May
2022

Co-Dx

Logix Smart™ Zika Virus (ZIKV) Test

REF ZIKV-K-004

LOGIX SMART™ ZIKV TEST
CO-DIAGNOSTICS, INC.

IVD

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

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1 MANUFACTURER AND AUTHORIZED REPRESENTATIVE

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2 INTENDED USE

The **Logix Smart™ Zika Virus** test kit is an in vitro diagnostic test, based on qPCR technology, intended for the detection of Zika virus in serum or plasma along with urine.

3 PRODUCT DESCRIPTION

The **Logix Smart™ Zika Virus** 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 Virus in serum or plasma (collected alongside with urine) from patients suspected of Zika fever or Zika disease during acute stages of the disease. **Logix Smart™ ZIKV Virus** test is recommended to be tested in serum or plasma, alongside with urine (Zika virus testing is essential to aid the control and spread of virus prior to pregnancy, transfusion or transplantation, or sexual relation). The **Logix Smart™ ZIKV Virus** test detects the virus within 40 cycles from serum, plasma, and urine specimen.

Each **Logix Smart™ Zika Virus** test kit consists of the following components:

- Master Mix which is complete and ready for use
- Positive Control to verify the performance of the master mix (PC)
- Nuclease Free Water to verify there is no contamination in the master mix

4 KIT COMPONENTS

See Table 1 for components found in the kit.

Table 1

Kit Components

Cap Color	Component	Symbol	Individual Catalog Number	Description	Amount
Brown	Logix Smart™ ZIKV Master Mix	MM	ZIKV-MM-004	Proprietary blend of co-primers and PCR reagents	1x500µL (100 reactions)
Red	Logix Smart™ ZIKV Positive Control	PC	ZIKV-PC-004	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, HANDLING, AND DISPOSAL

- The **Logix Smart™ ZIKV Virus** 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™ ZIKV Virus** 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™ ZIKV Virus** 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.
- 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.

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6 MATERIALS REQUIRED (NOT INCLUDED)

- Pipettes capable of transferring 5 µL
- Ice
- Vortex
- Centrifuge
- Real-time PCR System with FAM (green) or Cal Fluor 610 (orange) dyes or equivalent and accompanying tubes/plates and caps/films.
- The **Logix Smart™ Zika Virus** Test was validated within CoDx Box MIC manufactured by BioMolecular Systems. It is the recommended equipment to run the test.
- Biosafety cabinet, ideally BSL-2 facility.

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

7 BACKGROUND INFORMATION

Zika virus (ZIKV) is a Flaviviridae family virus. It is spread by daytime-active Aedes mosquitoes, such as A. aegypti and A. albopictus. The virus was first isolated in 1947 in monkeys and is named after the Zika Forest in Uganda. In 1952, the first human cases of Zika were detected and since then, outbreaks of Zika have been reported in Africa, the Americas, Asia, and the Pacific. Zika outbreaks have probably occurred in many other locations. Because the symptoms of Zika are like those of many other diseases, many cases may not have been recognized or properly reported.

Before its emergence in 2015 in Brazil, Zika virus was not thought to be endemically transmitted in the Americas. Since then, it has spread across South and into North America, including the Caribbean. As mentioned above, Zika virus is most commonly transmitted by mosquitoes; nevertheless, horizontal and vertical transmission in humans has been reported. The disease caused by the Zika virus, sometimes called Zika fever, presents with similar symptoms to other arboviral infections such as dengue and chikungunya. The symptoms include mild fever, skin rash, conjunctivitis, muscle and joint pain which normally last for 2 to 7 days. Birth defects and serious neurologic sequalae have been reported in association with Zika virus infection. There is no specific treatment, but symptoms are normally mild and can be treated with common fever medicines, rest, and drinking plenty of fluids (World Health Organization, 2016). Continued epidemiologic monitoring is needed as well as basic research into the pathogenesis, immunology, and biology of Zika fever and Zika virus for effective counter-measures and vaccines (Relich & Loeffelholz, 2017).

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In 2015, right after the reports of Zika infections in Brazil, 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 examining neonates born between January to November 2016 in the Northeast region of the country where Zika cases were prevalent. 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 Zika disease's 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).

Because Zika virus belongs to the family Flaviviridae and genus Flavivirus, it is related to the dengue, yellow fever, Japanese encephalitis, and West Nile viruses. The Zika virus is enveloped, icosahedral and has a nonsegmented, single-stranded, 10-kilobase, positive-sense RNA genome. It is most closely related to the Spondweni virus and is one of the two known viruses in the Spondweni virus clade.

8 ACCESSORIES (NOT INCLUDED)

8.1 Thermocycler

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

Two machines have been used and tested with the product, the Co-Dx Box™ thermocycler (Bio Molecular Systems), and the Eco 48 (Cole-Parmer). Of these, only the Co-Dx Box™ thermocycler (Bio Molecular Systems) has been validated with the current version of the product. Other validation exercises will include more testing more thermocyclers, as well as creating specific protocols for those thermal cyclers.

The Co-Dx Box™ thermocycler is recommended due to its ease of use, small size, durability, and fast report generation. The Co-Dx Box™ thermocycler software was developed by Bio Molecular 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 Co-Dx Box™ thermocycler reads fluorescence in real-time, generated from the PCR reagents loaded into Co-Dx Box™ PCR reaction tubes, amplifies the virus RNA by thermal cycling using magnetic induction, and displays output data through the integrated software. The Co-Dx 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 Co-Dx Box™ thermocycler. The Microsoft SurfaceTM Pro 4 System (MSPRO-4) is available for use with Co-Dx Box™ software in a windows-based operation system. The output

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device used with the Co-Dx 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™ ZIKV Virus**. 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™ ZIKV Virus** 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™ ZIKV Virus** test kit.

Please, always use the most recent version of this document as more information as added with future studies. For the most recent version go to http://codiagnostics.com/resources/instructions-for-use/ for free download of this manual.

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 Logix Smart™ ZIKV Test is provided with shipment. If not provided with shipment, the

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SDS can be retrieved from Co-Diagnostics website at the link: http://codiagnostics.com/products/diagnostic-solutions/

- 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 no collect samples, for nucleic acid PCR assays, in Heparin (green top tube) or EDTA (purple top tube) 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 analysis of results and troubleshooting other problems.

- CDC, Testing for Zika: https://www.cdc.gov/zika/symptoms/diagnosis.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

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.

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

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10.1 Sample Storage

Specimen 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. For storage longer than 7 days, samples should be frozen at -70°C. (World Health Organization, 2016).

10.2 Sample Handling

Reverse-transcription polymerase chain reaction (RT-PCR) analysis on clinical specimens from patients who are suspected or confirmed to be infected with Zika virus, 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 virus 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 x, 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 in a cold block, before starting setup.

11.2 No Template Control Set Up

- Thaw **Logix Smart™ ZIKV Virus** Master Mix on ice.
- Vortex, for no more than 3 seconds, and centrifuge the **Logix Smart™ ZIKV Virus** Master Mix.
- Put the **Logix Smart™ ZIKV Virus** Master Mix on ice.
- Aliquot 5µL of **Logix Smart™ ZIKV Virus** 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.

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- Add 5µL of extracted RNA sample to each well using a new tip between each sample.
- Thaw Logix Smart™ ZIKV Virus Positive Control (ZDC-PC-001) on ice.
- Vortex and centrifuge **Logix Smart™ ZIKV Virus Positive Control** for a few seconds.
- Add 5µL of Logix Smart™ ZIKV Virus 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 Set Up

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

Table 2

Thermocycler Conditions

Temperature	Time	Cycles	Capture
45˚C	15 minutes		N/A
95˚C	2 minutes		N/A
95˚C	3 seconds	45	N/A
55˚C	32 seconds		FAM (Green) and Cal Fluor Red 610 (CF610) (Orange)

- 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™ ZIKV Virus (ZIKV-K-004)** 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. See Table 3 for positive control ranges for cycle threshold values.

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12.1 Positive Controls

See Figure 1 for the positive control amplification curve.

Figure 1

Positive Control Amplification

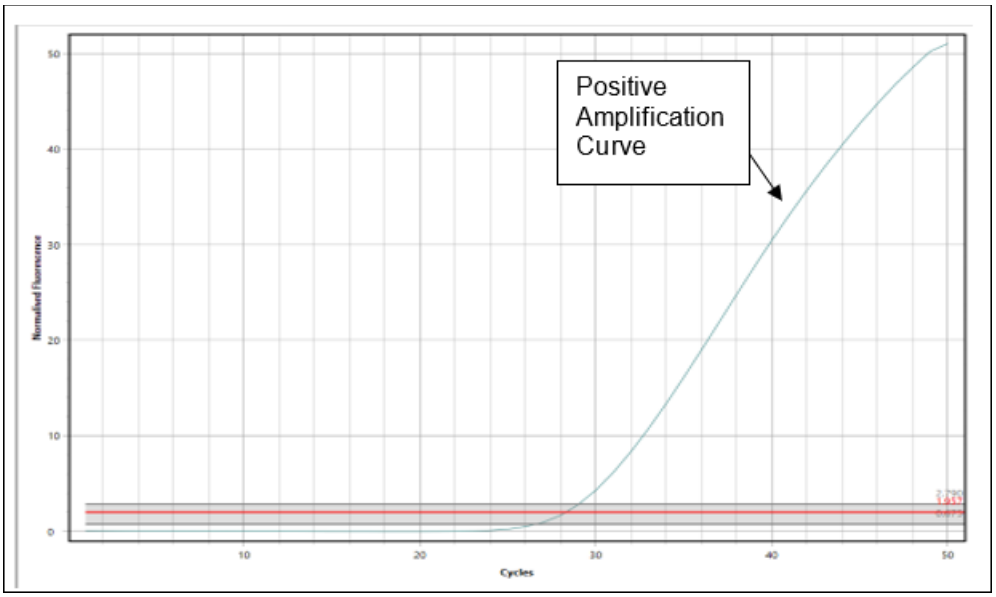

Table 3

Positive Control Ranges for Cycle Threshold Values

Item	Range for Positive Control Ct Values*
ZIKV (FAM)	24.00-28.50
IPC (CF610)	21.00-27.00

ZIKV (FAM): Zika Virus Marker
IPC (CF610): RNaseP Internal Positive Control Marker
*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 primer 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.

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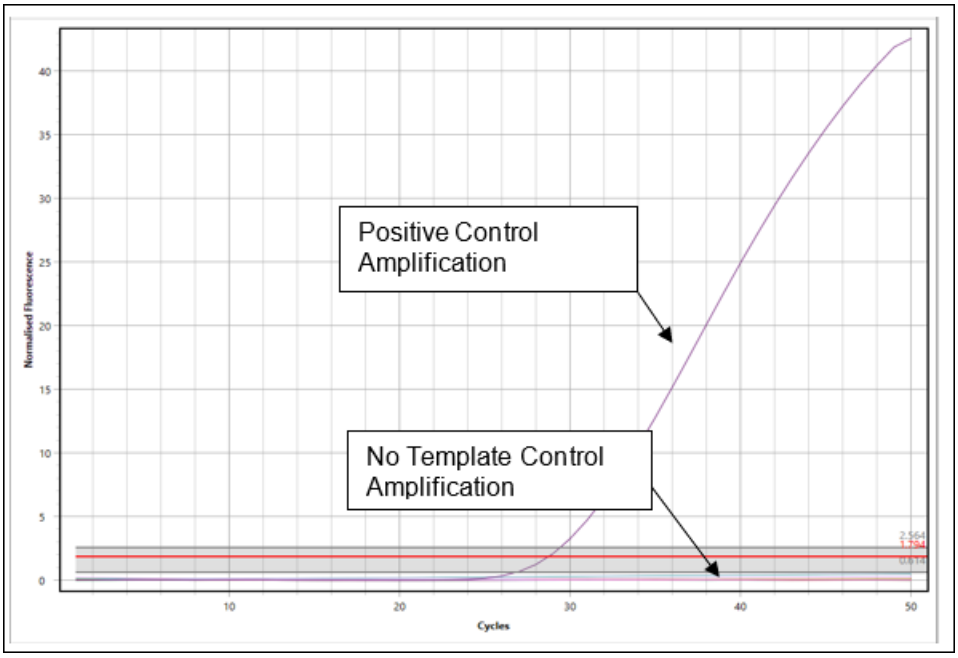
12.2 No Template Controls

The results of the No Template Control should show results like those seen in Figure 2.

Figure 2

No Template Control Amplification



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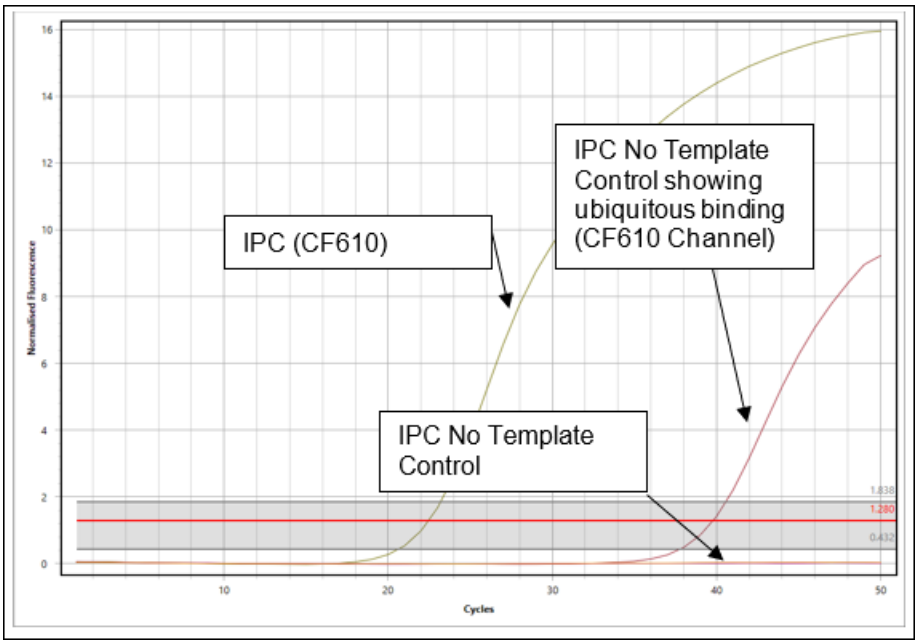
Occasionally, ubiquitous will cause amplification No Template Control in the IPC Channel (CF610) as seen in the Figure 3.

Figure 3

Ubiquitous Binding of No Template Control in the IPC CF610 Channel



If the No Template Control shows any amplification of ZIKV <40 cycles, the results are invalid, and the entire experiment must be repeated. Amplification of ZIKV in a No Template Control indicates contamination in one or more of the reagents or pipetting error. Amplification of ZIKV >40 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 and Figure 5)
- Negative: (Figure 7 and Figure 8)
  - Negative due to inadequate nuclear material: (Figure 10 and Figure 11)

A **Positive** result will show an amplification curve or cycle threshold value for ZIKV at or below 40 cycles. Amplification curves greater than 40 cycles for ZIKV 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:

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Figure 4

ZIKV Positive Sample in FAM

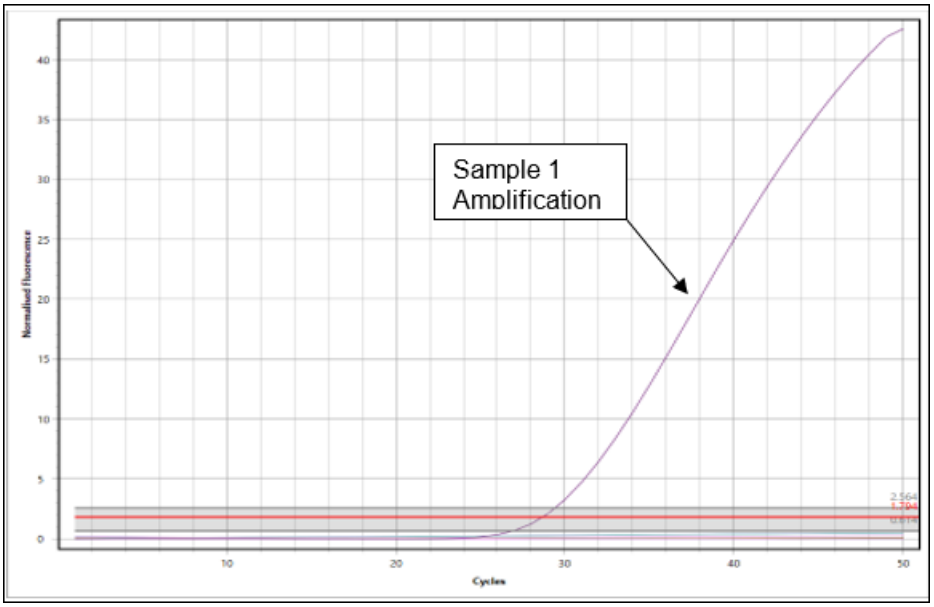
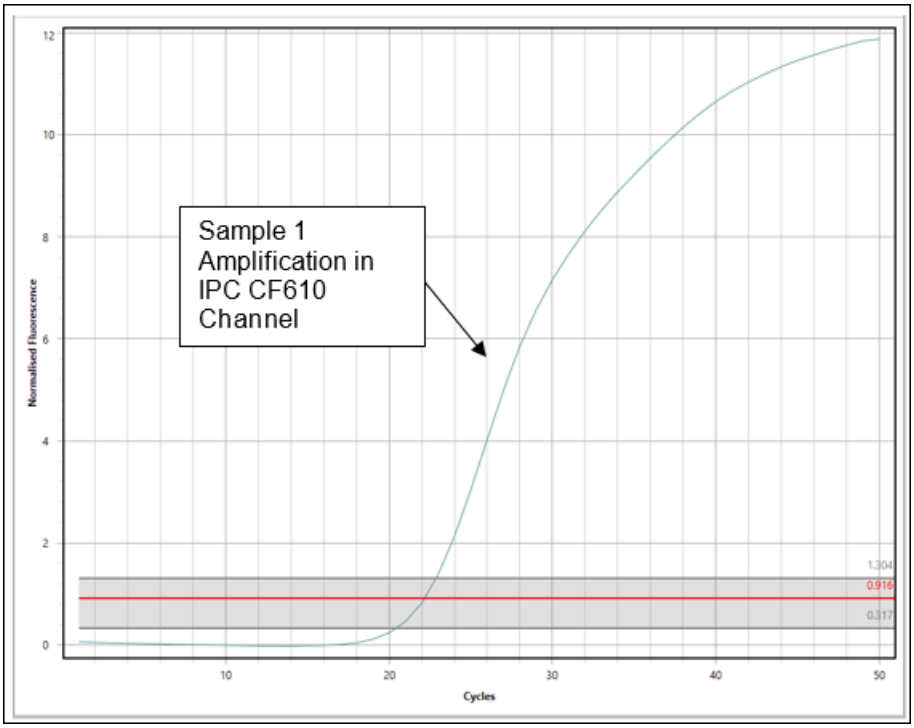


Figure 5

ZIKV Positive Samples in IPC CF610



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

Figure 6

ZIKV Positive Sample in FAM

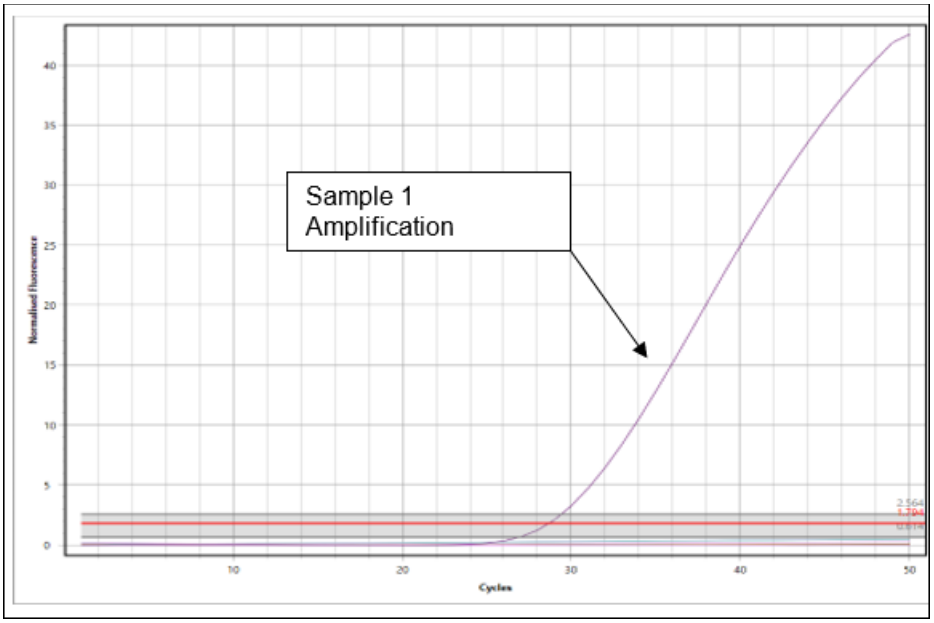
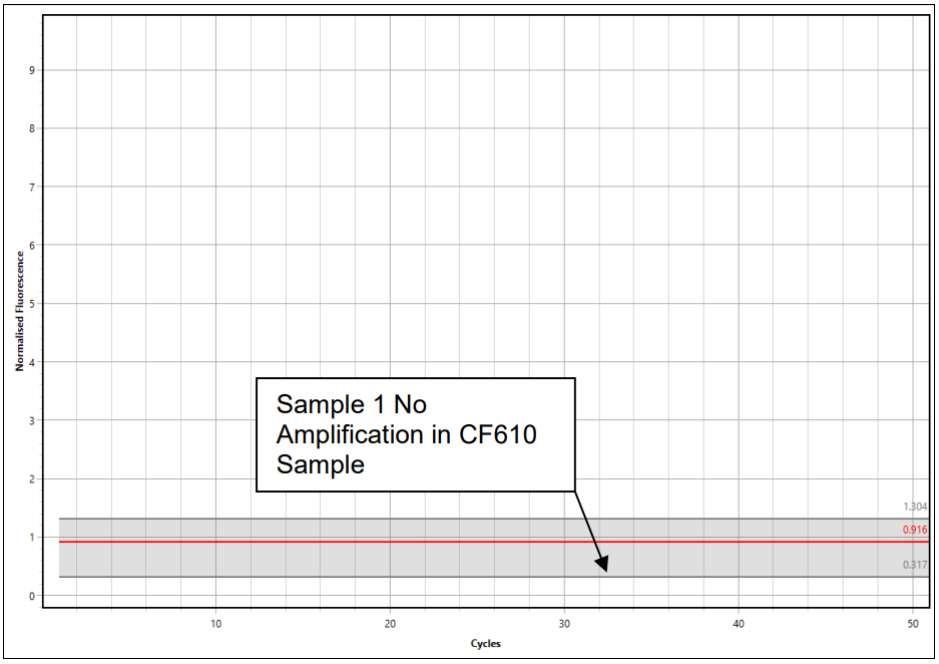


Figure 7

ZIKV No Amplification Sample in IPC CF610



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The presence of a curve for ZIKV indicates a positive result even when the RNase P (IPC) marker is negative. This will occur when the concentration of ZIKV 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; however, occasionally amplification greater than 40 cycles occurs in ZIKV or RNaseP channels. Any amplification curves greater than 40 cycles for ZIKV are outside of the detection limits for the assay. A negative sample result will have the curve shown in Figure 8.

Figure 8

ZIKV No Amplification Sample in FAM

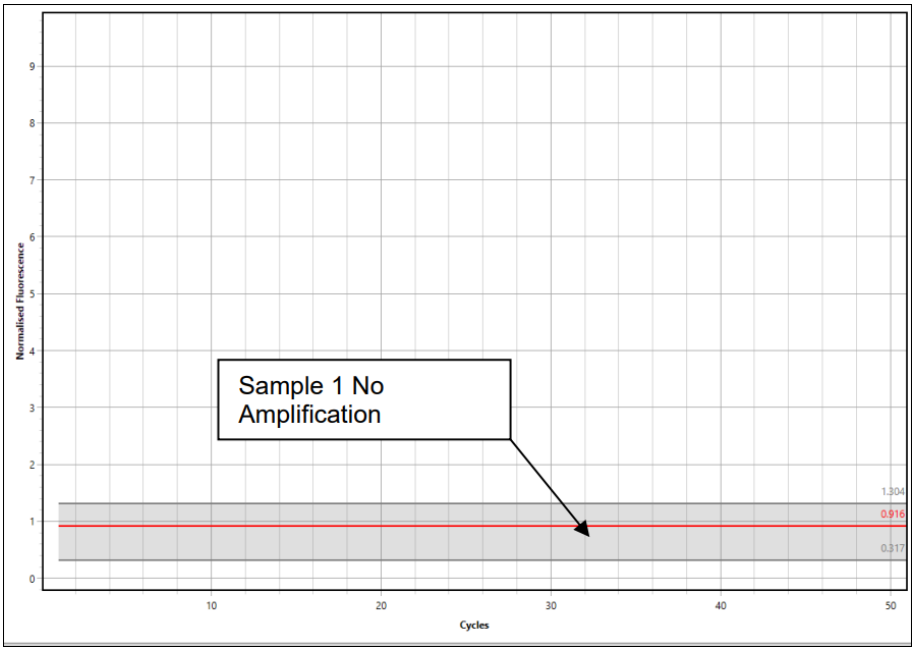

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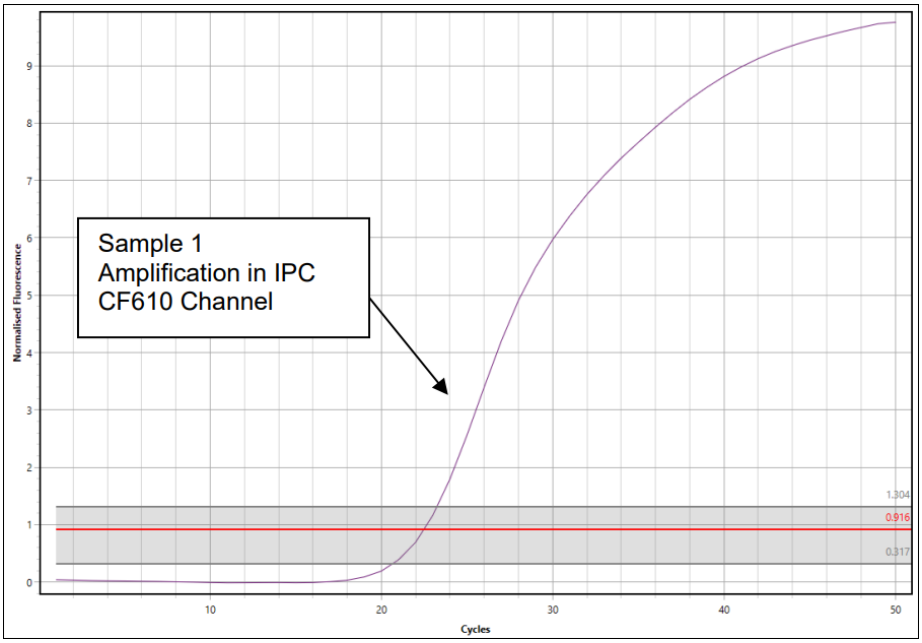
Figure 9

ZIKV Amplification Sample in IPC CF610



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

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

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Figure 10

Negative Due to Inadequate Nuclear Material (FAM)

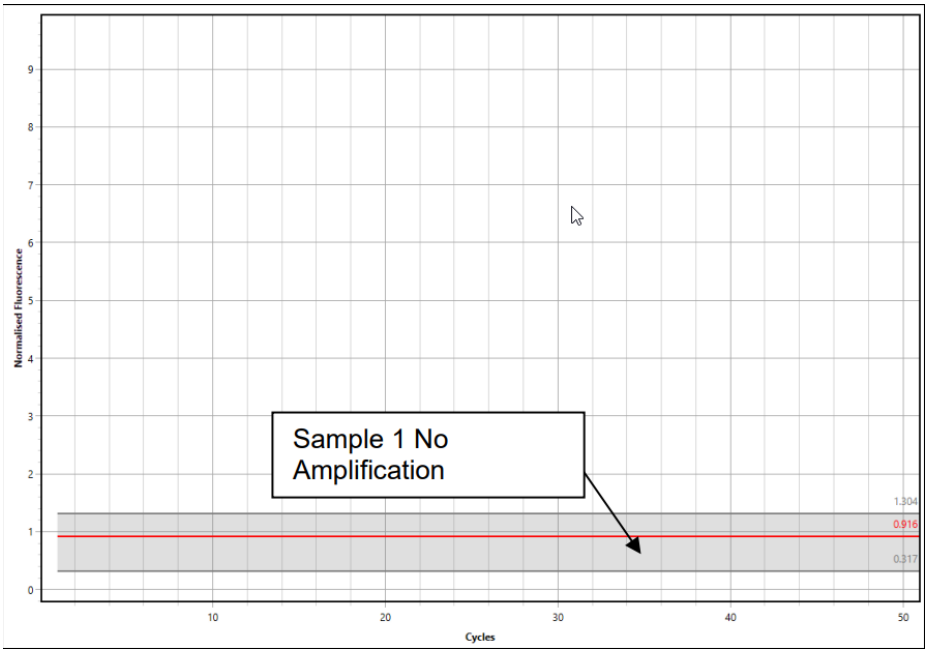
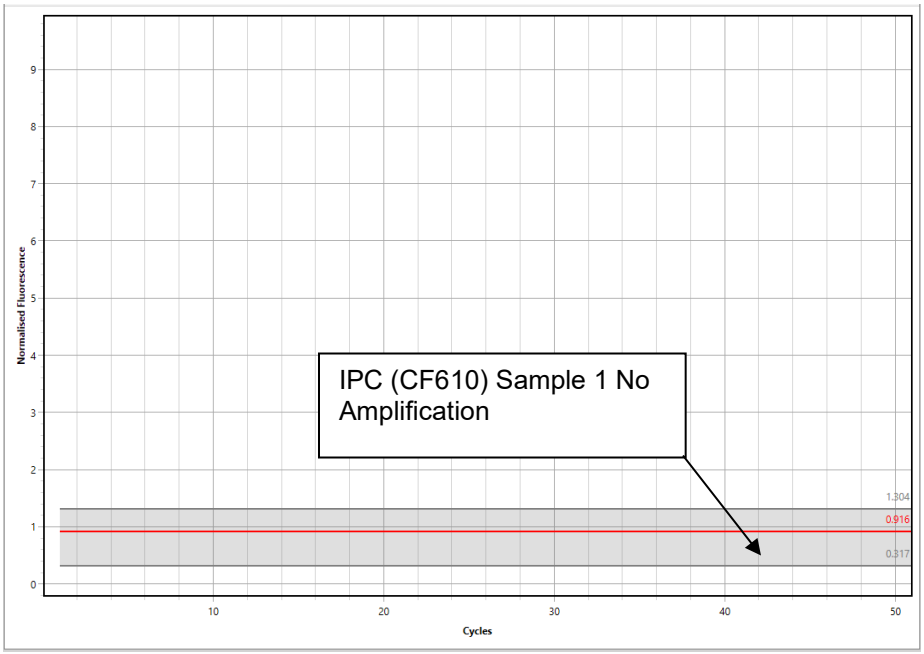


Figure 11

Negative Due to Inadequate Nuclear Material (IPC CF610)



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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) may not contain the human RNaseP gene. In such case, the two negative markers indicate a true negative result for Zika virus.

The interpretation of results with Ct values can be translated to Table 4.

Table 4

Interpretation of Results with Ct Values

<table>
<tr><th></th><th>Patient Sample</th><th>Positive Control (IPC)

Must always amplify with Ct value between 21.0 to 27.0. If IPC does not amplify, see troubleshooting</th><th>Negative Control (NTC)

Must always not amplify. If any amplification with NTC, see troubleshooting</th><th>Final Result</th></tr>
<tr><td rowspan="2">Serum</td><td>Zero or no Amplification</td><td rowspan="4"></td><td rowspan="4"></td><td>Negative (-)</td></tr>
<tr><td>Positive or Amplification</td><td>Positive (+)</td></tr>
<tr><td rowspan="2">Urine</td><td>Zero or no Amplification</td><td>Negative (-)</td></tr>
<tr><td>Positive or Amplification</td><td>Positive (+)</td></tr>
</table>

Negative result for Zika in Serum or Urine does not exclude the possibility of having the disease but it may happen to be the beginning of the infection where the virus is showing up only in Serum, but possible the viral load is still low and harder to detect. Or it may be late in the infection, after 10 days where the virus does not show up in blood any longer but can be detected in urine. Or it may be late in the infection, after 20 days, and the viral load is reduced because the immune system has been combating Zika efficiently. In this case only a serological exam will be able to detect Zika specific antibodies.

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 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.

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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 attest about the performance of the Master Mix, as well as validates 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 were 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

It 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 it is more likely that the same error could have happened to 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

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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 spiked with reference material from Zika Virus different strains. See Table 5.

Table 5

Diagnostics Accuracy Statistics from Contrived Samples

Statistic	Value	95% Confidence Interval
Sensitivity	98.84%	98.89% to 99.86%
Specificity	100.0%	97.83% to 100.0%
Positive Likelihood Ratio	-	-
Negative Likelihood Ratio	0.01	0.00 to 0.05
Positive Predictive Value	100%	-
Negative Predictive Value	98.82%	95.49% to 99.70%
Accuracy	99.41%	97.90% to 99.93%

*Data obtained from a pool of 341 analytical contrived samples

Analytical Evaluation found overall precision on 97.83% with less than 5% coefficient of variance in all analysis. Analytical Sensitivity was performed to determine the Limit of Detection which is the concentration with detection rate equal or better than 95%. See Table 6.

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Table 6

*Analytical Sensitivity (LoD) for Logix Smart™ ZIKV test kit (ZIKV-K-004)**

Specimen	Strain	Limit of Detection
Serum	Zika	**35 copies/µL**
Urine	Zika African Lineage (MR766)	**30 copies/µL**
	Zika Asian Lineage (PRVABC59)	**130 copies/µL**

*Data obtained from a pool of 341 analytical contrived samples

Analytical specificity was performed with wet-test and in silico analysis with microorganism of interest that could cross-reactive or interfere with the kit performance. Specificity also tested the performance of **Logix Smart™ Zika Virus** test with common interfering substances.

Logix Smart™ Zika Virus test showed 100% specificity 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.

Wet test has been performed with the following: West-Nile, Dengue (Type 1, 2, 3 and 4), Chikungunya, Influenza A H1, Influenza A H1N1, Influenza A H5, Influenza B, St. Louis Encephalitis, Measles, Epstein-Barr Virus, Borrelia Burgdorferi, Varicella Zoster Virus, Eastern Equine Encephalitis, Tick-Borne Encephalitis (TBEV).

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

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16 LEGEND OF PACKAGE SYMBOLS

See Table 7 for a legend of package symbols

Table 7

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
Σ 100	Contains sufficient for 100 tests/ reactions
	Protect from light
	Temperature limit
	Consult Instructions for Use
	Manufacturer
EC REP	Authorized representative in the European Community
IVD CE	CE-Marking for IVD in compliance to EU Directive 98/79/EC

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