+	-	-	
			+	+	+	

Anything before 42 cycles is considered a positive analyte reading (+). Anything at or after 42 cycles is considered a negative analyte reading (-).
*A positive result indicates a sample contains either Mpox or other orthopoxvirus DNA.

Note: Samples collected from individuals who were recently vaccinated for smallpox may show a positive result because of cross-reactivity with *Orthopoxvirus vaccinia virus*, which is present in some forms of the smallpox vaccine.

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13 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and we would like to be informed of any issues with the **Co-Dx Logix Smart™ Mpox (2-Gene) 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/.

13.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Currently, the expiration date of this product has been established as 12 months. Do not use expired reagents, because 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.

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.

To help prevent errors such as splashes, crossover contamination, and volume selection, it is essential that users have some molecular biology experience and be familiar with proper pipetting technique. 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 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

13.3.1 Co-Dx Logix Smart™ Mpox PC not Amplifying

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

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- **Co-Dx Logix Smart™ Mpox** MM or PC degradation (result of reagents being stored at a temperature above -16°C for an extended period)
- Use of expired reagents
- Wrong reagents being used

When this occurs, it is best to disregard the results from the samples and re-test by re-amplification. If the PC 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 PC occurs a third time after re-extraction and re-amplification, open a new **Co-Dx Logix Smart™ MPX** PC or MM, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02 visiting support@co-dx.com.

13.3.3 IPC RNaseP is not Amplifying in Samples

13.3.4 No amplification from the RNaseP 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
- Incorrect extraction
- Extraction system used is not compatible or has a step that eliminates RNaseP DNA

Note: Positive amplification in the Mpox channel indicates a positive analyte result despite the lack of concurrent amplification in the IPC channel. The IPC amplification is dependent on the presence of human genomic DNA (gDNA) in the extraction sample, the amount of which is governed by the type of the sample and the extraction procedure used. Samples obtained from culture or sterile/pure sites (e.g., CSF, urine, or cell lysates) may not contain the human RNaseP gene.

When this occurs, the results should be interpreted as invalid 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 a third time an investigation should be conducted to identify possible causes for the error. If the cause for the error is clear, the test can either be singled out as invalid due to either PCR inhibitors being present or not enough nuclear material being present. If the cause for an error is

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unclear, contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02 or contact us at support@co-dx.com.

13.3.5 **No Template Control** (NC) Showing Amplification

13.3.5.1 Amplification of MPX 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.

When this occurs, 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 support@co-dx.com.

14 REFERENCES

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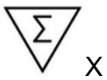
15 LEGEND OF PACKAGE SYMBOLS

See Table 9 for the legend of package symbols.

Table 9

Legend of Package Symbols

Icon	Definition
REF	Catalog number
LOT	Batch Code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
	Contains sufficient for X tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Non-Sterile product – Do not sterilize
	Manufacturer
RUO	Research Use Only

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