Sep
2021

Co-Dx

Logix Smart™ ZDC (Zika, dengue, chikungunya) Kit

LOGIX SMART™ ZDC (ZIKV, DENV, CHIKV) Kit

CO-DIAGNOSTICS, INC.

REF **ZDC-K-001**

IVD

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

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1 INTENDED USE

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

2 KIT COMPONENTS

Lid Color	Component	Symbol	Catalog Number	Description	Amount
Brown	Logix Smart™ ZDC Master Mix	MM	ZDC-MM-001	Proprietary blend of CoPrimers™ and PCR reagents	1x500µL (100 reactions)
Red	Logix Smart™ ZDC Positive Control	PC	ZDC-PC-001	Proprietary blend of target templates	1x500µL (100 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	DNase/RNase-free water	1x500µL (100 reactions)

- Kit Catalog Number is ZDC-K-001. Contact Sales at (801) 438-1036 ext. 01 to order.

3 LOGIX SMART™ ZDC STORAGE, HANDLING, & DISPOSAL

- The **Logix Smart™ ZDC** kit is shipped on dry ice. The components of the kit 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 kit, laboratory should follow internal procedures for quality control.
- All components should be stored below -20°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 **Logix Smart™ ZDC** test kit remains frozen at -20°C.
- Protect **Master Mix** from light.
- Expired products should not be used, as the integrity of the components can not be guaranteed.
- The product is not a biological waste. See Safety Data Sheets for hazard classification. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

4 WARNINGS AND PRECAUTIONS

WARNING!

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

- Integrity
- Correct labelling
- 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 and *in vitro* diagnostic procedures.
- Patient samples should always be treated as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, 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 between areas.

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- 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 **Logix Smart™ ZDC** test kit is provided with the shipment. If not provided with shipment the SDS can be retrieved from Co-Diagnostics website at the link: http://co-dx.com/resources/safety-data-sheets/
- 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 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 to the laboratory area to contamination.
- Do not use components of the kit that have passed expiration date.
- Discard sample and assay waste according to your local safety regulations.

5 BACKGROUND INFORMATION

5.1 Zika virus (ZIKV)

- **About:** Zika was first isolated in 1947 in monkeys found in the Zika Forest in Uganda. The first human cases were discovered in 1952 and since then outbreaks throughout Africa, the Americas, Asia, and the Pacific have been reported. Zika virus was not thought to be endemically transmitted in the Americas until the emergence in Brazil in 2015. There was a striking increase in reports of congenital microcephaly cases, which triggered a declaration of an international public health emergency (Araújo, et al., 2018). In this study Araújo et al. found direct correlation between microcephaly cases and Zika occurrences. Another study conducted in 2016 demonstrated that ZIKV infects and destroys human neuronal stem cells grown as neurospheres and brain organoids. These observations helped solidify the link between fetal ZIKV infection and the development of microcephaly (Relich & Loeffelholz, 2017). Due to this serious neurological sequelae found in 2018, the World Health Organization (WHO) issued the annual review of diseases where the priority for R&D investments for Zika has been raised (World Health Organization, 2018).
- **The Virus:** is an enveloped, single stranded (+) RNA virus part of the *Flaviviridae* family.
- **Transmission:** It is an arbovirus spread by the bite of infected mosquitos of the genus *Aedes*. *Aedes* mosquitos usually bite during the day (early morning and late afternoon/evening). Zika virus is also transmitted from mother to fetus during pregnancy, through sexual contact, transfusion of blood and blood products, and organ transplant (Zika virus, 2018) .
- **Signs and Symptoms:** Mild fever, skin rash, conjunctivitis, and muscle and joint pain.
- **Detection:** Infection can only be confirmed by laboratory testing of blood or other bodily fluids, such as urine, cerebrospinal fluid (CSF), or semen. Detection of Zika virus by means of real-time RT-PCR should be done early (up to 10 days) after illness onset. Antibody assays

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commonly cross-react with closely related flaviviruses and detection of neutralizing antibodies by plaque reduction assays is troublesome and can only be done in specialized laboratories.

5.2 Dengue virus (DENV)

- **About:** According to the World Health Organization Severe dengue was first recognized in the 1950s during the dengue epidemics in the Philippines and Thailand. Today, severe dengue affects most Asian and Latin American countries and has become a leading cause of hospitalization and death among children and adults in these regions (Dengue and severe dengue, 2019). There are 4 known serotypes of dengue (DENV 1-4). Immunity is acquired for long periods for each type, although the immunity to one type does not prevent the infection from the other types. What may cause in hyperendemic areas infection of dengue virus as frequent as up to four time per individual. The illness spectrum varies from asymptomatic, classic dengue fever, and severe or hemorrhagic dengue fever, which can be fatal (Wilder-Smith & Gubler, 2008). There is no specific treatment for dengue or severe dengue, but early detection and access to proper medical care lowers fatality rates below 1%. In many countries dengue fever is a reportable disease and must be registered with the responsible authority.
- **The virus:** is an enveloped, single stranded (+) RNA virus part of the Flaviviridae family.
- **Transmission:** Like Zika virus, dengue is an arbovirus spread by the bite of infected mosquitos of the genus *Aedes*. *Aedes* mosquitos usually bite during the day (early morning and late afternoon/evening). Patients who are already infected with the virus can transmit the infection (for 4-5 days; maximum 12) via bite from uninfected *Aedes* mosquitoes after their first symptoms appear (Dengue and severe dengue, 2019).
- **Signs and Symptoms:** Most common symptoms range from mild flu-like symptoms to rashes, hemorrhagic manifestations, and easy bruising. Symptoms usually occur 4-7 days after a mosquito bite and last 3-10 days.
- **Detection:** Infection of dengue can be confirmed by laboratory testing of blood (serum and plasma). Detection of dengue virus by means of real-time RT-PCR should be done within the first week after symptoms appear.

5.3 Chikungunya virus (CHIKV)

- **About:** Chikungunya is a mosquito (mainly by *Aedes albopictus*) spread disease that leads to painful rheumatic symptoms. The lineages of chikungunya are divided into two branches: West African (WA) and East/Central/South African (ECSA). The West African strains are more related to small outbreaks locally in Africa. The strains from ECSA lineage are the ones that spread largely through the world (Silva & Dermody, 2017).
- **The virus:** is a small enveloped, spherical, single stranded (+) RNA virus of the *Alphavirus* genus of the family *Togaviridae*.
- **Transmission:** Like Zika and dengue virus, chikungunya is an arbovirus spread by the bite of infected mosquitos of the genus *Aedes*. *Aedes* mosquitos usually bite during the day (early morning and late afternoon/evening).

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- **Signs and Symptoms:** The most common symptoms are fever, joint pain and swelling, headaches, muscle pain, and rashes. Symptoms usually occur 3-7 days after a mosquito bite. Patients can develop a post-acute or chronic arthropathy lasting 21 to 90 days in acute cases, and three months to more than two years in chronic cases (PAHO/WHO, 2017).
- **Detection:** Infection of chikungunya can be confirmed by laboratory testing of blood (serum and plasma). Detection of chikungunya virus by means of real-time RT-PCR should be done within 8 days after symptoms appear. Following in the viremic phase, IgM and IgG will be detectable towards the end of the week after start of symptoms.

5.4 Multiplex (ZIKV, DENV, and CHIKV)

Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any real-time RT-PCR-based test system that accumulation of mutations over time may lead to false negative results. This also makes the development of a vaccine particularly complicated. Make sure to always use the most current version of the **Logix Smart™ ZDC** test kit and avoid use of expired test kit components.

Transmission and symptoms of all three viruses are similar. Therefore, fast and accurate laboratory diagnosis is essential for case management. The **Logix Smart™ ZDC** is a useful tool to detect all three viruses simultaneously, Making for a faster diagnosis and treatment for the patient.

5.5 Patient Sample 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 and WHO websites in the following addresses:

- CDC, testing for Zika: https://www.cdc.gov/zika/symptoms/diagnosis.html
- CDC, dengue specimens: https://www.cdc.gov/ncezid/dvbd/specimensub/dengue-shipping.html
- CDC, chikungunya virus: https://www.cdc.gov/chikungunya/hc/diagnostic.html
- World Health Organization (WHO), Laboratory testing for Zika virus infection: https://apps.who.int/iris/bitstream/handle/10665/204671/WHO_ZIKV_LAB_16.1_eng.pdf;jsessionid=2935A1D6A4788EA7148C5431A506941F?sequence=1
- WHO, dengue: https://www.who.int/denguecontrol/en/
- WHO, chikungunya: https://www.who.int/emergencies/diseases/chikungunya/en/

5.5.1 Sample Selection for:

5.5.1.1 **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 up to 20 days**. Relich & Loeffelholz recommends that due to the onset of the disease being difficult to determine due to patients being asymptomatic, that to have a robust result and avoid the problem of variability of viral load and days from the onset of the disease that ideally serum and urine should be tested at the same time. In the case of suspected neurological effects cerebrospinal fluid (CSF) may also be tested.

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5.5.1.2 **DENV:** Dengue virus can be detected using qPCR **in serum and plasma up to 7 days** after onset of symptoms. After this period a nucleic acid assays can be performed together with serological tests.

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

5.5.2 **Sample Storage:** Samples are best kept refrigerated at 2-8°C and tested within 48 hours. If there is a delay of more than 48 hours before testing whole blood, serum should be separated and stored separately. The WHO recommends that all other types of specimens may be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C. (World Health Organization, 2016).

5.5.3 **Sample Handling:** real-time RT-PCR analysis on clinical samples from patients who are suspected or confirmed to be infected with Zika, dengue, or chikungunya viruses should be conducted under Biosafety Level 2 (BSL-2) conditions as described in the *WHO Laboratory Biosafety Manual, 3rd ed*. Any testing for the presence of Zika, dengue, or chikungunya viruses 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 PRODUCT DESCRIPTION

The **Logix Smart™ ZDC** test kit is an *in vitro* diagnostic test, based on real-time polymerase chain reaction technology. It tests for the presence or absence of ribonucleic acid (RNA) of Zika, dengue types 1-4, and chikungunya viruses. Specifically, in serum or plasma (collected alongside with urine), or cerebrospinal fluid (CSF) from patients suspected of Zika, and serum or plasma from patients suspected of dengue, or chikungunya viral infections. Serology test confirmation may be needed if onset of infection has passed the early stages of these diseases.
The **Logix Smart™ ZDC** test includes an internal control to identify possible qPCR inhibition, confirm the integrity of the reagents, and verify the quality of sample extraction. The **Logix Smart™ ZDC** test also includes a positive control which includes three synthetic RNA molecules carrying sequences that are homologous to Zika (ZIKV), dengue (DENV), and chikungunya (CHIKV) viruses and are targeted by this multiplex assay. Positive controls represent a source of cross-contamination. Precautions should be taken to prevent and minimize the risk.

CoPrimers™ included in the **Logix Smart™ ZDC** test include the following:

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

CoPrimers™ of the **Logix Smart™ ZDC** test are based on current sequence alignments of zika virus, chikungunya virus, and all four dengue virus subtypes (1-4). This allows for the RNA detection and differentiation/multiplexing of all assays (ZIKV, DENV, CHIKV), but does not differentiate between the subtypes of dengue.

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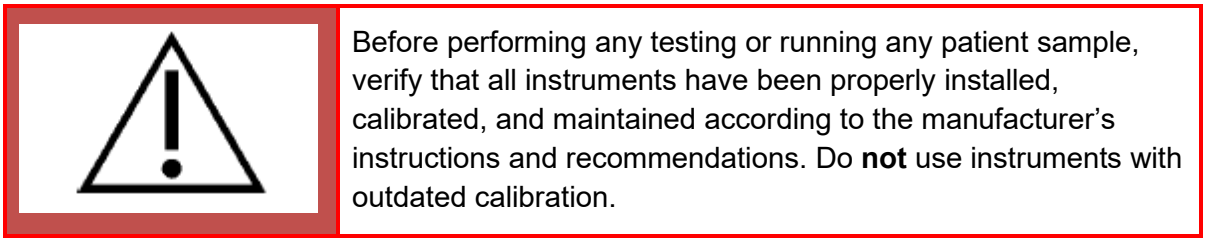
The test is a one-step reverse transcription qPCR test, with an internal positive control from RNA to cDNA, PCR amplification of the targets, and the internal positive control cDNA, and simultaneous detection of PCR amplicons by fluorescent dye labelled CoPrimers™. The kit consists of a master mix with proprietary CoPrimer™ mixture (brown lid tube), all positive controls (red lid tube), and Nuclease-Free water (clear lid tube). All kit components are manufactured ready to use immediately upon arrival.

7 MATERIALS AND DEVICES (REQUIRED BUT NOT PROVIDED)

- Appropriate 4-channel real-time PCR instrument, compatible with the fluorophores used in this test.
    - Two real-time PCR instruments have been used and tested with the product, the CoDx Box thermocycler (Bio Molecular Systems), and the Eco 48 (Cole-Parmer). Of these, only the CoDx 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.
- 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.



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 instruments with outdated calibration.

8 PROCEDURE

The World Health Organization recommends recording the full name, date of birth, contact information, and the time and date of collection of the patient sample. Additionally, the following information could also be collected:

- Symptoms, date of onset, duration of symptoms, contact with known Zika virus cases (and type of contact e.g. breastfeeding, sexual partner);
- Comprehensive travel history (dates, place, duration of visit); and

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- Vaccination history, especially any vaccinations for flaviviruses including yellow fever virus, Japanese encephalitis virus, and dengue virus.

8.1 Patient Sample Collection

Logix Smart™ ZDC can be used to detect one or all the listed viruses. It is recommended to collect samples that will detect all viruses simultaneously. If the user wishes to not test for all viruses simultaneously or would like additional information on when to collect, refer to *section 5.5 above*.

- **ZDC Multiplex:** Collect serum or plasma, and urine sample (collected at the same time as the serum sample). Cerebrospinal fluid (CSF) can also be used, when testing for Zika only.

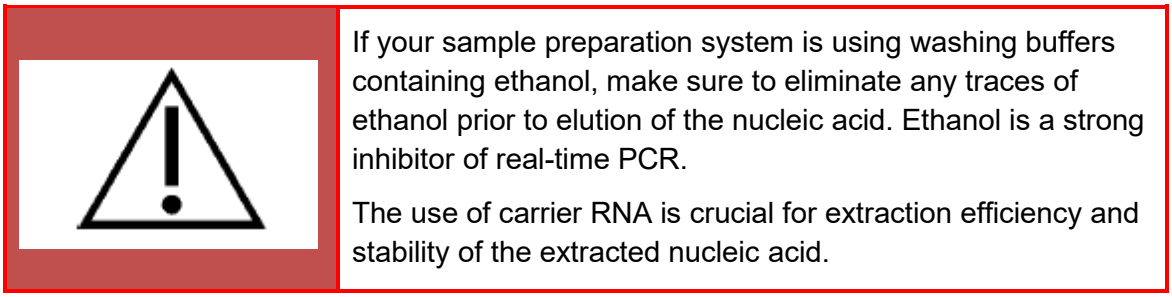
8.2 Sample Preparation

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

- 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 **Logix Smart™ ZDC** must be validated by the user.

Extraction of RNA using the QIAamp® Viral RNA Mini Kit must be performed following the manufacturer's instructions using 140 µL of sample, and a modified elution using 60 µL of buffer AVE. It is highly recommended prior to the elution of nucleic acids, to ensure the removal of all ethanol. 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.



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.

8.3 Logix Smart™ ZDC Reagent Setup

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning, that disinfects and degrades nucleic acids.
- All **Logix Smart™ ZDC** master mix, positive control, no template control (nuclease-free water), and sample tubes should be vortexed for 3 seconds, and briefly spun down before using to ensure properly mixed reagents, and to remove any condensation or residue from the lids.
- Thaw all reagents and samples on **ice**, or on a cold block, before starting setup.

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8.4 Reaction Setup

8.4.1 Every reaction setup should include enough reaction wells for the number of patient samples and at least one positive and one no template control (NTC) (**# patient samples + 2 = total reaction wells needed**). Example: 5 patient samples to test + 1 PC well + 1 NTC well = 7 total reaction wells.

8.4.2 All pipetting should be done on **ice,** if possible. Pipetting of positive control and sample elution is recommended to be done in a separate area, if possible, or at a separate time, then the master mix and nuclease-free water. Change pipette tips in-between patient sample elution and change pipette tips after pipetting each component. Pipet positive control last if possible, to avoid contamination events.

8.4.3 Pipet 5 µL of **Master Mix** into each well being used in an appropriate optical plate or optical reaction tube (example: CoDx Box real-time PCR instrument uses 48-well reaction tubes).

8.4.4 Pipet 5 µL of patient sample (elution from nucleic acid extraction) or 5 µL of a control (**No Template Control** (nuclease-free water) and **Positive Control**) to the appropriate well(s). At least one positive and one NTC control must be included in each run.

8.4.5 Seal the reaction plate with an optical adhesive film or the reaction tubes with appropriate lids.

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

8.5 PCR Instrument Setup

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

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

8.5.2 Define the following settings:

Reaction Volume	10 µL
Ramp Rate	Default
Passive Reference	None

8.5.3 Program PCR instrument with the cycling conditions below:

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

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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.

8.5.2 Define the fluorescence detectors (dyes):

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

- 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.

Verification and validation studies performed for **Logix Smart™ ZDC (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 while conducting the experiment.

9.1 Validity of Diagnostic Test Runs

9.1.1 Valid Diagnostic Test Run

- Check to see that both the positive and no template control passed.

9.1.1.1 The following control conditions must be met:

Control Type	Control Name	Purpose of Control	ZIKV	DENV	CHIKV	Internal Control (RNaseP)
ZDC Positive Control	ZIKV (FAM™)	Verifies the performance of the master mix	+	+	+	+
	DENV (CF®560)					
	CHIKV (Q®670)					
	RNaseP (CF®610)					
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-	-	-

- If controls pass, interpret the sample results.

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9.1.2 Invalid Diagnostic Test Run

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

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 ZIKV, DENV, or CHIKV at or below 45 cycles. Amplification curves greater than 45 cycles for ZDC are in the uncertainty zone. The presence of a curve for positive sample in all or any of the ZIKV, DENV, or CHIKV indicates a positive result. 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 occurs in ZDC or RNaseP channels. 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 result if any of the controls fail. See troubleshooting.

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The interpretation of results can be translated to the following table:

Marker	ZIKV	DENV	CHIKV	Patient Internal Positive Control (RNaseP)	Logix Smart™ Positive Control	No Template Control (NTC) Logix Smart™ Master Mix + Nuclease-Free Water	Result
Instrument Reading	+	+	+	Pass			**ZDC +**
	-	-	-				ZDC -
	+	-	-				**ZIKV +** DENV- CHIKV-
	-	+	-				ZIKV- **DENV+** CHIKV -
	-	-	+				ZIKV- DENV- **CHIKV+**
	+	+	-				**ZIKV+** **DENV+** CHIKV-
	-	+	+				ZIKV- **DENV+** **CHIKV+**
	+	-	+				**ZIKV+** DENV- **CHIKV+**
	Any Result			Fail	Pass		**Inconclusive: See Troubleshooting**
				Pass	Fail	Pass	
					Pass	Fail	

Note: Anything before 45 cycles is considered a positive reading (+). Anything after 45 cycles is considered a negative reading (-). When possible, always check that the medical history and/or symptoms match with the result prior to treatment.

10 TROUBLESHOOTING

Co-Diagnostics Inc. values customer feedback and wants to be informed of any issues with the **Logix Smart™ ZDC kit,** even if the recommended steps for troubleshooting solves the issue. To give feedback please fill out the Customer Feedback Form by visiting https://codiagnostics.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 kit 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 **Logix Smart™ ZDC Positive Control** not amplifying

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

- Pipetting errors (pipetting control into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,
- **Logix Smart™ ZDC Master Mix** or **Logix Smart™ ZDC 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 patient samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the positive control, after re-extraction and re-amplification, happens a third time, open a new **Logix Smart™ ZDC Positive Control** or **Master Mix**, and retest. If still failing, please contact Co-Diagnostics Inc. Technical Support by calling (801) 438-1036 ext. 02.

10.3.2 **RNaseP (Internal Positive Control (IPC))** not amplifying in patient samples

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

- Not enough nuclear material in the patient sample,
- PCR inhibitors such as: ethanol and heparin,

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- the extraction was performed incorrectly,
- or the extraction kit used is not compatible or has a step that eliminates RNaseP DNA.
- Note: Positive amplification in ZIKV, DENV, or CHIKV channel indicates positive 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, amount of 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, etc.) may not contain the human RNaseP gene. In such case, the two negative markers indicate a true negative result for Zika, dengue types 1-4, or chikungunya.

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

10.3.3 **No Template Control** showing amplification

- Amplification of ZDC 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 NTC 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 NTC, 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.

11 LIMITATIONS

- 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: http://codiagnostics.com/resources/instructions-for-use/
- Use of this product is to be limited to trained and instructed personnel 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 that 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 is required for optimal results.
- Do not use the **Logix Smart™ ZDC** kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction prior to using this assay.

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

12 LIMIT OF DETECTION

Diagnostic Evaluation is based on only contrived samples with serum, plasma and urine used for matrix.

Table 1 Limit of Detection for Logix Smart™ ZDC

Marker	Specimen	Strain	Estimated LOD
Zika virus	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	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	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**

For more information on performance evaluation, refer to **PID-1027: Logix Smart ZDC-001 Performance Data**.

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13 QUALITY CONTROL

In accordance with the Co-Diagnostics Incorporated ISO 13485-certified Quality Management System, each lot of **Logix Smart™ ZDC** kit is tested against predetermined specifications to ensure consistent product quality.

14 TECHNICAL ASSISTANCE

For technical assistance, please contact our Technical Support using one of the following methods:

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

15 REFERENCES

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16 TRADEMARKS AND DISCLAIMERS

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Product not available in all countries.

17 LEGEND OF PACKAGE SYMBOLS

Icon	Description
IVD	*In vitro* diagnostic medical device
REF	Catalog number
LOT	Batch Code
CAP	Cap color
COMP	Component
CONT	Content/Volume
NUM	Number
	Use-by-date
	Contains sufficient for 100 tests/ reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	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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