Instructions for Use

LyteStar™
Arbovirus ZCD RT-PCR Kit 1.0

LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0

For detection of Dengue, Chikungunya and Zika from human serum specimens

For use with

CFX96™ (BioRad)
CFX Opus 96 (BioRad)
Magnetic Induction Cycler (Mic; Bio Molecular Systems)
*ab*CyclerQ (AITbiotech)

IVD For *in vitro* diagnostic use

REF Product No.: 881103

96 reactions

Please refer to Storage and Shelf Life in this IFU

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Content

1. Intended Use

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is intended for the simultaneous and specific detection of four serotypes of Dengue (DENV 1-4), Chikungunya and Zika virus RNA in human serum specimens. The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is a three-target assay targeting the three-prime untranslated region (3'UTR) for Dengue virus (DENV), the non-structural protein 2 (*NSP2*) gene for Chikungunya virus (CHIKV) and the envelope (*E*) gene for Zika virus (ZIKV).

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is for professional use only.

2. Kit Components

Catalog no.	881103
Master A	2 x 360 µl
Master B	4 x 300 µl
Internal Control (IC)	800 µl
Positive Control (PC)	400 µl
PCR grade water	500 µl

3. Storage and Shelf Life

- The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 has a shelf life of 12 months from the manufacturing date.
- Store all reagents at -15°C to -25°C upon arrival.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay. Master B should be frozen in aliquots, if they are to be used intermittently.
- Mix Master A thoroughly by vortex mixing, and centrifuge briefly before use.
- Protect Master B from light.
- All frozen reagents should be completely thawed to room temperature before use. Immediately return unused portions to the freezer for storage.

4. Quality Control

In compliance with AstronDX Technologies' EN ISO 13485 certified Quality Management System, each lot of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is tested against pre-determined specifications to ensure consistent product quality.

5. Product Use Limitations and Precautions

- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR procedures.
- Specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Avoid microbial and nuclease (DNAse/RNAse) contamination of the specimen and the components of the kit.
- Always use DNAse/RNAse-free disposable pipette tips with aerosol barriers.
- Always wear protective disposable powder-free gloves when handling kit components.
- Use separated and segregated working areas for (i) specimen preparation, (ii) reaction set-up and (iii) amplification/detection activities.
- Workflow in the laboratory should proceed in unidirectional manner.
- Always wear disposable gloves in each area and change them before entering different areas.
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.
- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not use components of the kit that have passed their expiration date.

- Discard sample and assay waste according to your local safety regulations.
- Wash hands thoroughly after handling specimens and test reagents.
- Do not use kits from different lots together.
- Do not use an expired kit.
- In case of damage to the packaging and leaking vials, do not use the kit (possible contamination or deterioration that can cause false interpretation).
- Despite thorough selection of the oligonucleotides (primers and probes) to specifically target highly conserved regions of the Chikungunya virus (CHIKV) RNA, cross-reactivity with RNA of some genotypes of the closely related O'nyong nyong virus (ONNV) may still occur.

6. Product Warranty

AstronDX Technologies guarantees the performance of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 for applications as described in the manual. The user must determine the suitability of the product for the particular intended use. Should the product fail to perform satisfactorily in the described applications, please contact AstronDX Technologies Technical Support (**16. Technical Support**) for trouble-shooting.

AstronDX Technologies reserves the right to change, alter, or modify any product to enhance its performance and design.

7. Product Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles/face masks. For more information, please consult the appropriate material safety data sheets (MSDSs).

8. Introduction

Arboviruses are a group of viruses primarily transmitted to humans and other vertebrates via arthropod vectors, particularly mosquitoes and ticks. Dengue (DENV), Chikungunya (CHIKV) and Zika (ZIKV) virus are all arboviruses transmitted by the mosquito vectors *Aedes aegypti* and *Aedes albopictus*. Arboviruses can be organized into four groups; *Flaviviridae, Togaviridae, Bunyaviridae,* and *Reoviridae*. Dengue and Zika virus are closely related and belong to the genus *Flavivirus* in the family *Flaviviridae* while Chikungunya virus belongs to the genus *Alphavirus* in the family *Togaviridae* [1].

DENV was initially discovered in 1943 during a yellow fever outbreak, followed by a series of epidemics that have occurred in many countries, particularly in tropical and subtropical regions. As of March 2023, DENV is responsible for over 380,000 cases including 113 deaths, reported globally [2]. In 2019, several countries in Southeast Asia, including Indonesia, Myanmar, and Thailand, reported a significant increase in DENV cases, leading to outbreaks [3]. Based on the Department of Health in Malaysia, the cumulative number of Dengue cases reported to date in 2023 is 36,997 cases, in comparison to the 12,941 cases reported in the same period in 2022, with a total of 22 deaths [3]. Individuals infected with DENV may experience no signs/asymptomatic or may have severe symptoms including high fever, severe headache, severe eye pain, joint pain, muscle and/or bone pain, rash, and mild bleeding manifestation [4]. DENV has four serotypes (1-4), and infection with one serotype provides lifelong immunity against that serotype, however the individual is still at risk of secondary infection with other serotypes [4].

Zika virus (ZIKV) was first isolated in 1947 in a rhesus monkey from the Zika Forest of Uganda, and caused the first reported human case in Nigeria five years later. It was not considered a significant public health concern until a major outbreak affected approximately 73% of the Micronesia population in 2007 [5]. The outbreak raised concerns about the potential for the ZIKV to cause severe neurological complications, including Guillain-Barré syndrome, neuropathy, and myelitis. As of December 2021, a total of 89 countries have documented evidence of ZIKV as an autochthonous mosquito-borne virus [6]. ZIKV has two major lineages: African and Asian. African lineage viruses have been isolated sporadically since 1947, while Asian lineage viruses have been isolated in Malaysia (1951) and the Pacific Islands (2007). The 2015-2016 epidemic in the Americas was caused by a strain of the Asian lineage, known as the American strain [6].

In 1952, the first Chikungunya virus (CHIKV) outbreak occurred in Tanzania, East Africa, and established the first recognition of CHIKV. Epidemics then spread to regions with established populations of the *Aedes aegypti* and *Aedes albopictus* mosquitos, such as Thailand, Malaysia, Singapore, Italy, France, and the Caribbean islands, with a common diagnosis of sudden onset of fever, skin rashes, severe joint pain followed by persistent rheumatic symptoms [7]. As of March 2023, CHIKV infection has been reported in almost 115,000 cases, globally, [8]. CHIKV has three major genotypes, namely Asian, West African and East Central South Africa.

Individual cases of arbovirus infections may be misdiagnosed due to similarity of symptoms. Therefore, a highly sensitive and specific differential test is vital to precisely identify the cause of the infection at an early stage. The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 was developed as a three-target assay, targeting the 3' untranslated region (3'UTR) for Dengue virus (DENV), the non-structural protein

2 (*NSP2*) gene for Chikungunya virus (CHIKV), and the envelope (*E*) gene for Zika virus (ZIKV).

[1] Madewell Z. J. (2020). Arboviruses and Their Vectors. Southern medical journal, 113(10), 520–523. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8055094/pdf/smj-113-520.pdf

[2] Dengue worldwide overview (2019). European Centre for Disease Prevention and Control. Available at: https://www.ecdc.europa.eu/en/dengue-monthly (Last cited on 06 June 2023)

[3] World Health Organization (WHO) Western Pacific Region (2017). Dengue Situation Update 671. Available at: https://cdn.who.int/media/docs/default-source/wpro---documents/emergency/surveillance/dengue/dengue_20230706.pdf?sfvrsn=b4a28654_63

[4] European Centre for Disease Prevention and Control. Factsheet about Dengue. Available at: https://www.ecdc.europa.eu/en/dengue-fever/facts

[5] World Health Organization (WHO) (2016). The history of Zika Virus. Available at:https://www.who.int/news-room/feature-stories/detail/the-history-of-zika-virus. (Last cited on 07 June 2023)

[6] World Health Organization (WHO) (2022). Zika Epidemiology Update February 2022. Available at: https://www.who.int/publications/m/item/zika-epidemiology-update---february-2022

[7] World Health Organization (WHO) (2022). Chikungunya Fact Sheet. Available at: https://www.who.int/news-room/fact-sheets/detail/chikungunya. (Last cited on 07 June 2023)

[8] European Centre for Disease Prevention and Control. Chikungunya Worldwide View. Available at: https://www.ecdc.europa.eu/en/chikungunya-monthly (Last cited on 06 June 2023

9. Product Description

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is an *in vitro* diagnostic test system, based on real-time PCR technology, for the qualitative detection of Dengue virus (DENV), Chikungunya virus (CHIKV) and Zika virus (ZIKV) specific RNA. The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 consists of a single tube targeting three genes; the 3'UTR specific for DENV, *NSP2* gene specific for CHIKV and *E* gene

specific for ZIKV. The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit. The Internal Control template used in the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is an *in vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

Real-time RT-PCR technology utilizes reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA), polymerase chain reaction (PCR) for the amplification of specific target sequences, and target-specific probes for the detection of the amplified DNA. The probes are labelled with a fluorescent reporter and quencher dyes. The probes used for specific amplification of the 3'UTR of DENV RNA, *NSP2* gene of CHIKV RNA, and *E* gene of ZIKV RNA are labelled with the fluorophore FAM, Tex615 and Cy5™, respectively. The probe specific to the target of the Internal Control (IC) is labelled with the fluorophore HEX. Using probes linked to distinguishable dyes enables the parallel detection of DENV, CHIKV, ZIKV specific RNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument.

The oligonucleotides included in the three assays were designed, modified, and based on the sequences/targets published in the articles listed below:

Target	**Publication**
DENV	Mansuy *et al*. (2018)
CHIKV	Wagoner *et al*., (2016)
ZIKV	Broeders *et al*., (2020)
Internal Control	Deer *et al*., (2010)

The test consists of three processes in a single tube assay:

- Reverse transciption of target and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- The template of the Internal Control (IC)

- The template of the Positive Control (PC)
- PCR grade water (for setting up of “No Template Control”, NTC)

Master A and Master B reagents contain all components (buffer, enzymes, primers and probes) to allow PCR mediated reverse transcription, amplification and target detection of the 3’UTR of DENV specific RNA, *NSP2* gene of CHIKV specific RNA, *E* gene of ZIKV specific RNA, and Internal Control in one reaction setup.

The Positive Control (PC) contains *in vitro* transcripts of synthesized target genes of DENV, CHIKV and ZIKV.

The Internal Control used in the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is an *in vitro* transcribed RNA of an artificial sequence with no homology to any known genomes.

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- CFX96™ (BioRad)
- CFX Opus 96 (BioRad)
- Magnetic Induction Cycler (Mic: Bio Molecular Systems)
- *ab*CyclerQ (AITbiotech)

10. Material and Devices required but Not Provided

- Appropriate real-time PCR instrument
- Appropriate nucleic acid extraction system or kit
- 1.5 ml microcentrifuge tubes (with safe-lock or screw cap)
- Microcentrifuge (with speed ≥ 13,000 rpm)
- Pipettes, adjustable (range: 10 µl, 100 µl, 200 µl, 1000 µl)
- Pipette tips (with aerosol barriers)
- Disposable gloves (powder-free)
- Heating block for lysis of specimens during extraction
- Vortex mixer
- Appropriate 96-well reaction plates or reaction tubes with corresponding (optical) closing material

11. Specimen Storage

- Suitable specimens include whole blood, serum, plasma, urine, cerebrospinal fluid, semen, and saliva.

- Follow specimen transport and storage conditions outlined in the following guidelines:

  - World Health Organization (2000). Guidelines for the collection of clinical specimens during field investigation of outbreaks. https://apps.who.int/iris/handle/10665/66348

  - Pan American Health Organization (2023). Recommendations for laboratory detection and diagnosis of arbovirus infections in the Region of the Americas. https://iris.paho.org/handle/10665.2/57555

  - World Health Organization (2011). Comprehensive guidelines for prevention and control of dengue and dengue hemorrhagic fever. Revised and expanded edition. https://apps.who.int/iris/handle/10665/66348

  - World Health Organization (2022). Interim Guidance on Laboratory Testing for Zika Virus and Dengue Virus Infections. https://www.who.int/publications/i/item/WHO-ZIKV_DENV-LAB-2022.1

  - Centers for Disease Control and Prevention (2023). Chikungunya Virus Clinical Evaluation & Disease - Diagnostic Testing. https:///www.cdc.gov/chikungunya/hc/clinicalevaluation.html

12. Instructions for Use

12.1. Sample Preparation

Extracted RNA is the starting material for the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0. The quality of the extracted RNA has a profound impact on the performance of the whole test system. It has to be ensured that the nucleic acid extraction system used is compatible with real-time PCR technology.

The following nucleic acid extraction kits / systems are suitable for use with the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0:

- SpinStar™ Viral Nucleic Acid Kit (AstronDX Technologies)
- MagCore® Plus II Automated Nucleic Acid Extractor (RBC Bioscience)
- QIAamp® MiniElute Virus Spin Kit (Qiagen)
- QIAamp® Viral RNA Mini Kit (Qiagen)
- QIAsymphony® (Qiagen)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 has to be validated by the user.

If using a spin column-based sample preparation procedure including washing buffers containing ethanol, an additional centrifugation step for 10 min at approximately 17000 × g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid is highly recommended.

NOTE

Ethanol is a strong inhibitor in real-time PCR. If your sample preparation system is using washing buffers containing ethanol, you need to make sure to eliminate any traces of ethanol prior to elution of the nucleic acid.

12.2. Master Mix Setup

1. All reagents and samples should be thawed completely, mixed (by gentle vortex mixing) and centrifuged briefly before use. Prepare a marginal excess (additional 0.5 reaction) of the required Master Mix volume.

2. The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as (i) a PCR inhibition control or as (ii) a control of the sample preparation procedure (nucleic acid extraction) and PCR inhibition control.

   (i) If the IC is used as a PCR inhibition control, but not as a control for the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	7.5 µl	90 µl
Master B	12.5 µl	150 µl
IC	1.0 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

(ii) If the IC is used as a control for the sample preparation procedure and as a PCR inhibition control, the IC has to be added during the nucleic acid extraction procedure.

No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture.

The volume of the IC which has to be added depends always and only on the elution volume. It represents **10% of the elution volume**. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC must be added into the specimen/lysis buffer mixture.

If the IC was added during the sample preparation procedure, the Master Mix is set up according to the following pipetting scheme:

Number of Reactions	1	12
Master A	7.5 µl	90 µl
Master B	12.5 µl	150 µl
Volume Master Mix	**20 µl**	**240 µl**

NOTE

⚠ ***Never add the Internal Control directly to the specimen.***

12.3. Reaction Setup

1. Pipette 20 µl Master Mix into each required well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.

2. Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive Control; or water as No Template Control, NTC).

3. Make sure at least one Positive Control and one NTC are used per run.

4. Thoroughly mix the samples and controls with the Master Mix by pipetting up and down.

5. Close the 96-well reaction plate with an appropriate optical adhesive film and the reaction tubes with appropriate caps.

6. Centrifuge the 96-well reaction plate at 1,000 x g (~3,000 rpm) for 30s.

Reaction Setup	
Master Mix	20 µl
Sample or Control	10 µl
Total Volume	**30 µl**

13. Programming the Real-Time PCR Instrument

13.1 Settings

• Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default

13.2 Fluorescent Detectors (Dyes)

• Define the following fluorescent detectors:

Detection	Detector Name	Reporter	Quencher
DENV (3'UTR) specific RNA	DENV	FAM	IBFQ
CHIKV (*NSP2* gene) specific RNA	CHIKV	Tex615*	IBRQ
ZIKV (*E* gene) specific RNA	ZIKV	Cy5	IBRQ
Internal Control	IC	HEX	IBFQ

*When using *ab*CyclerQ, select TexasRed for CHIKV

13.3 Temperature Profile and Dye Acquisition

• Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature	Time
Reverse-transcription	Hold	1	-	50 °C	10:00 min
Denaturation	Hold	1	-	95 °C	2:00 min
Amplification	Cycling	45	-	95 °C	5 sec
			√	60 °C	30 sec

√ Signal acquisition: activate FAM, Tex615, Cy5 and HEX channels in all runs.

14. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the manual of the respective instrument. For detailed instructions regarding data analysis of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (**16.Technical Support**).

14.1. Validity of Diagnostic Test Runs

14.1.1 Valid Diagnostic Test Runs (Qualitative)

For a **valid** diagnostic test run (qualitative), the following control conditions must be met:

Control ID	FAM / Tex615 / Cy5 Detection Channel	HEX Detection Channel
Positive Control	POSITIVE	POSITIVE
Negative Control	NEGATIVE	POSITIVE

14.1.2 Target CT values of PC and IC

	Positive Control *(DENV)*	Positive Control *(CHIKV)*	Positive Control *(ZIKV)*	Internal Control
Target CT value	< 35 cycles	< 35 cycles	< 35 cycles	≤ 40 cycles*

*Required for unknown samples that do not amplify in FAM, Tex615, and Cy5 channels

Note: The above CT target values are exclusively given for **monitoring the integrity of the product and validated assay conditions** and should be achieved **ONLY for the provided Positive Control (PC) and Internal Control (IC)** when used as per the instructions given under section 12.3. Reaction set up. **The target CT values for PC MUST NOT be misinterpreted as the diagnostic cut-off values for clinical samples.**

14.1.3 Invalid Diagnostic Test Runs (Qualitative)

A **qualitative** diagnostic test run is **invalid**, (i) if the run has not been completed or (ii) if any of the control conditions for a **valid** diagnostic test run are not met.

In case of an **invalid** diagnostic test run, **repeat testing by using the remaining purified nucleic acids** or start from the original samples again.

14.2 Interpretation of Results

FAM *DENV*	Tex615 *CHIKV*	Cy5 *ZIKV*	HEX *Internal Control*	Result Interpretation
+	+	+	+*	Dengue, Chikungunya and Zika specific RNA detected. *Positive for Dengue, Chikungunya and Zika*
-	+	+	+*	Chikungunya and Zika specific RNA detected. *Positive for Chikungunya and Zika*
+	-	+	+*	Dengue and Zika specific RNA detected. *Positive for Dengue and Zika*
+	+	-	+*	Dengue and Chikungunya specific RNA detected. *Positive for Dengue and Chikungunya*
+	-	-	+*	Dengue specific RNA detected. *Positive for Dengue*
-	+	-	+*	Chikungunya specific RNA detected. *Positive for Chikungunya*
-	-	+	+*	Zika specific RNA detected. *Positive for Zika*
-	-	-	+	Dengue, Chikungunya and Zika specific RNA not detected. The samples do not contain detectable amounts of Dengue, Chikungunya and Zika specific RNA.
-	-	-	-	PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

Note: For Dengue 3'UTR (FAM channel), Chikungunya *NSP2* gene (Tex615 channel) and Zika *E* gene (Cy5 channel) "+" refers to amplification curve detected at CT ≤ 45 cycles. "-" refers to no amplification / no CT obtained.

* Detection of the Internal Control in the HEX channel is not required for positive results in the FAM/Tex615/Cy5 detection channels. A high Dengue, Chikungunya and/or Zika viral load in the sample can lead to reduced or absent Internal Control signals.

14.2.1 Threshold Settings for Cycler Software

Cycler	Threshold			
	FAM DENV Channel	Tex615 CHIKV Channel	Cy5 ZIKV Channel	HEX IC Channel
CFX96™	100 RFU	100 RFU	100 RFU	100 RFU
CFX Opus 96	100 RFU	100 RFU	100 RFU	100 RFU
Mic qPCR	Auto	Auto	Auto	Auto
*ab*CyclerQ	Auto	Auto	Auto	Auto

14.2.2 CT Cut-Off Values for Clinical Samples

	FAM DENV Channel	Tex615 CHIKV Channel	Cy5 ZIKV Channel
CT Cut-Off Value	< 45 cycles	< 45 cycles	< 45 cycles

15. Performance Evaluation

The analytical performance evaluation of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 was accomplished using quantified DENV, CHIKV and ZIKV specific RNA.

15.1 Analytical Sensitivity

The analytical sensitivity (limit of detection: LoD) of LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is defined as the concentration of DENV, CHIKV and/or ZIKV RNA molecules that can be detected with a positivity rate of ≥ 95%. The analytical sensitivity was determined in consideration of a selected nucleic acid extraction method, by analyzing samples with known concentration.

A dilution series of the AmpliRun® ZIKV/DENV/CHIKV Control (Plasma) was prepared by spiking into human serum extracted with the MagCore® Viral Nucleic Acid Extraction Kit (High Sensitivity). Extracted DENV, CHIKV, and ZIKV RNA was analyzed with LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0. Results were analyzed by Probit analysis (Table 1, Table 2 and Table 3).

Nucleic Acid Extraction Procedure:

MagCore® Viral Nucleic Acid Extraction Kit (High Sensitivity) (RBC Bioscience)
Sample volume: 400 µl
Elution volume: 60 µl

The analytical sensitivity of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 in consideration of MagCore® nucleic acid extraction method was determined at 3.04 copies/µl for Dengue 3'UTR target, 0.78 copies/µl for Chikungunya *NSP2* gene target, and 3.57 copies/µl for Zika *E* gene target (p≤ 0.05).

Table 1. PCR results used for the calculation of the analytical sensitivity of Dengue 3'UTR target for the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
23.8	12	12	100
7.53	12	12	100
3.77	12	11	91.7
2.38	12	12	100
0.75	12	3	25.0
0.24	12	2	16.7
0.075	12	0	0
0.024	12	0	0
0.0076	12	0	0

Table 2. PCR results used for the calculation of the analytical sensitivity of Chikungunya *NSP2* gene target for the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
17.6	12	12	100
5.57	12	12	100
2.79	12	12	100
1.76	12	12	100
0.56	12	12	100
0.176	12	5	41.7
0.056	12	3	25.0
0.017	12	1	8.3
0.0056	12	0	0

Table 3. PCR results used for the calculation of the analytical sensitivity of Zika *E* gene target for the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 in consideration of a particular extraction method and in combination with the CFX Opus 96 platform (BioRad).

Concentration (copies/µl)	No. of Samples Tested	No. of Samples Positive	Hit rate (%)
16.8	12	12	100
5.32	12	12	100
2.66	12	12	100
1.68	12	10	83.3
0.53	12	3	25.0
0.17	12	3	25.0
0.053	12	1	8.3
0.017	12	0	0
0.0053	12	0	0

15.2 Analytical Specificity

The analytical specificity of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 is ensured by the thorough selection of the oligonucleotides (primers and probes). The oligonucleotides were checked by sequence comparison analysis against publicly available sequences to ensure that the applied primer/probes in LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 specifically detect Dengue, Chikungunya and Zika virus.

The specificity of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 was evaluated by testing genomic RNA/DNA extracted from other pathogens likely to be present in the same sample material as Dengue, Chikungunya and Zika virus, or that cause similar symptoms to these viruses (Table 4).

Table 4. Microorganisms tested to demonstrate the analytical specificity of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0.

LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0				
Organisms	***DENV (FAM channel)***	***CHIKV (Tex615 channel)***	***ZIKV (Cy5 channel)***	***Internal Control (HEX channel)***
Borrelia burgdorferi	negative	negative	negative	valid
Rickettsia monacensis	negative	negative	negative	valid
Epstein-Barr Virus (EBV)	negative	negative	negative	valid
Parvovirus B19 (PAB19)	negative	negative	negative	valid
Yellow Fever Virus (YFEV)	negative	negative	negative	valid
Japanese Encephalitis Virus (JEV)	negative	negative	negative	valid
West Nile Virus (WNV)	negative	negative	negative	valid
Ross River Virus	negative	negative	negative	valid
Dengue 1	positive	negative	negative	valid
Dengue 2	positive	negative	negative	valid
Dengue 3	positive	negative	negative	valid
Dengue 4	positive	negative	negative	valid
Chikungunya Virus (CHIKV)	negative	positive	negative	valid
Zika Virus (ZIKV) (Asia lineage)	negative	negative	positive	valid
Zika Virus (ZIKV) (Africa lineage)	negative	negative	positive	valid

The LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 did not cross-react with any pathogen or genotype/subtype other than its own target.

15.3 Diagnostic Sensitivity and Specificity

The clinical performance of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0, in regards to diagnostic sensitivity and specificity, was evaluated through retrospective studies performed at various public health laboratories. The diagnostic sensitivity and specificity of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 was compared to the clinical site's reference method.

In comparison to the reference tests, the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 achieved a diagnostic sensitivity of 98.3% for the detection of the Dengue virus and 100% for the detection of the Chikungunya virus, and a diagnostic specifiticity of 100% for the detection of both Dengue and Chikungunya virus.

As of September 2018, Malaysia has only recorded eight Zika cases, all of which occurred in 2016 [1]. At least 3 of these cases were epidemiologically connected to the outbreak in Singapore, which was considered to be the first minor epidemic of ZIKV, and the outbreak reported at least 455 cases within 3 months [2]. Due to the low prevalence of Zika cases in Malaysia, and the lack of positive clinical specimens in the country, evaluation of the clinical performance of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0, for the detection of Zika, by means of patient samples alone is not possible at this time. Given the lack of clinical samples, proficiency studies to validate the performance of the LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0 in accurately detecting Zika virus samples has been performed, to the extent possible.

[1] Ministry of Health (2019). “Updated Zika Alert” Dan Arahan Pentadbiran Untuk Pemantauan Dan Pengurusan Jangkitan Virus Zika Semakan Tahun 2019. https://www.moh.gov.my/index.php.database_stores/store_view_page/31/347

[2] Woon, Y.L., Lim, M. F. Tg Abd Rashid, T.R., Thayan, R., Chidambaram, S.K., Syed Abdul Rahim, S. S., Mudin, R. N., Sivasampu, S. (2019). Zika virus infection in Malaysia: an epodemiological, clinical, and virological analysis. BMC Infectious Diseases, 19(1). https://doi.org/10.1186/s12879-019-3786-9

		Reference Method			
		DENV (n=58)	CHIKV (n=30)	DENV (n=40)	CHIKV (n=30)
		Positive		Negative	
LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0	Detected	57	30	0	0
	Not Detected	1	0	40	30
	Total	**58**	**30**	**40**	**30**
Overall Concordance		**98.3 %** 57 / 58 Sensitivity	**100 %** 30 / 30 Sensitivity	**100 %** 40 / 40 Specificity	**100 %** 30 / 30 Specificity

To calculate the diagnostic sensitivity of LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0:

$$\text{Diagnostic Sensitivity [\%]} = \frac{\text{“correct positive”}}{\text{(“correct positive” + “false positive”)}} \times 100$$

To calculate the diagnostic specificity of LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0:

$$\text{Diagnostic Specificity [\%]} = \frac{\text{“correct negative”}}{\text{(“correct negative” + “false negative”)}} \times 100$$

16. Technical Support

For customer support, please contact our Technical Support:

e-mail: **techsupport@astrondx.com**
phone: **+603 7931 6760**

17. Appendix

Explanation of Symbols

Symbol	Explanation
	*In vitro* diagnostic medical device
	Product Number
	Batch Code
	Manufacturer
	Date of Manufacture
	Contains sufficient for “n” tests/rxns
	Temperature limitation
	Version
	Use-By Date

18. Ordering Information

Products	Packing (reactions)	Order No.
LyteStar™ Arbovirus ZCD RT-PCR Kit 1.0	96	881103
SpinStar™ Viral Nucleic Acid Extraction Kit 1.0	100	811803
MagCore® Viral Nucleic Acid Extraction Kit, High Sensitivity (200µl/400µl), CART CODE 203	96	MVN400-06

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