2019

CO-DIAGNOSTICS INC.

Logix Smart™ ZDC (Zika, dengue, chikungunya)

REF ZDC-K-001

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

CO-DIAGNOSTICS, INC.

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

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2 INTENDED USE

The **Logix Smart™ ZDC (Zika, dengue, chikungunya)** kit is an *in vitro* diagnostic test, based on qPCR technology, intended for the detection of the Zika, dengue types 1-4, and chikungunya viruses in serum, plasma, or CSF collected along with urine for detection of Zika, and serum or plasma for detection of dengue or chikungunya during the early stages of these diseases. Serology test confirmation may be needed if onset of infection has passed the early stages of these diseases.

3 PRODUCT DESCRIPTION

The **Logix Smart ZDC** test kit is a single-step reverse transcription real-time PCR reaction that can be broken down into 3 stages: sample preparation, reverse transcription, and the polymerase chain reaction with real-time monitoring. It tests the presence or absence of ribonucleic acid (RNA) of Zika, dengue types 1-4, and chikungunya viruses in serum or plasma (collected alongside with urine) from patients suspected of Zika, dengue, or chikungunya viral infections during acute stages of the disease. The **Logix Smart ZDC** test detects the virus within 45 cycles from serum, plasma, and urine specimen.

Each **Logix Smart ZDC** test kit consists of the following components:

- Ready-to-use Master Mix, complete with RNaseP internal positive control to verify sample quality
- Positive Control (PC), to verify the performance of the master mix
- Nuclease-Free Water as a negative control, to verify master mix is free from contamination

4 KIT COMPONENTS

Table 1 Kit Components

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Black	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 positive primers	1x500µL (100 reactions)
Clear	Nuclease Free Water	NTC	GEN-NF-001	Water free of DNase/RNase activity	1x500µL (100 reactions)

5 STORAGE

- 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.
- All components should be stored immediately at or below -20°C to prevent degradation of reagents.
- Always work with each **Logix Smart ZDC** component on ice. Make aliquots, if necessary, to avoid multiple freeze/thaw cycles.
- 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 to ensure that the **Logix Smart ZDC** test kit remains frozen at -20°C.
- Stability data for the product is currently being collected and results will be published and new Instructions for Use updated to reflect the stability conditions.

6 MATERIALS REQUIRED (NOT INCLUDED)

- Pipettes capable of transferring 5µL
- Ice
- Vortex
- Centrifuge
- Real-time PCR System with FAM (green), Cal Fluor Red 610 (Orange), Cal Fluor Orange 560 (yellow) and Quasar 670 (Red) dyes or equivalent and accompanying tubes/plates and caps/films.
- The **Logix Smart ZDC** test kit was validated with the CoDx Box™ (manufactured for Co-Diagnostics by BioMolecular Systems). It is the recommended equipment to run the test.
- 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 well maintained. Do **not** use instruments with outdated calibration.

7 BACKGROUND INFORMATION

Zika virus (ZIKV) is part of the *Flaviviridae* family. It is an arbovirus spread by the *Aedes* mosquito species. The most common symptoms include mild fever, skin rash, conjunctivitis, and muscle and joint pain. It was first isolated in 1947 in monkeys found in the Zika Forest in Uganda. The first human cases were 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). This same study conducted in 2016 in Brazil 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 the serious neurological sequelae this year (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).

Dengue virus (DENV) is part of the *Flaviviridae* family. It is an arbovirus spread by the *Aedes* mosquito species. The 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. There are 4 genotypes/serotypes of Dengue virus (DENV1, DENV2, DENV3, and DENV4). 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%.

Chikungunya virus (CHIKV) is part of the *Togaviridae* family. It is an arbovirus spread by the *Aedes* mosquito species. 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). The lineages of chikungunya 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).

8 ACCESSORIES (NOT INCLUDED)

8.1 Thermocycler

Co-Diagnostics, Inc. can either directly or through reagent rental programs provide the CoDx Box thermocycler machines (manufactured for Co-Diagnostics, Inc. by BioMolecular Systems). The **Logix Smart ZDC** test kit can also be used in other real-time PCR systems as long as the parameters to run the test are set as established for the **Logix Smart ZDC** test kit.

Two machines 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.

The CoDx Box thermocycler is recommended due to its ease of use, small size, durability, and fast report generation. The CoDx Box thermocycler software was developed by BioMolecular Systems solely for Co-Diagnostics, Inc., and it has been verified for use with Co-Diagnostics, Inc. real-time PCR products, simplifying result interpretation. The CoDx Box thermocycler reads fluorescence in real-time, generated from the PCR reagents loaded into CoDx Box PCR reaction tubes, amplifies the virus RNA by thermal cycling using magnetic induction, and displays output data through the integrated software. The CoDx Box thermocycler is available with 48 reaction wells and either 2 or 4 channels.

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

8.2 Extraction Kit

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.

- QIAamp Viral Mini Kit, Qiagen, cat No. 52904, for 50 extractions
- QIAamp Viral Mini Kit, Qiagen, cat. No. 52906, for 250 extractions

Other kit options include: sbeadex™ Livestock (LGC, Cat. No. 65000), QIAamp Min Elute Virus Spin Kit (Qiagen, Cat No. 57704), ReliaPrep™ Blood gDNA Kit (Promega, A5081), MagNA Pure Compact RNA Isolation extraction kit (Roche, Cat. No. 04802993001), Nuclisens (bioMérieux, Inc.) extraction kit., even though no test performance studies have been performed with the current iteration of the **Logix Smart ZDC** test kit.

Please, always use the most recent version of this document as more information as added with future studies. This can be downloaded for free at: http://codiagnostics.com/resources/instructions-for-use/

9 WARNINGS AND PRECAUTIONS

WARNING!

Users should pay attention to the following:

- Use sterile pipette tips with filters.
- Use standard precautions when handling any patient samples, as they may contain infectious agents.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents.
- Always use nuclease free water, provided with this kit.
- 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://codiagnostics.com/resources/safety-data-sheets/
- To prevent contamination, it is required to use Good Laboratory Practices for Molecular Biology, which requires a unidirectional workflow and the separation of negative and positive materials.
- 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.

10 SAMPLE INFORMATION

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/

10.1 Collection Recommendations

Zika, dengue, and chikungunya have been detected in whole blood (also serum and plasma). The WHO recommends the use of whole blood, serum, to be collected along with a matching urine sample from patients to be tested (World Health Organization, 2016).

Testing for ZIKV: According to Relich & Loeffelholz (2017), ZIKV RNA can be detected in serum with real time RT-PCR tests from 2 to 7 days after onset of symptoms. After 7 days, the viral load in the blood starts to decrease. The viral RNA can be detected in urine up to 20 days, although it has happened to be detected in urine in more than 20 days. Zika virus was found in semen 2 months after onset of symptoms. The same study also recommended that to have a robust result and solve the problem of variability of viral load and days from the onset of the disease, especially because onset of the disease can be difficult to determine as some people are asymptomatic, ideally serum and urine should be tested at the same time.

Testing for DENV: Dengue virus can be found in serum and plasma up to 5 days after onset of symptoms. After this period a nucleic acid assays can be performed together with serological tests, as due to the lower viral load the risk of false negatives is higher.

Testing for CHIKV: Chikungunya virus can be detected 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, as the risk of false negatives is higher due to the lower viral load.

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

10.2 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).

10.3 Sample Handling

Reverse-transcription polymerase chain reaction (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).

11 PROCEDURE

11.1 Real Time RT-PCR Setup

- All real-time RT-PCR master mix, positive control, no template control (nuclease-free water), and sample tubes should be briefly spun down before using, to remove any condensation or residue from the lids, especially after mixing or after being in storage.
- Thaw all reagents and samples on **ice**, or on a cold block, before starting setup.

11.2 No Template Control Set Up

- Thaw **Logix Smart ZDC Master Mix (ZDC-MM-001, Master Mix, or MM)** on ice.
- Vortex **Master Mix** for no more than 3 seconds, and then centrifuge.
- Put the **Master Mix** on ice.
- Aliquot 5µL of **Master Mix** into PCR tubes on ice.
- Add 5µL **Nuclease-Free Water (GEN-NF-001)** to the appropriate well(s).

11.3 Patient Sample and Positive Control Set Up

- Prepare extracted patient samples and the positive control in a separate space from the master mix and nuclease-free water, to avoid contamination.
- Thaw extracted, purified RNA on ice (if frozen).
- Vortex and centrifuge extracted RNA for a few seconds.
- Add 5µL of extracted RNA sample to each well using a new tip between each sample.
- Thaw **Logix Smart ZDC Positive Control (ZDC-PC-001, Positive Control, or PC)** on ice.
- Vortex and centrifuge **Positive Control** for a few seconds.
- Add 5µL of **Positive Control** to appropriate well(s).
- Place caps on the tubes according to the real-time system being used.
- Put plate/tubes in real-time PCR machine and start the run.

11.4 Thermocycler Setup

Program the thermocycler to the conditions found in **Table 2** with a total reaction volume of 10µL:

Table 2 Thermocycler Conditions

Temperature	Time	Cycles	Capture
45˚C	15 minutes		N/A
95˚C	2 minutes		N/A
95˚C	3 seconds	50	N/A
55˚C	32 seconds		FAM (Green) Cal Fluor Red 610 (Orange) Cal Fluor Orange 560 (Yellow) Quasar 670 (Red)

- When the run is finished, ensure that the run file is saved.
- Check to see that both the positive and negative controls passed.
- If controls pass, interpret the sample results. If controls fail, the run is invalid. Document the run and initiate troubleshooting.

12 DATA ANALYSIS

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.

12.1 Positive Controls

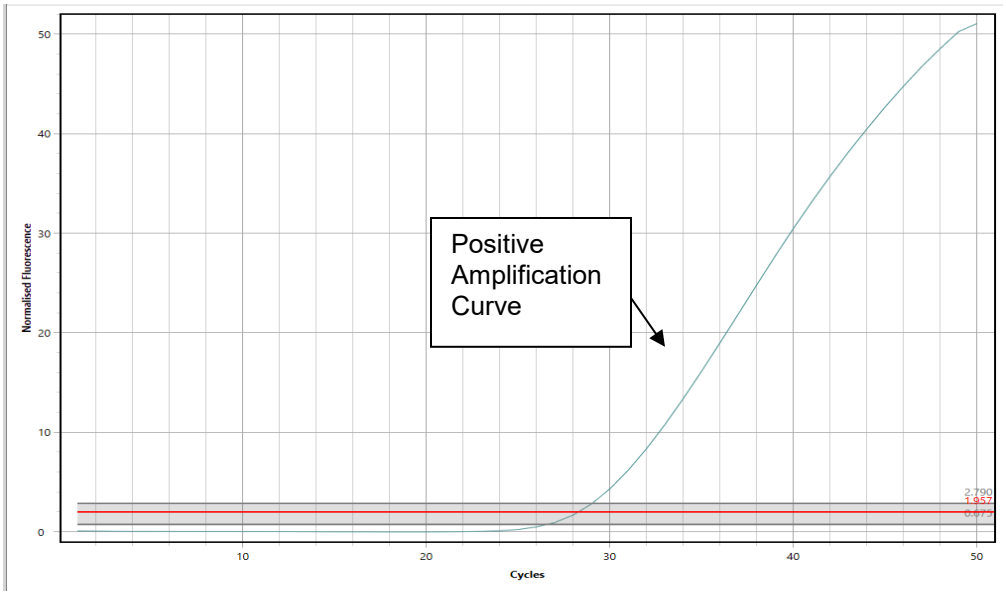

Figure 1: Positive Control Amplification

Table 3: Positive Control Ranges for Cycle Threshold Values

Range for Positive Control Ct Values*	
ZIKV (FAM)	26-29
DENGUE (CF560)	34-39
CHIKV (Q670)	24-27
IPC (CF610)	20-24
NTC	>45 or No Amplification

ZIKV (FAM): Zika Virus Marker
DENV (CF560): Dengue Virus Marker
CHIKV (Q670): Chikungunya Virus Marker
IPC (CF610): RNaseP Internal Positive Control Marker
NTC: No Template Control
*Ct values may vary by ± 2 cycles based on instrument differences.

If the positive control does not show amplification, then the tests are invalid. Loss of amplification for a positive control is indicative of master mix degradation which may result from reagents being at temperatures above -20˚C for more than one hour or being used past the expiration date. Pipetting error may also account for lack of positive control amplification by pipetting control into the wrong well, missing a well, or pipetting an inadequate amount of reagent into a reaction well.

12.2 No Template Controls

The results of the No Template Control should show results like those seen below:

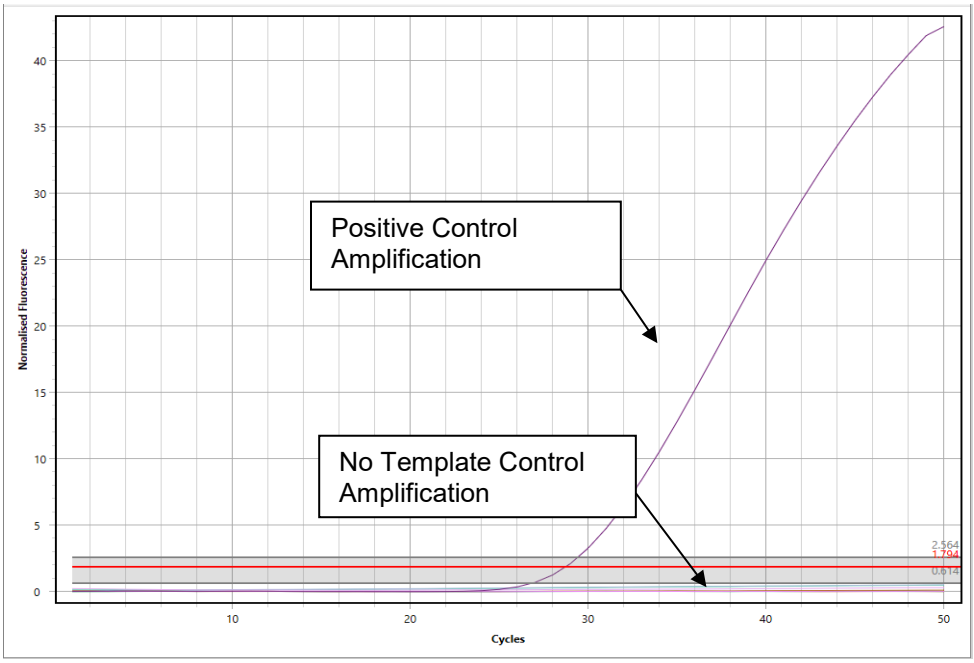

Figure 2: No Template Control Amplification

Occasionally, ubiquitous binding will cause amplification No Template Control in the IPC Channel (CF610) as seen in the figure below:

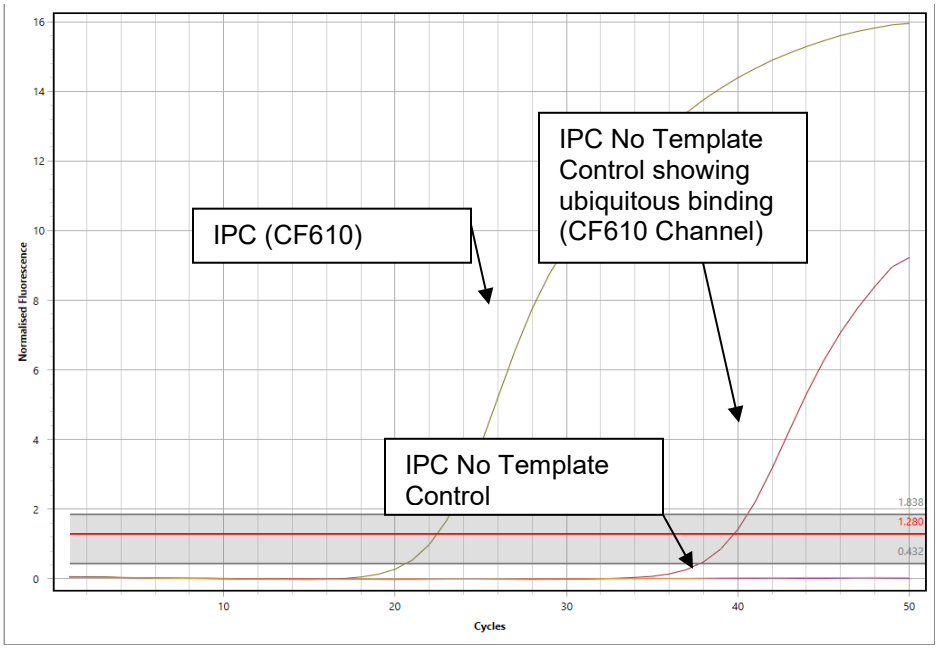

Figure 3: Ubiquitous Binding of No Template Control in the IPC CF610 Channel

If the No Template Control shows any amplification of ZDC <45 cycles the results are invalid and the entire experiment must be repeated. Amplification of ZDC in a No Template Control indicates contamination in one or more of the reagents or pipetting error. Amplification of ZDC >45 cycles is outside the detectable range and is considered negative.

12.3 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes (figures may vary based on machine used and quantity of MM and sample):

- Positive (Figure 4/5 and Figure 6/7)
- Negative (Figure 8/9)
- Negative Due to Inadequate Nuclear Material (Figure 10/11)

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 outside of the detection limits for the assay. A positive sample will have the following curves in the target's respective channel AND IPC CF610 channel:

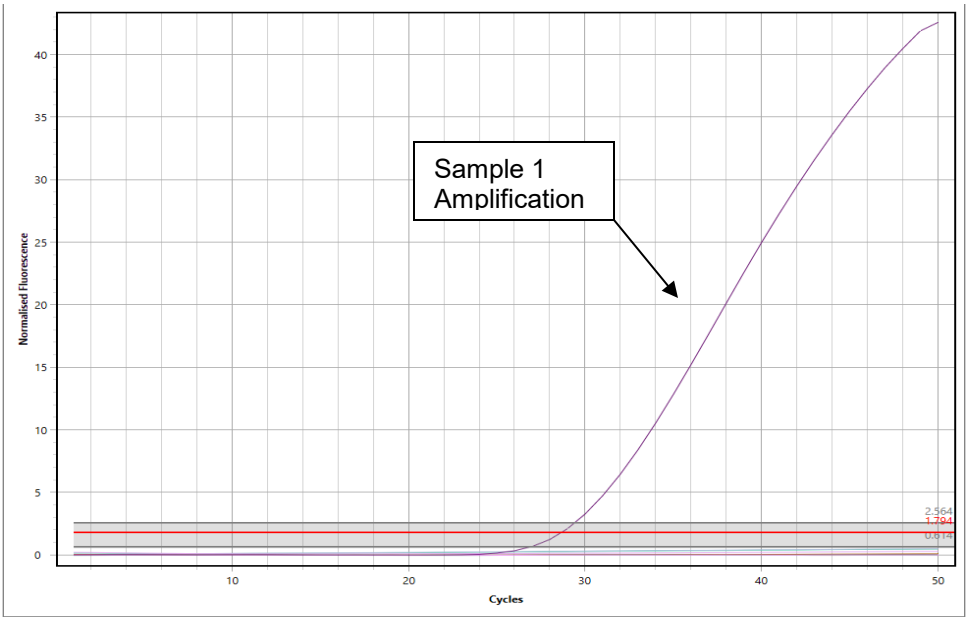

Figure 4: ZDC Positive Sample in FAM/CF560/Q670

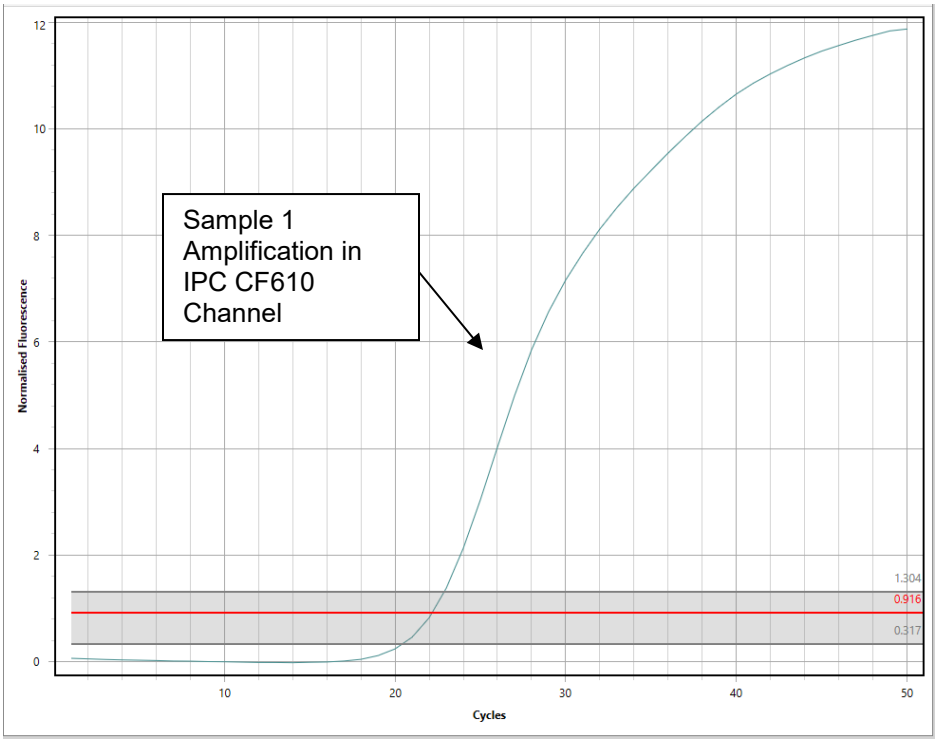

Figure 5: ZDC Positive Sample in IPC CF610

The presence of a curve for positive sample in FAM/CF560/Q670 indicates a positive result. The amplification of the IPC (CF610) shows that the extraction was successful.

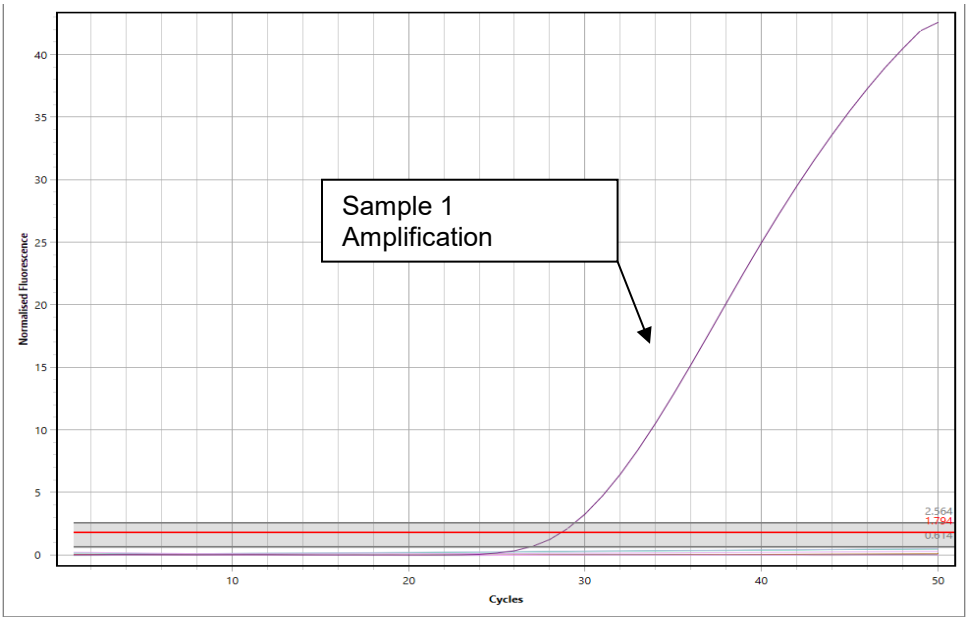

Figure 6: ZDC Positive Sample in FAM/CF560/Q670

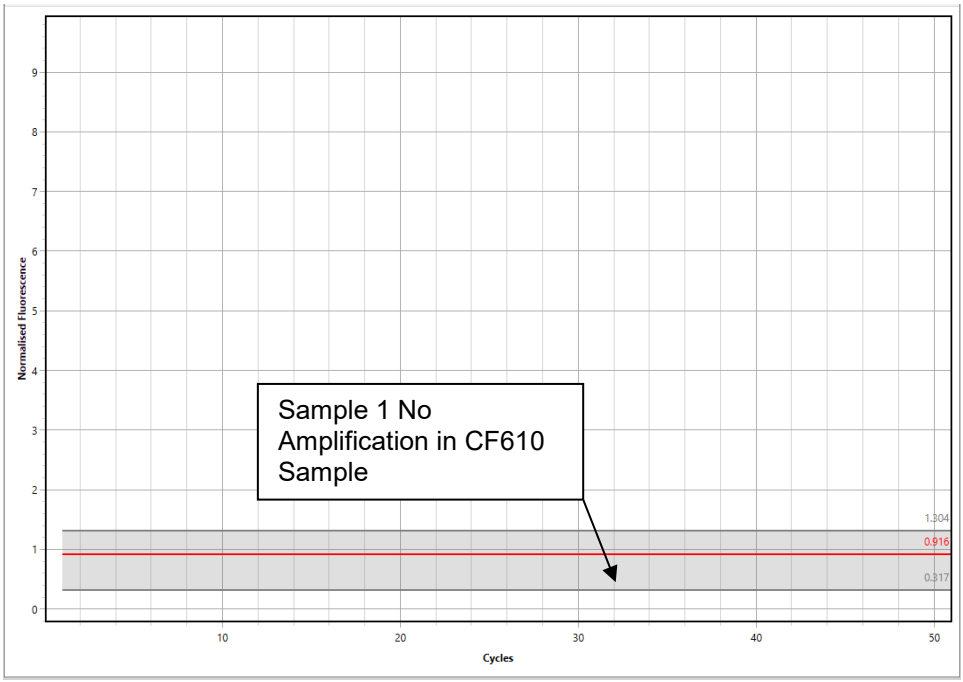

Figure 7: ZDC No Amplification Sample in IPC CF610

The presence of a curve for ZDC indicates a positive result even when the RNaseP (IPC) marker is negative. This will occur when the concentration of ZDC is greater than the concentration of RNaseP or when using cell lysates or extremely pure/sterile samples.

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. A negative sample result will have the following curve:

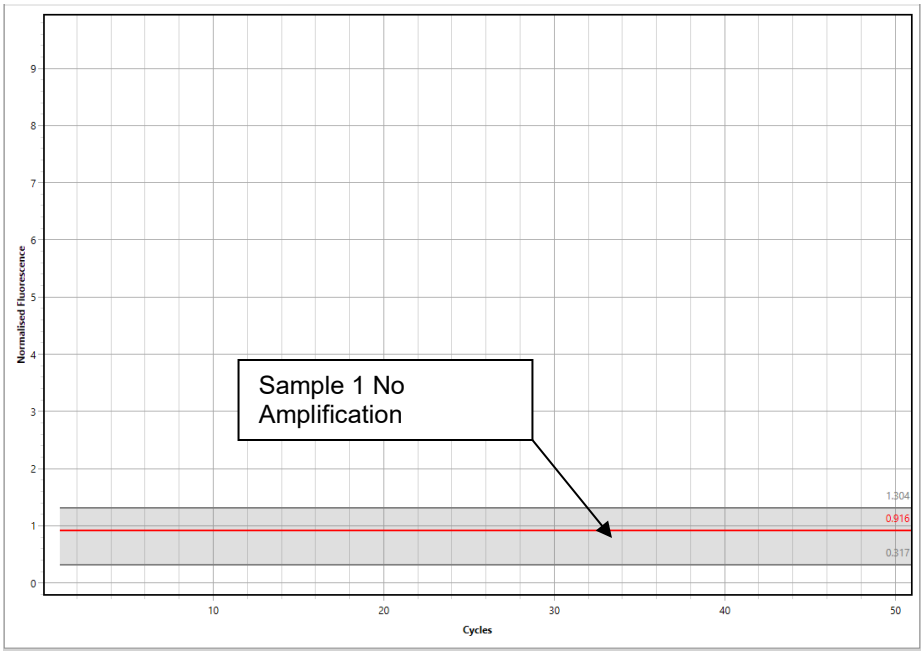

Figure 8: ZDC No Amplification Sample in FAM/CF560/Q670

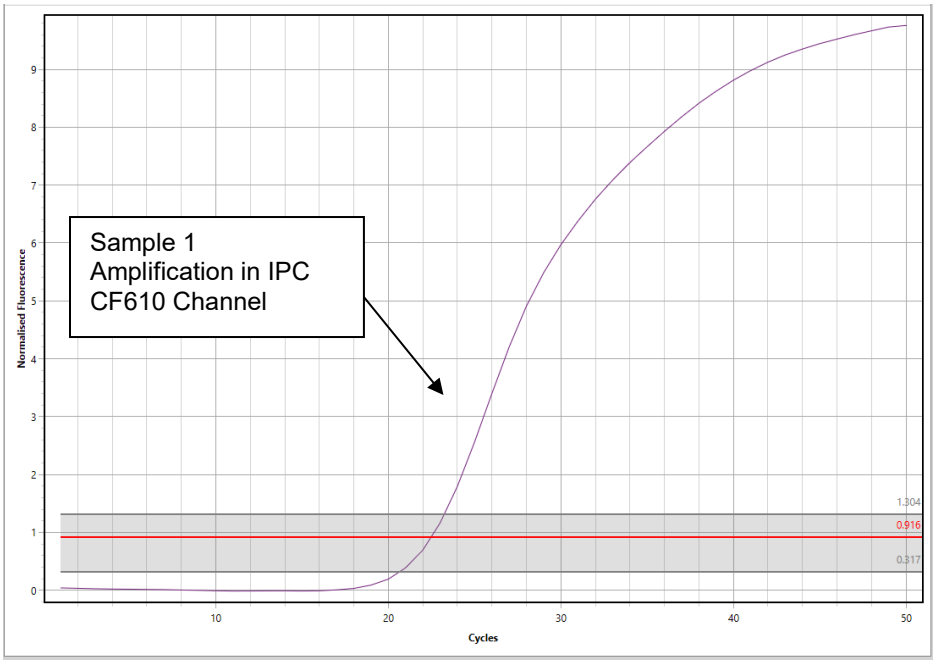

Figure 9: ZDC Amplification Sample in IPC CF610

The absence of a curve for ZDC indicates a negative result ONLY when the RNaseP (IPC) marker is positive.

A **Negative Due to Inadequate Nuclear Material** result will have the curve in *Figure 10*:

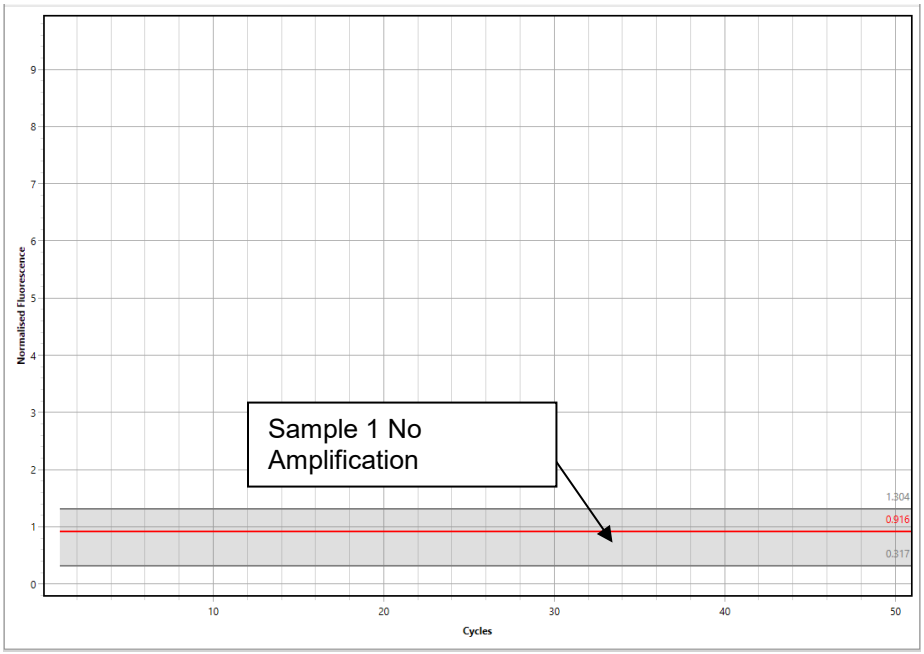

Figure 10: Negative Due to Inadequate Nuclear Material (FAM/CF560/Q670)

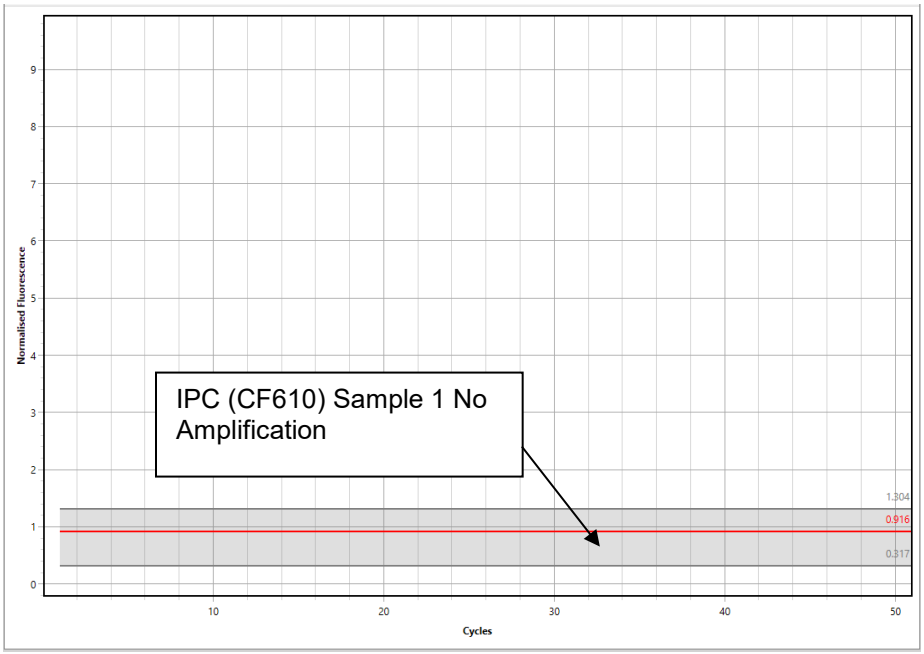

Figure 11: Negative Due to Inadequate Nuclear Material (IPC CF610)

If the RNaseP (IPC) control marker is also negative, the result is negative due to inadequate nuclear material. This can result from human error in sample preparation, sample degradation, or inadequate sampling. The test may be repeated with a new sample or called negative due to inadequate nuclear material.

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.

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

Table 4 Interpretation of Results with Ct Values

Marker	Instrument reading	Specimen	Positive Control reading	No Template Control reading	Final Result	Rationale
ZIKV (FAM)	Negative (-)	No amplification at < 45 cycles in any specimen: serum, plasma, CSF or urine.	**Positive Control (PC)** Must always amplify with Ct value disclosed in **Table 3**. If IPC does not amplify, see troubleshooting	**No Template Control (NTC)** Must always not amplify. If any amplification with NTC, see troubleshooting	Negative (-)	ZIKV RNA has not been detected in any of the samples.
	Positive (+)	Amplification at < 45 cycles in any specimen: serum, plasma, CSF or urine.			Positive (+)	ZIKV RNA has been detected with 99% certainty* for serum/plasma, 92% for urine and 95% for CSF.
DENV (CF560)	Negative (-)	No amplification at < 45 cycles in any specimen: serum or plasma.			Negative (-)	DENV RNA has not been detected in any of the samples.
	Positive (+)	Amplification at < 45 cycles in any specimen: serum or plasma.			Positive (+)	DENV RNA has been detected with 100% certainty* for serum/plasma.
CHIKV (Q670)	Negative (-)	No amplification at < 45 cycles in any specimen: serum or plasma.			Negative (-)	CHIKV RNA has not been detected in any of the samples.
	Positive (+)	Amplification at < 45 cycles in any specimen: serum or plasma.			Positive (+)	CHIKV RNA has been detected with 100% certainty* for serum and 99% for plasma.

*The certainty disclosed here refers to the negative predictive value (NPV), which is the certainty of a positive result being positive. For NPV results for Logix Smart ZDC refer to **Table 6**

13 TROUBLESHOOTING

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 stablished 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.

13.2 User Errors

Polymerase Chain Reaction (PCR) Assay is a technique that uses temperature cycling, and a DNA polymerase to amplify a single or a few copies of a segment of DNA or RNA. 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 must have calibration up to date for the pipettes and thermocyclers, when applicable.

A 90 minutes 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

The Positive Control and No Template Control are validated, manufactured and tested along with the Master Mix. The purpose of these controls is to confirm the performance of the Master Mix, as well as to validate the user technique used during the experiment. If the user has a poor use of the techniques required to perform a molecular biology assay, it is more likely that it will be shown by the Positive Control not amplifying or the No Template Control showing amplification.

13.3.1 Positive Control not amplifying

No amplification from the positive control suggests that the PCR is not working. This could be the result of one or multiple factors, such as: pipetting errors, master mix degradation, positive control degradation, or the wrong reagents being used. Without further evidence, it is best to disregard the results from the patient samples and retest. 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.

13.3.2 No Template Control showing amplification

If the negative control template amplifies, this means that for some reason (e.g. contamination from pipetting, splashes on the PCR plate, user contamination) an error caused the No Template Control (Nuclease-Free Water) to be contaminated with the positive control or with the sample. Because this indicates the likelihood that the same error could have happened to contaminate the sample, the results cannot be trusted, and the test must be invalidated. 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.

14 PERFORMANCE EVALUATION

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

Table 5 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**

Table 6 Diagnostics Accuracy of Logix Smart ZDC

	Zika Virus				Dengue		Chikungunya	
	Serum	Plasma	Urine	CSF	Serum	Plasma	Serum	Plasma
Sensitivity	97.20%	93.22%	91.43%	96.00%	97.96%	99.33%	98.97%	94.36%
Specificity	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%
Accuracy	1.00	0.99	0.96	0.98	1.00	1.00	1.00	0.99
PPV	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
NPV	0.99	0.99	0.92	0.95	1.00	1.00	1.00	0.99
MCC	0.98	0.96	0.92	0.95	0.99	0.99	0.99	0.96

Note: PPV, Positive Predictive Value; NPV, Negative Predictive Value; MCC, Matthews correlation coefficient

Analytical specificity was performed with wet-test and in-silico analysis with microorganism of interest that should be detected and the relevant microorganism that should not cross-react or interfere with this product's performance. Specificity also tested the performance of the **Logix Smart ZDC** test with common interfering substances.

Logix Smart ZDC showed 100% specificity while not cross-reacting with other microorganisms, nor having performance altered by these microorganisms or interfering substances. The only substance acting as interference was Heparin, which is a well-known PCR inhibitor. Warnings about heparin have been included in the warning section of this document.

A wet-test has been performed with the following microorganism: West-Nile Virus (WNV), measles, Epstein-Barr Virus, Varicella Zoster Virus, Eastern Equine Encephalitis, St. Louis Encephalitis, tick-borne encephalitis (TBEV), influenza A H1, influenza A H1N1, influenza A H5, influenza B, and Borrelia Burgdorferi.

In-silico analysis has been performed with the following microorganism: Lassa Virus (LASV), Leptospira, Rickettsiales, and Spondweni Virus (SPOV)

15 REFERENCES

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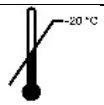
16 LEGEND OF PACKAGE SYMBOLS

IVD	*In vitro* diagnostic medical device
REF	Catalogue 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
	Non-Sterile product – Do not sterilize
	Manufacturer
EC REP	Authorized representative in the European Community
IVD CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC