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Co-Dx®

Instructions for Use
Co-Dx™ Logix Smart® Zika, Dengue, and Chikungunya (ZDC) Kit

REF ZDC-K-001

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Co-Dx™ Logix Smart® ZIKV, DENV, CHIKV (ZDC) Kit
CO-DIAGNOSTICS, INC.

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

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1 INTENDED USE

The **Co-Dx™ Logix Smart® ZIKV, DENV and CHIKV (ZDC)** kit is an *in vitro* diagnostic multiplex test, based on real-time polymerase chain reaction (PCR) technology, for the simultaneous qualitative detection of the Zika virus (ZIKV), dengue virus types 1-4 (DENV), and the chikungunya virus (CHIKV) virus-specific ribonucleic acid (RNA).

2 PRODUCT DESCRIPTION AND TEST PRINCIPLES

The **Co-Dx Logix Smart ZDC** kit is an *in vitro* diagnostic test, based on real-time PCR technology. It tests for the presence or absence of RNA in ZIKV, DENV, and CHIKV, specifically, in serum or plasma, urine, or cerebrospinal fluid (CSF) from patients suspected of ZIKV, and serum or plasma from patients suspected of DENV, or CHIKV infections. Serology test confirmation may be needed if onset of infection has passed the early stages of these diseases.

The **Co-Dx Logix Smart ZDC** kit includes an internal control to identify possible Quantitative Polymerase Chain Reaction (qPCR) inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The **Co-Dx Logix Smart ZDC** kit also includes a Positive Control (PC) which includes three synthetic RNA molecules carrying sequences that are homologous to the ZIKV, DENV, and CHIKV viruses, which are targeted by this multiplex assay. PCs represent a source of cross-contamination. Precautions should be taken to prevent and minimize the risk.

Co-Primers® in the **Co-Dx Logix Smart ZDC** kit include the following:

- Co-Primers that are targeting ZIKV are labelled with the fluorescein amidites (FAM™) fluorophore.
- Co-Primers that are targeting DENV are labelled with the CAL Fluor® Orange 560 fluorophore.
- Co-Primers that are targeting CHIKV are labelled with the Quasar® 670 fluorophore.
- Co-Primers that are targeting the Internal Positive Control (IPC) DNA are labelled with CAL Fluor® Red 610 fluorophore.

Co-Primers of the **Co-Dx Logix Smart ZDC** kit are based on current sequence alignments of ZIKV, and DENV. This allows for the RNA detection and differentiation/multiplexing of all assays (ZIKV, DENV, CHIKV), but does not differentiate between the subtypes of DENV.

The test is a one-step, reverse transcription, PCR test for the simultaneous detection of those targets, using fluorescently labeled Co-Primers. The kit consists of MM with a proprietary Co-Primer mixture, all PCs, and negative control (NC). All kit components are manufactured ready to use immediately upon arrival.

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2.1 Principles of Operation

Begin the test by selecting the sample type, followed by collecting the sample using the appropriate procedures and conditions. Identify the sample by following the laboratory quality system and current regulations. Store the sample properly until it is tested in the same facility, or it is shipped to the assigned laboratory.

The **Co-Dx Logix Smart ZDC** kit assay is a multiplexed, single-step, real-time reverse transcription PCR test that can be broken down into the following three stages:

- Sample preparation
- Reverse transcription
- PCR with real-time monitoring

The assay also includes an IPC that acts as an extraction control to confirm the performance of the extraction.

The sample preparation for PCR requires the samples to be processed to break apart cells and viruses to expose the genetic material. For this process, a commercially available extraction system is used. In this process, the nucleic acids are isolated and purified from the lower respiratory tract fluids (e.g., bronchoalveolar lavage, sputum, tracheal aspirate), or the upper respiratory tract fluids (e.g., nasopharyngeal, anterior nasal, and oropharyngeal swabs).

The purified nucleic acid is then plated with the master mix (MM), 5 µL of each. The MM is pre-mixed and contains the necessary components to perform both the reverse transcription and PCR. This eliminates the need for MM to be prepared ahead of time by the user.

The plated reactions will then be put in the thermocycler using the following cycling conditions:

- 15 minutes at 45°C
- 2 minutes at 95°C
- 50 cycles x [3s at 95°C, 32s at 55°C]

The 15-minute step at 45°C is the reverse transcription step, where the complementary deoxyribonucleic acid (cDNA) is created from the RNA template.

The 2-minute step at 95°C is to inactivate the reverse transcriptase and acts as the initial denaturation step for PCR, which is then followed by the thermocycling for the PCR.

During the PCR, the FAM™ labeled forward Co-Primer acts as both the forward primer and the probe. During the annealing/extension phase of the PCR, the 5’

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nuclease activity of Taq polymerase degrades the Co-Primer's portion that has annealed to the targeted sequence, causing spatial separation between the fluorophore and the quencher, generating a fluorescent signal. With each cycle, additional fluorophore molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at the end of each cycle by the real-time thermocycler.

See Table 1 for the **Co-Dx Logix Smart ZDC** kit components.

Table 1

Kit Components

Cap Color	Component	Symbol	Description	Individual Catalog Number
Brown	**Co-Dx Logix Smart ZDC** Master Mix	MM	Proprietary blend of Co-Primers and PCR reagents	TUBE-LZD-0001 (for 1x500 µL [100 reactions])
Clear	**Co-Dx Logix Smart ZDC** Negative Control	NC	Nuclease-Free Water	TUBE-LZD-0002 (for 1x500 µL [100 reactions])
Red	**Co-Dx Logix Smart ZDC** Positive Control	PC	Proprietary blend of target templates	TUBE-LZD-0003 (for 1x500 µL [100 reactions])

The product code for the **Co-Dx Logix Smart ZDC** kit is ZDC-K-001. Contact our Sales team at (801) 438-1036 ext. 1 to order.

3 REAGENT STORAGE AND HANDLING

See the following information for reagent storage and handling:

- The **Co-Dx Logix Smart ZDC** kit 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 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.

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- 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 Product Information - Co-Dx.
- 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 Product Information - Co-Dx.

4 MATERIALS REQUIRED (BUT NOT INCLUDED)

The following is a list of materials and devices required but not provided with this kit:

- An appropriate 4-channel real-time PCR instrument, compatible with the fluorophores used in this test.

  Note: Two real-time PCR instruments have been used and tested with the product, the Co-Dx Box thermocycler (Bio Molecular Systems), and the Eco 48 (Cole-Parmer). Of these items, only the Co-Dx Box thermocycler (Bio Molecular Systems) has been validated with the current version of the product.

  Other validation exercises will include testing more thermocyclers, as well as creating specific protocols for those thermocyclers.

- An appropriate nucleic acid extraction system or kit
- A vortex mixer
- A centrifuge with a rotor for 2 mL reaction tubes
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Ice
- A biosafety cabinet, ideally Biosafety Level 2 (BSL-2) facility

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WARNING!

Before performing any testing or running any patient sample, verify that all instruments have been properly installed, calibrated, and maintained according to the manufacturer's instructions and recommendations. Do **not** use instructions with outdated calibration.

5 WARNINGS AND PRECAUTIONS

WARNING!

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

- Integrity
- Correct labelling
- Frozenness upon arrival

Users should adhere to the following guidelines:

- Limit use of this product to personnel instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- Treat patient samples as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, a lab coat, and eye protection when handling patient samples. Always wear gloves when handling kit 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 each time you move 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 SDS for safe handling. The SDS for the **Co-Dx Logix Smart ZDC** kit is provided with the shipment. If the SDS is 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)

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- Do not collect samples for nucleic acid PCR assays, in Heparin (green top tube), or EDTA (purple top) tubes as these components are well-known PCR inhibitors. Preferably collect whole blood in the serum separator tubes.
- 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 of laboratory area contamination.
- Do not use components of the kit that have passed the expiration date.
- Discard sample and assay waste according to your local safety regulations.

6 SAMPLE COLLECTION, HANDLING, TRANSPORTATION, AND STORAGE

Sample selection, collection, storage, and handling play an essential part on the performance of nucleic acid assays. Valuable information is presented here to help laboratories develop better result analysis procedures and troubleshoot other problems.

- For more information visit the Centers for Disease Control (CDC) and World Health Organization (WHO) websites in the following addresses:
- CDC, testing for ZIKV: https://www.cdc.gov/zika/symptoms/diagnosis.html
- CDC, DENV specimens: https://www.cdc.gov/ncezid/dvbd/specimensub/dengue-shipping.html
- CDC, chikungunya virus: https://www.cdc.gov/chikungunya/hc/diagnostic.html
- WHO, Laboratory testing for ZIKV infection: https://apps.who.int/iris/bitstream/handle/10665/204671/WHO_ZIKV_LAB_16.1_eng.pdf;j
- WHO, dengue: https://www.who.int/denguecontrol/en/
- WHO, chikungunya: https://www.who.int/emergencies/diseases/chikungunya/en/

6.1 Sample Collection

6.1.1 **ZIKV:** According to Relich & Loeffelholz (2017), ZIKV RNA can be detected using qPCR in serum from 2 to 7 days after onset of symptoms. After 7 days, the viral load in the blood starts to decrease. ZIKV RNA can be detected using qPCR in urine for up to 20 days. Relich & Loeffelholz recommends that, due to the onset of the disease being difficult to determine (due to patients being asymptomatic), and the variability of the viral load over time, serum and urine should be tested simultaneously for a more robust result. In the case of suspected neurological effects, CSF may also be tested.

6.1.2 **DENV:** DENV can be detected using PCR in serum and plasma for up to 7 days after the onset of symptoms. After this period, a nucleic acid assay can be performed together with serological tests.

6.1.3 **CHIKV:** CHIKV can be detected using qPCR in serum or plasma for up to 8 days after onset of symptoms. After this period, nucleic acid assays can be performed together with serological tests.

6.2 Sample Handling

Real-time Polymerase Chain Reaction (rRT-PCR) analysis on clinical samples from patients who are suspected or confirmed to be infected with ZIKV, DENV, or should be conducted under BSL-2 conditions as described in the *WHO Laboratory Biosafety Manual, 3rd ed*. Any testing for the presence of ZIKV, DENV, or CHIKV should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances (World Health Organization, 2016).

6.3 Sample Transportation

Transport specimens known or believed to contain the ZIKV, DENV, or CHIKV viruses on dry ice as a Biological Substance Category B, UN3373. Always abide by international laws as outlined in the WHO Guidance on Regulations for the Transport of Infectious Substances 2015-2016. (CDC, 2020). Transport specimens, that are frozen overnight, with enough ice to keep them frozen through the duration of travel if ground transportation is necessary. After collection of the sample and transfer to the clinical lab, the sample will receive an entry into the laboratory system.

6.4 Sample Storage

Keep samples refrigerated at a temperature between 2°C and 8°C and test within 48 hours. If there is a delay of more than 48 hours before testing whole blood, separate serum, and store separately. Based on the WHO's recommendations, keep specimen types at -20°C for up to 7 days. For storage longer than 7 days, keep specimens frozen at -70°C. (World Health Organization, 2016).

7 PROCEDURE

The WHO recommends recording the patient's full name, date of birth, contact information, and the time and date the sample was collected. Additionally, the following information could be collected:

- Symptoms, date of onset, duration of symptoms, contact with known ZIKV cases, and type of contact (e.g., sexual contact)
- Comprehensive travel history (dates, place, duration of visit)
- Vaccination history, especially any vaccinations for flaviviruses including yellow fever virus, Japanese encephalitis virus, and the DENV virus

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7.1 Sample Preparation

The quality of the extraction of the RNA from the samples is essential for the performance of **Co-Dx Logix Smart ZDC** kit. The extraction protocol should be performed by following the manufacturer's instructions or an internally validated protocol. The extraction method validated with **Co-Dx Logix Smart ZDC** kit and recommended by Co-Diagnostics, Inc. is the QIAamp® Viral RNA Mini kit (QIAGEN) and includes the following products:

- Cat No. 52904 for 50 extractions
- Cat. No. 52906 for 250 extractions

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with **Co-Dx Logix Smart ZDC** kit must be validated by the user.

Perform extraction of RNA using the QIAamp® Viral RNA Mini kit by following the manufacturer's instructions, using 140 µL of sample and a modified elution, with 60 µL of buffer AVE. It is highly recommended, prior to the elution of nucleic acids, to ensure all ethanol is removed. For column-based kits that include washing with buffers containing ethanol, an additional centrifugation step (see extraction procedure) using a new collection tube, is recommended.

WARNING!

If your sample preparation system is using washing buffers containing ethanol, remove all 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.

7.2 Set up the Co-Dx Logix Smart ZDC Reagent

Perform these steps to set up the reagent:

7.2.1 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.

7.2.2 Vortex all **Co-Dx Logix Smart ZDC** MM, PC, NC, and all sample tubes for 3 seconds.

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7.2.3 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.

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

7.3 Set Up the Reaction

Perform the steps below to set up the reaction.

7.3.1 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 displays the minimum number of wells needed for 5 samples.

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

7.3.2 Pipette 5 µL of MM into each well collected.

7.3.3 Pipette 5 µL of the NC into the appropriate wells (in addition to the 5 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.

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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.
- Pipette the PC last, if possible, to avoid contamination events.

7.3.4 Pipette 5 µL of sample or PC into the appropriate well.

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

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

7.4 PCR Instrument Setup

7.4.1 If using a Co-Dx Box from Co-Diagnostics Inc., contact the Laboratory at (801) 438-1036 ext. 3 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 when using another device, follow the settings outlined to program the PCR instrument.

7.4.2 To achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined in Table 2.

Table 2

Setting Definitions

Item	Setting
Reaction Volume	10 µL
Passive Reference	None

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

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Table 3

PCR Instrument 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

7.4.2.1 Ensure that PCR instrument being used is compatible with 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. 2.

7.4.2.2 Define the fluorescence detectors (dyes) as listed in Table 4.

Table 4

Fluorescence Detectors (Dyes) Definitions

Target	Detector Name	Reporter	Quencher
ZIKV specific RNA	ZIKV	FAM™	BHQ® - 1
DENV specific RNA	DENV	CAL Fluor® Orange 560	BHQ® - 1
CHIKV specific RNA	CHIKV	Quasar® 670	BHQ® - 2
RNaseP specific RNA (IPC)	RNaseP	CAL Fluor® Red 610	BHQ® - 2

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

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8 DATA ANALYSIS

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

Verification and validation studies performed for **Co-Dx Logix Smart ZDC** kit **(ZDC-K-001)** were conducted following Good Laboratory Practices for Molecular Biology assays (Viana & Wallis, 2011). If these conditions are not met, the performance will show higher variability, due to user errors that occur while conducting the experiment.

8.1 Validity of Diagnostic Test Runs

8.1.1 Valid Diagnostic Test Run

8.1.1.1 Verify that both the PC and the NC passes.

8.1.1.2 The control conditions outlined in Table 5 must be met. If controls pass, interpret the sample results.

Table 5

Required Control Conditions

Control Type	Control Name	Purpose of Control	ZIKV	DENV	CHIKV	Internal Control (RNaseP)
Co-Dx Logix Smart ZDC Positive Control (PC)	ZIKV (FAM™)	Verifies the performance of the master mix	+	+	+	+
	DENV (CF®560)					
	CHIKV (Q®670)					
	RNaseP (CF®610)					
Co-Dx Logix Smart ZDC Negative Control (NC)	Nuclease-Free Water	Verifies the reagents are free of contamination	-	-	-	-

8.1.2 Invalid Diagnostic Test Run

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

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8.2 Interpretation of Results

Once the controls have passed, the patient 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 ZIKV, DENV, or CHIKV at or below 45 cycles. Amplification curves greater than 45 cycles for ZDC are in the uncertainty zone. The amplification of the RNaseP shows that the extraction was successful.

A negative result will show no amplification for ZIKV, DENV, or CHIKV; however, occasionally amplification greater than 45 cycles can occur. Any amplification curves greater than 45 cycles for ZDC are outside of the detection limits for the assay. The absence of a curve for ZDC indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

An inconclusive result will occur if any of the controls fail. See the troubleshooting section of this document.

The interpretation of results can be translated to Table 6.

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Table 6

Interpretation of Results

<table>
<tr><th>Marker</th><th>ZIKV</th><th>DENV</th><th>CHIKV</th><th>Patient Internal Positive Control (RNaseP)</th><th>Co-Dx Logix Smart Positive Control (PC)</th><th>Co-Dx Logix Smart Negative Control (NC)</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>Zika, dengue, and chikungunya virus RNA +</td></tr>
<tr><td>-</td><td>-</td><td>-</td><td>Zika, dengue, and chikungunya virus RNA -</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">-</td><td rowspan="3">-</td><td>Zika virus RNA +</td></tr>
<tr><td>Dengue virus RNA -</td></tr>
<tr><td>Chikungunya virus RNA -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">+</td><td rowspan="3">-</td><td>Zika virus RNA -</td></tr>
<tr><td>Dengue virus RNA +</td></tr>
<tr><td>Chikungunya virus RNA -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">-</td><td rowspan="3">+</td><td>Zika virus RNA -</td></tr>
<tr><td>Dengue virus RNA -</td></tr>
<tr><td>Chikungunya virus RNA +</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">+</td><td rowspan="3">-</td><td>Zika virus RNA +</td></tr>
<tr><td>Dengue virus RNA +</td></tr>
<tr><td>Chikungunya virus RNA -</td></tr>
<tr><td rowspan="3">-</td><td rowspan="3">+</td><td rowspan="3">+</td><td>Zika virus RNA -</td></tr>
<tr><td>Dengue virus RNA +</td></tr>
<tr><td>Chikungunya virus RNA +</td></tr>
<tr><td rowspan="3">+</td><td rowspan="3">-</td><td rowspan="3">+</td><td>Zika virus RNA +</td></tr>
<tr><td>Dengue virus RNA -</td></tr>
<tr><td>Chikungunya virus RNA +</td></tr>
<tr><td colspan="3" rowspan="3">Any Result</td><td>Fail</td><td colspan="2">Pass</td><td>Inconclusive: See Troubleshooting</td></tr>
<tr><td rowspan="2">Pass</td><td>Fail</td><td>Pass</td><td rowspan="2"></td></tr>
<tr><td>Pass</td><td>Fail</td></tr>
</table>

An analyte result will be considered positive (+) if a Cq value of < 45, or negative (-) if a Cq value of ≥ 45 cycles. When possible, always check that the medical history and/or symptoms match with the result prior to treatment.

9 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Co-Dx Logix Smart ZDC** kit **,** even if the recommended steps for troubleshooting

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solves the issue. To give us feedback, complete the Customer Feedback Form by visiting https://co-dx.com/contact/feedback/.

9.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently being tested. Currently, the expiration date of this product has been established as 12 months.

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

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

9.3 Invalid Results/Inconclusive Results

9.3.1 **Co-Dx Logix Smart ZDC** PC Not Amplifying

If a PC does not amplify, it could be due to one or multiple of the following factors:

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

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- Using degraded **Co-Dx Logix Smart ZDC** MM or PC (a result of reagents being at temperatures above -16°C for an extended period)
- Using expired reagents
- Using the wrong reagents

Without further evidence, it is best to disregard the results from the patient samples and re-test by re-amplification. If the PC fails a second time, 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, after re-extraction and re-amplification, occurs a third time, open a new **Co-Dx Logix Smart ZDC** PC or MM, and retest. If the test still fails, contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 2.

9.3.2 RNaseP IPC Not Amplifying in Patient Samples

No amplification from the RNaseP channel could be the result of one or multiple of the following factors:

- Not enough nuclear material is in the patient sample.
- PCR inhibitors such as ethanol and heparin are present.
- Extraction was performed incorrectly.
- Extraction kit used is not compatible or has a step that eliminates RNaseP DNA.

Note: Positive amplification in ZIKV, DENV, or CHIKV 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 and the amount (which is governed by the type of the patient sample and the extraction procedure used). Samples obtained from culture or sterile/pure sites (e.g., CSF, urine, cell lysates) may not contain the human RNaseP gene. In such case, the two negative markers indicate a true negative result for ZIKV, DENV, or CHIKV.

IPC negative patient results cannot be trusted and re-testing by re-amplification should be performed. If the IPC fails a second time, 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 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

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contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 2 for help.

9.3.3 Negative Control (NC) Showing Amplification

Amplification of **Co-Dx Logix Smart ZDC** in an NC indicates one of the following:

- Contamination in one or more of the reagents
- Incorrect placement of a plate or tube into the real-time PCR instrument
- Pipetting errors

None of the results can be trusted when the NC shows amplification and re-testing by re-amplification should be performed. If the NC fails a second time, 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, occurs a third time, open a new NC and retest. If it is still failing, contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 2.

10 LIMITATIONS

Limitations include the following:

- Strict compliance with this document is required for optimal results. Always use the most recent version of this document which can be downloaded for free at: Product Information - Co-Dx
- Use of this product is to be limited to personnel trained in real-time PCR techniques and IVD procedures.
- 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 purity, integrity, and performance of the reagents prior to testing on patient samples.
- Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results.
- Do not use the **Co-Dx Logix Smart ZDC** kit 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 ZIKV, DENV, and CHIKV genome covered by this test kit may result in failure to detect the presence of the pathogens.

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- As with any diagnostic test, results of the **Co-Dx Logix Smart ZDC** kit are to be interpreted with consideration of all clinical and laboratory findings.

11 ANALYTICAL EVALUATION

11.1 Limit of Detection (LoD) and Inclusivity

The Limit of Detection (LoD) was established using a probit analysis. The LoD testing was performed using two manufactured lots of the Co-Dx Logix Smart ZDC kit, over 3 testing days for Zika, dengue types 1-4, and chikungunya viruses. Reference materials were spiked pre-extraction when using a live/intact virus, and extracted viral genomic material was spiked post-extraction. A summary of the LoD results is displayed in Table 7.

Table 7

*Limit of Detection for **Co-Dx Logix Smart ZDC***

Marker	Specimen	Strain	Estimated LoD
Zika virus (ZIKV)	Serum (spiked post-extraction)	Asian lineage, PRVABC59	3.19 x10^4 copies/mL
		African, MR766	3.23 x10^4 copies/mL
	Plasma (spiked pre-extraction)	Asian lineage, PRVABC59	1.52 x10^4 copies/mL
	Urine (spiked post-extraction)	Asian lineage, PRVABC59	6.23 x10^4 copies/mL
	CSF (spiked post-extraction)	Asian lineage, PRVABC59	4.83 x10^4 copies/mL
Chikungunya virus (CHIKV)	Serum (spiked post-extraction)	S27 Petersfield	1.03 x10^3 copies/mL
	Plasma (spiked pre-extraction)	R91064	4.27 x10^3 copies/mL
Dengue virus type 1-4 (DENV)	Plasma (spiked post-extraction)	Quantitative Synthetic Dengue virus type 1 RNA	2.11 x10^5 copies/mL
		N/A IDT (synthetic RNA template)	8.21 x10^4 copies/mL
		Dengue Type 2, New Guinea C	9.08 x10^4 copies/mL
		Dengue Type 3, H87	5.05 x10^4 copies/mL
		Dengue Type 4, H241	2.69 x10^5 copies/mL
	Plasma (spiked pre-extraction)	Dengue Type 1, Hawaii	4.03 x10^2 PFU/mL
		Dengue Type 2, New Guinea C	7.27 x10 PFU/mL
		Dengue Type 3, H87	1.91 x10^2 PFU/mL
		Dengue Type 4, H241	6.13 x10^2 PFU/mL

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11.2 Inclusivity – In Silico

Alignments have been performed with the oligonucleotide Co-Primer sequences of the ZDC Co-Primers with publicly available nucleic acid sequences for ZDC in the GenBank, NCBI, and associated databases to demonstrate the predicted inclusivity of the **Co-Dx Logix Smart ZDC** test. In silico Analysis and BLASTn analysis queries of

the ZDC Co-Primers were performed against public domain nucleotide sequences. The database search parameters were as follows:

- The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, and TSA patent sequences; phase 0, 1, and 2 HTGS sequences; and sequences longer than 100 Mb.
- The database is non-redundant. Identical sequences have been merged into one entry while preserving the accession, GI, title, and taxonomy information for each entry.
- The database is reviewed consistently to detect potential mutations in the targeted region.
- The search parameters automatically adjust for short input sequences and the expect threshold is 1000.
- The match and mismatch scores are 1 and -3, respectively.
- The penalty to create and extend a gap in alignment is 5 and 2, respectively.

Inclusivity results were as follows:

- **ZIKV.RepSeq.5621.F12:** 0 mismatches on 536 sequenced ZIKV strains. 1 mismatch on 156 ZIKV strains. No strains with 2+ mismatches
- **ZIKV.RepSeq.5621.R3:** 0 mismatches on 474 ZIKV strains. 1 mismatch on 124 ZIKV strains. 2 mismatches on 25 strains. 3 mismatches on 106 strains. None with 4+ mismatches.
- **ZIKV.5621Rev2.R8:** 0 mismatches on 115 ZIKV strains. 1 mismatch on 0 strains. 2 mismatches on 4 strains. 3 mismatches on 485 strains.
- **DNV.T4.F14**: Dengue Virus Type 4 – 293/380 with 100% match. 49/380 with 1 mismatch. 25/380 with 2 mismatches. 13/380 with 3+ mismatches
- **DNV.T4.R10:** Dengue Virus Type 4 – 385/444 with 100% match. 7/444 with 1 mismatch. 52/444 with 2 mismatches. 0/444 with 3+ mismatches
- **DNV.T3.F17**: Dengue Virus Type 3 – 1118/1136 with 100% match. 1/1118 with 1 mismatch. 14/1118 with 2 mismatches. 2/1118 with 3 mismatches
- **DNV.T3.R20:** Dengue Virus Type 3 – 1295/1343 with 100% match. 3/1343 with 1 mismatch. 30/1343 with 2 mismatches. 15/1343 with 3+ mismatches

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- **DNV.T2.F6**: Dengue Virus Type 2 – 1772/1796 with 100% match. 1/1796 with 1 mismatch. 3/1796 with 2 mismatches. 20/1796 with 3 mismatches
- **DNV.T2.R2:** Dengue Virus Type 2 – 1626/2061 with 100% match. 434/2061 with 1 mismatch. 1/2061 with 2 mismatches. 0/2061 with 3+ mismatches
- **DNV.T1.F1:** Dengue Virus Type 1 – ~2050/2150 with 100% match. ~35/2150 with 1 mismatch. ~50/2150 with 2 mismatches. 6/2150 with 3+ mismatches
- **DNV.T1.R2:** Dengue Virus Type 1 – 2239/2256 with 100% match. 5/2256 with 1 mismatch. 9/2256 with 2 mismatches. 3/2256 with 3 mismatches
- **CHIK.L2.F15**: Chikungunya Virus – 538/847 with 100% match. 304/847 with 1 mismatch (mostly Asian strains). 5/847 with 2 mismatches.
- **CHIK.L2.R7:** Chikungunya Virus – 798/832 with 100% match. 34/847 with 1 mismatch.

11.3 Exclusivity

11.3.1 Exclusivity – In Silico

In Silico Exclusivity Analysis and BLASTn analysis queries of the ZDC Co-Primers were performed against public domain nucleotide sequences. The BLASTn database search parameters were as follows:

- The nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences, but excludes EST, STS, GSS, WGS, and TSA patent sequences; phase 0, 1, and 2 HTGS sequences; and sequences longer than 100 Mb.
- The database is non-redundant. Identical sequences have been merged into one entry while preserving the accession, GI, title, and taxonomy information for each entry.
- The database is reviewed consistently to detect potential mutations in the targeted region.
- The search parameters automatically adjust for short input sequences and the expect threshold is 1000.
- The match and mismatch scores are 1 and -3, respectively.
- The penalty to create and extend a gap in alignment is 5 and 2, respectively.

11.3.2 Exclusivity results are as follows:

- **DNV.T4.F14:** DNV Type 1, 2 with as little as 4 mismatches. Nothing else with less than 7 mismatches.
- **DNV.T4.R10:** DNV Type 3 with as little as 0 mismatches. Nothing else with less than 7 mismatches.

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- **DNV.T3.F17:** DNV Type 1 with as little as 3 mismatches. Japanese Encephalitis with as little as 4 mismatches. Nothing else with less than 7 mismatches.
- **DNV.T3.R20:** DNV Type 1 with as little as 1 mismatch. Nothing else with less than 7 mismatches.
- **DNV.T2.F6:** DNV Type 4 with as little as 0 mismatches. DNV Type 1 with as little as 3 mismatches. DNV Type 3 with as little as 4 mismatches Nothing else with less than 7 mismatches.
- **DNV.T2.R2:** No other DNV Types showed up as matches. Nothing else with less than 7 mismatches.
- **DNV.T1.F1:** DNV Types 3,4 with as little as 1 mismatch. DNV Type 2 with as little as 3 mismatches. Nothing else with less than 7 mismatches.
- **DNV.T1.R2:** DNV Type 3 with as little as 0 mismatches. No other DNV types showed up. As low as 3 mismatches with O'nyong-nyong virus. Nothing else with less than 8 mismatches.
- **CHIK.L2.F15:** 4 occurrences of 1 mismatch with O'nyong-nyong virus – a virus in Eastern Africa very similar to Chikungunya.
- **CHIK.L2.R7:** As low as 3 mismatches with O'nyong-nyong virus.
- **ZIKV.RepSeq.5621.F12:** No other organisms match with E-Value>1.
- **ZIKV.RepSeq.5621.R3:** 2-4 mismatches on many strains of DNV types 2 and 3.
- **ZIKV.5621Rev2.R8:** 2 mismatches on 1 strain of Ilheus Virus. 2 mismatches on 1 strain of Barkedji Virus. 3-4 mismatches on: Murray Valley Encephalitis Virus, Koutango Virus, Japanese Encephalitis Virus, Usutu Virus, West Nile Virus, Spondweni Virus.

Co-Primers have a slightly different cross-reactivity risk profile than traditional primers. Due to the low Tm's of the Priming and Capture sequences, Co-Primers are more susceptible to mismatches. Our internal experiments show that a single mismatch on either forward or reverse causes a noticeable delay in amplification, with more mismatches causing significant suppression of signal. Three or more mismatches on the forward and reverse combined, are expected to result in no detectable amplification.

The results suggest that the **Co-Dx Logix Smart ZDC** test is not predicted to cross-react to any of the non-target organisms. Cross-reactivity with DNV markers is not material to this test since all 4 DNV Co-Primer pairs are part of a single 4-type-DENV assay.

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11.3.3 Exclusivity – Wet Testing

Wet testing was performed using negative and positive contrived samples To assess the exclusivity and competitive inhibition, respectively. For both negative and positive samples, non-target organism was spiked at a concentration of 2,000 copies/µl in the sample (10,000 copies/reaction). Positive samples were spiked with Vircell RNA controls: Zika (Asian lineage), Chikungunya, and Dengue (types 1-4) at the following concentrations: 1000 copies/µl ZIKV, 1000 copies/µl CHIKV, and 500 copies/µl of each DENV type (1-4). The results are summarized in Table 8Table 8.

Table 8

Exclusivity and Competitive Inhibition Results Summary

Non-Target Organism	Negative Cross Reactivity			Positive Cross-Reactivity		
Average Cq	**ZIKV (Cq)**	**CHIKV (Cq)**	**DENV (Cq)**	**ZIKV (Cq)**	**CHIKV (Cq)**	**DENV (Cq)**
B. burgdorferi	0	0	0	34.15	28.40	40.57
Epstein-Barr virus	0	0	0	36.21	29.64	43.83
Influenza A H3	0	0	0	34.15	28.14	39.65
Influenza A H5	0	0	0	33.42	28.16	40.15
Influenza A H1N1	0	0	0	33.67	28.17	41.26
Influenza A H1	0	0	0	33.86	28.24	40.24
Influenza B	0	0	0	34.17	28.33	40.50
Measles	0	0	0	33.43	28.12	40.36
West Nile Virus	0	0	0	33.20	28.34	41.64
East Equine Enc. Virus	0	0	0	34.01	28.33	41.16
St Louis Enc. Virus	0	0	0	33.82	28.26	40.84
Vari-Zoster Virus	0	0	0	33.55	28.14	40.37
TBEV	0	0	0	33.91	28.11	40.62

The results do not suggest that the **Co-Dx Logix Smart ZDC** test is cross-reactive to any of the non-target organisms that were tested, specifically negative samples did not show any false positives, other than Influenza B and St. Louis Encephalitis virus in the yellow channel. When retested, neither showed amplification in any of the samples. Positive samples in the presence of non-target organism genetic material also did not reduce the ability for the **Co-Dx Logix Smart ZDC** test to produce positive results. See Table 9 for microorganisms included in the cross-reactivity assessment.

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Table 9

Microorganism Included in the Cross-Reactivity In Silico Assessment

High priority pathogens from the same genetic family	High priority organisms likely in the circulating area	Other microorganisms of importance
Human coronavirus 229E	Adenovirus	Influenza C
Human coronavirus OC43	Human Metapneumovirus (hMPV)	Parechovirus
Human coronavirus HKU1	Parainfluenza virus 1-4	*Corynebacterium diphtheriae*
Human coronavirus NL63	Influenza A & B	*Legionella non-pneumophila*
SARS-coronavirus	Enterovirus	*Bacillus anthracis* (Anthrax)
MERS-coronavirus	Respiratory syncytial virus	*Moraxella catarrhalis*
	Rhinovirus	*Neisseria elongata*
	Chlamydia pneumoniae	*Neisseria meningitides*
	Haemophilus Influenza	Leptospirosis
	Legionella pneumophila	*Chlamydia psittaci*
	Mycobacterium tuberculosis	*Coxiella burnetii* (Q-Fever)
	Streptococcus pneumoniae	*Staphylococcus aureus*
	Streptococcus pyogenes	
	Bordetella pertussis	
	Mycoplasma pneumoniae	
	Pneumocystis jirovecii (PJP)	
	Pooled human nasal wash – to represent diverse microbial flora in the human respiratory tract	
	Candida albicans	
	Pseudomonas aeruginosa	
	Staphylococcus epidermidis	
	Staphylococcus salivarius	

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12 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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14 LEGEND OF PACKAGE SYMBOLS

See Table 10 for a legend of the package symbols.

Table 10

Legend of Package Symbols

Icon	Description
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Batch code
	Use-by-date
Σ x	Contains sufficient for x tests/reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use document
	Manufacturer
EC REP	Authorized representative in the European community
CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC

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