TaqPath™ Zika Virus Kit (ZIKV)
Instructions for Use
Thermo Fisher Scientific

For in vitro Diagnostic Use
For Prescription Use Only
For Use Under an Emergency Use Authorization (EUA) Only

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Contents

1. Intended Use

The TaqPath™ Zika Virus Kit (ZIKV) is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum and urine (collected alongside a patient-matched serum specimen) from individuals meeting Centers for Disease Control and Prevention (CDC) Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated), by laboratories in the United States that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of Zika virus RNA, which is generally detectable in serum and/or urine during the acute phase of infection and up to 14 days following onset of symptoms, if present. Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude Zika virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

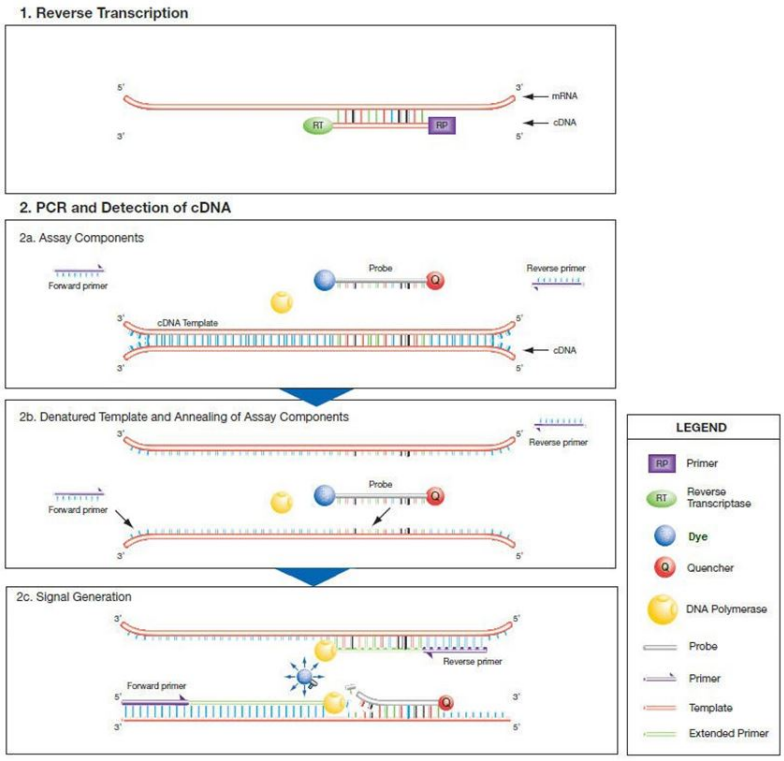
The TaqPath™ Zika Virus Kit (ZIKV) is intended for use by trained clinical laboratory personnel who have received specific training on the use of the TaqPath™ Zika Virus Kit (ZIKV) on the Applied Biosystems® QuantStudio Dx Real-time PCR instrument and software system. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Test Principle

The TaqPath™ Zika Virus Kit (ZIKV) is a one-step real-time reverse transcription polymerase chain reaction assay for the qualitative detection of RNA from the Zika virus. The assay is in a lyophilized format and includes all reagents needed for RT-PCR in a single tube. The TaqPath™ Zika Virus Kit (ZIKV) includes a primer and probe set designed to detect RNA from the Zika virus in serum and urine specimens. The assay also includes a primer and probe set designed to detect Peptidylprolyl Isomerase A (PPIA) RNA. PPIA RNA is present in human matrices (serum or urine) and serves as an extraction, reverse transcription and PCR amplification positive control.

RNA is isolated from serum and urine samples using the KingFisher™ Flex Purification System (KingFisher) and MagMAX™ Pathogen RNA/DNA Kit. During the one-step RT-PCR reaction, the RNA is converted to cDNA and then subsequently amplified by the Applied Biosystems® QuantStudio Dx Real-time PCR instrument and software system. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the QuantStudio Dx Real-time PCR instrument.

Figure 1. Reverse Transcription and PCR



3. Product Description

The TaqPath™ Zika Virus Kit (ZIKV) is a lyophilized real-time reverse transcription polymerase chain reaction assay for qualitative detection of RNA from the Zika virus in serum or urine (collected alongside a patient-matched serum specimen). Each assay consists of two primers and one dye labeled probe. RNA is purified from the MagMAX™ Pathogen RNA/DNA Kit.

4. Kit Components

The TaqPath™ Zika Virus Kit (ZIKV) is packaged in a single pouch which contains twelve (12) lyophilized strip tubes. Each strip is comprised of eight (8) assay tubes which contain lyophilized one-step RT- PCR reagents: primers, probes, reverse transcription and amplification reagents, reverse transcriptase and PPIA endogenous control. The kit also contains twelve (12) flat cap strips for sealing the assay tubes following sample addition, and a desiccant pouch to adsorb moisture.

5. Shipment and Storage

The TaqPath™ Zika Virus Kit (ZIKV) is shipped in a light blocking pouch at ambient temperature. The pouch can be stored at room temperature upon receipt. No cold-chain management is required. Store unused strip tubes in desiccated conditions with no light exposure at room temperature.

6. Materials Required but Not Provided

- Real-Time PCR Instrument:
  - QuantStudio™ Dx Real-Time PCR Instrument with 96-well fast block (Thermo Fisher Scientific; Catalog#: 4480299)

- ZIKV Positive Control:
  - Zika Virus Culture Fluid (ZeptoMetrics Corporation; Catalog#: 0810525CF)
    or
  - ZIKV Inactivated Virus Suspension, Puerto Rico 2015 isolate (Vircell S.L.; Catalog#: MC018)

- Automated Nucleic Acid Extraction System and Materials:
  - KingFisher™ Flex Purification System, with 96 Deep-well Head (Thermo Fisher Scientific; Catalog#: 5400630)
  - MagMAX™ Express-96 Deep Well Plates (Thermo Fisher Scientific; Catalog#: 4388476)
  - MagMAX™ Express-96 Standard Plates (Thermo Fisher Scientific; Catalog#: 4388475)
  - MagMAX™ Express-96 Deep Well Tip Combs (Thermo Fisher Scientific; Catalog#: 4388487)

- Nucleic Acid Extraction Kit:
  - MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific; Catalog#: 4462359)

- General Laboratory Equipment:
  - Microcentrifuge, capable of 16,000 × *g* (Eppendorf, Part no. 5415D; or equivalent)
  - Microplate Shaker (Thermo Fisher Scientific, Catalog#: 88880023; or equivalent
  - Centrifuge with a rotor for microtiter plates
  - Vortex mixer
  - Single- and multichannel pipettes

- General Laboratory Materials and Consumables:
  - 100% ethanol, ACS reagent grade or equivalent
  - 100% isopropanol, ACS reagent grade or equivalent
  - Nuclease-free water
  - 1.5 mL microcentrifuge tubes
  - 2 mL microcentrifuge tubes
  - Pipette tips with filters

7. General Warnings and Precautions

- The assay is for in vitro diagnostic use under the FDA Emergency Use Authorization Only.
- Urine and serum specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipet tips with aerosol barriers. Tips used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with the local, state, and federal regulations.
- Safety Data Sheets are available upon request.

8. Warnings and Precautions for TaqPath™ Zika Virus Kit (ZIKV)

The TaqPath™ Zika Virus Kit (ZIKV) workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or biological safety cabinet. TaqPath™ Zika Virus Kit (ZIKV) components are lyophilized and contained in prefilled strip tubes. No cold-chain management is required. Store unused strip tubes in desiccated conditions at room temperature.

9. Samples and Controls

Patient serum and urine samples must be collected according to appropriate laboratory guidelines. RNA from patient samples is purified from the MagMAX™ Pathogen RNA/DNA Kit.

Positive and negative test controls must be examined prior to the interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Cutoff values for valid test results are as follows:

- A positive Zika virus control Ct value is ≤ 38.00
- PPIA endogenous control Ct value is ≤ 38.00
- Nuclease-Free Water (negative control) Ct value is > 38.00

10. Instructions for Use

a. RNA extraction: The workflow is initiated by adding serum or urine samples, magnetic bead mix, and lysis solution to a MagMAX™ Express-96 Deep Well Plate. The prepared plate is loaded onto the KingFisher™ Flex Purification System (with 96 Deep-Well Head), which is programmed to execute the following automated steps:
   (i) Sample lysis in a guanidinium thiocyanate-based solution.
   (ii) Attachment of nucleic acid on the surface of the magnetic beads.
   (iii) Capture of the magnetic bead/nucleic acid complexes by magnets.
   (iv) Wash steps to remove proteins, cell debris, and potential PCR inhibitors.
   (v) Elution of nucleic acid into an aqueous buffer.

b. RT-PCR: Eluted samples are added to the TaqPath™ Zika Virus Kit (ZIKV) strip containing lyophilized RT-PCR reagents for detection of ZIKV and PPIA target sequences. The assay strip is flicked or vortexed to mix, briefly centrifuged, then placed in the QuantStudio™ Dx Real-Time PCR Instrument.

Data analysis: Data exported from the QuantStudio™ Dx Real-Time PCR Instrument is evaluated for the presence of Zika virus in the sample.

10.1 Preparing Positive Zika Control & Extract RNA using the MagMAX™ Pathogen RNA/DNA Kit

- Prepare the ZIKV Positive Control. In the case of the ZIKV Inactivated Virus Suspension (Vircell S.L.; Catalog#: MC018) dilute to 1×10^6 copies/mL using the Carrier RNA as a diluent (Carrier RNA is included in the MagMAX™ Pathogen RNA/DNA Kit)
- Prior to initiating the procedure, label MagMAX™ Express-96 Deep Well Plates as “sample plate” (one plate), “wash 1” (two plates), and “wash 2” (two plates).
- Label MagMAX™ Express- 96 Standard Well Plates as “elution plate” (one plate) and “tip comb” (one plate).
- Mark the wells on all plates that will receive patient samples, and positive and negative control samples.

<u>Preparing Wash Solutions</u>

1. Prepare Wash Solution 1 by adding 125 mL of 100% isopropanol to the Wash Solution 1 Concentrate bottle.
2. Prepare Wash Solution 2 by adding 232mL 100% ethanol to the Wash Solution 2 Concentrate bottle.
3. Mix both wash solutions by inverting the bottles 3-4 times. Store both solutions at room temperature for up to 6 weeks.

Prepare Lysis/Binding Solutions

Prepare the required amount of Lysis/Binding Solution on each day of use.

4. Determine the volume (in mL) of Lysis/Binding Solution Concentrate, carrier RNA, and 100% isopropanol to mix, based on the total number of patient samples to be processed, plus one negative and one positive control sample. Representative volumes are shown in Table 1.
5. Combine the components then mix by vortexing. Store the Lysis/Binding Solution at room temperature for up to 8 hours.

Table 1. Lysis/Binding Solution Preparation

Number of Samples	Lysis/Binding Solution (mL)	Carrier RNA (mL)	100% Isopropanol (mL)
1	0.35	0.0023	0.35
2	0.7	0.0046	0.70
3	1.05	0.0069	1.05
4	1.4	0.0092	1.40
5	1.75	0.0115	1.75
6	2.1	0.0138	2.10
7	2.45	0.0161	2.45
8	2.8	0.0184	2.80
9	3.15	0.0207	3.15
10	3.5	0.0230	3.50

Prepare Bead Mix

6. Determine the volume of Nucleic Acid Binding Beads and Lysis Enhancer to mix based on the total number of samples. Representative volumes are in Table 2.
7. Mix the Nucleic Acid Binding Beads by vortexing.
8. On ice, combine the required volumes of Nucleic Acid Binding Beads and Lysis Enhancer, then mix by vortexing. Store the Bead Mix on ice for up to 4 hours.

Table 2. Bead Mix Preparation

Number of Samples	Nucleic Acid Binding Beads (µL)	Lysis
1	1	10
2	2	20
3	3	30
4	4	40
5	5	50
6	6	60
7	7	70
8	8	80
9	9	90
10	100	100

Prepare Processing Plates

9. Add 300 µL of Wash Solution 1 to each well in the two deep-well plates labeled as “wash1”.
10. Add 450 µL of Wash Solution 2 to each well in the two deep-well plates labeled as “wash 2”.
11. Add 50 µL of Elution Buffer to the standard-well plate labeled as “elution plate”.
12. To the deep-well plate labeled as “sample plate” add the following in the indicated order:
    1) 700 µL of Lysis/Binding Solution.
    2) 20 µL Bead Mix.
    3) 10 µL of the ZIKV positive control to the appropriate well (one replicate).
    4) 300 µL of nuclease-free water to the positive control wells and negative control wells. One replicate of negative control is required for each run.
    5) 300 µL of sample to the appropriate wells.
    6) Seal and cover the plate(s).

Process Samples on the KingFisher™ Flex Purification System

13. Power on the KingFisher Flex Purification System and select the. “MagMAX_Pathogen_High_Volume_1” protocol.
14. Start the “MagMAX_Pathogen_High_Volume_1” protocol.
15. Load the plates onto the processor as directed.
16. Store the elution plate on ice for immediate use in RT-PCR or seal the plate and store at -20°C for up to 1 month, or at -80°C for long-term storage.

10.2 TaqPath™ Zika Virus Kit (ZIKV) RT-PCR

1) If necessary, thaw the elution plate on ice.
2) Remove the cap of the 8-well strip. Do not reuse the cap on the strip.
3) Per RNA sample, add 25µL to the corresponding well of the assay strip. Apply a new cap to seal tubes.
4) Mix by flicking the strip several times or by vortexing. Briefly centrifuge the assay strip after mixing.
5) Place the assay strip onto the QuantStudio™ Dx Real-Time PCR Instrument. Run the assay using the following cycle parameters:

Table 3. PCR Cycling Parameters

Steps	Thermal Cycling Conditions	
Reverse Transcription	HOLD	50°C for 10 min
Activation	HOLD	95°C for 2 min
PCR Amplification	40 cycles	95°C for 3 sec
		60°C for 30 sec

Analyze the data

6) Analyze data using the automatic baseline and threshold settings in the QuantStudio™ Dx Real-Time PCR Instrument software.
7) Determine the Ct (cycle threshold) and standard deviation (if needed) for each assay. Review Ct data for the positive and negative control samples, and the PPIA control assay. Export run data as an Excel file.

10.3 Validity of Diagnostic Runs

For a diagnostic test run to be considered valid, the results for positive and negative controls listed in Table 4 must be observed:

Table 4. Required Diagnostic Run Control Results

Sample ID	Detection Channels	
	VIC™ (PPIA)	**FAM™ (ZIKV)**
Negative Control	NEGATIVE	NEGATIVE
Zika Positive Control	NEGATIVE	POSITIVE
*NEGATIVE defined as Ct >38.00 *POSITIVE defined as Ct ≤38.00		

In the case of an invalid diagnostic run, all associated patient samples are considered invalid. Fresh patient samples and controls must be tested.

10.4 Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. Table 5 correlates the TaqPath™ Zika Virus Kit (ZIKV) with the result interpretation.

Note: Positive results are indicative of current infection.

Table 5. Result Interpretation

TaqPath Result (+/-)	Zika Positive Control	PPIA Control	Negative Control (Water)	Interpretation of Results
+	+	+	-	Zika RNA is detected in sample
+	+	-	-	Zika RNA is detected in sample
-	+	+	-	**Zika RNA is not detected in sample
+	+	+	+	*Invalid- Repeat testing is required
+	-	+	+	*Invalid- Repeat testing is required
+	+	-	+	*Invalid- Repeat testing is required
+	-	-	+	*Invalid- Repeat testing is required
+	-	+	-	*Invalid- Repeat testing is required
+	-	-	-	*Invalid- Repeat testing is required
-	-	-	-	*Invalid- Repeat testing is required
-	-	+	-	*Invalid- Repeat testing is required
-	+	-	-	*Invalid- Repeat testing is required
-	-	-	+	*Invalid- Repeat testing is required
-	+	-	+	*Invalid- Repeat testing is required
-	-	+	+	*Invalid- Repeat testing is required
-	+	+	+	*Invalid- Repeat testing is required

Note: positive detection signal defined as Ct ≤38.00; negative detection signal defined as Ct >38.00.Detection of the PPIA control is not required for positive results in the FAM™ detection channel. For example, high viral detection signals can reduce or eliminate detection signals in other channels.

*Invalid results may be due to: operator error, instrument failure, PCR inhibitors, or reagent failure. If repeat testing is invalid, then all associated patient samples are considered invalid. Fresh patient samples and controls must be tested.

**A patient-matched serum specimen is currently required for serological follow up testing of negative RT-PCR results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

If the repeat testing is valid, continue to evaluate patient samples as stated in the table.

11. Assay Limitations

- The use of this assay as an *In vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- The TaqPath™ Zika Virus Kit (ZIKV) was established for human urine and serum samples only. Other specimen types have not been evaluated and should not be tested with this assay.

- Human urine and serum samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Specimen collection after nucleic acid can no longer be found in the specimen matrix
  - Using unauthorized extraction or assay reagents
  - The presence of RT-PCR inhibitors
  - Mutation in the Zika virus
  - Failure to follow instructions for use

- False-positive results may arise from:
  - Cross contamination during specimen handling or preparation
  - Cross contamination between patient samples
  - Specimen mix-up
  - RNA contamination during product handling

- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The TaqPath™ Zika Virus Kit (ZIKV) cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with Zika virus, and should not be the sole basis of a patient management decision.
- A patient matched serum specimen is required for serological follow up testing of all negative RT-PCR results, per the CDC testing algorithm. (Found at http://www.cdc.gov/zika/index.html).
- Laboratories are required to report all positive results to the appropriate public health authorities.

12. Conditions of Authorization for Laboratories

The TaqPath™ Zika Virus Kit (ZIKV) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm. However, to assist clinical laboratories running the TaqPath™ Zika Virus Kit (ZIKV), the relevant Conditions of Authorization are listed below.

- Authorized laboratories will include with reports of the results of the TaqPath™ Zika Virus Kit (ZIKV) the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) using the KingFisher™ Flex Purification System (KingFisher) and MagMAX™ Pathogen RNA/DNA Kit or with other authorized extraction methods.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) on the Applied Biosystems® QuantStudio Dx Real-time PCR instrument, or other authorized instruments.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) on human serum, or urine (collected with a patient-matched serum specimen), or other authorized specimen types.

- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will collect information on the performance of the test and report to DMD/OIR/CDRH (via email CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher any suspected occurrence of false positive or false negative results of which they become aware.

- All laboratory personnel using the test should be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

- Thermo Fisher, its authorized distributor(s), and authorized laboratories, will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

13. Performance Characteristics

Analytical performance of the TaqPath™ Zika Virus Kit (ZIKV) was evaluated by determining limit of detection (LOD), characterizing the impact of interfering substances and cross-reactivity, as described in the following sections:

Analytical Sensitivity- Limit of Detection

LOD was determined through three (3) studies:

i. LOD determination testing using heat inactivated Zika Virus (Strain: PRVABC59)
ii. LOD determination testing using live Zika Virus (Strain: PRVABC59)
iii. FDA Sensitivity Study

LOD- Heat Inactivated Zika Virus (Strain: PRVABC59)

Sensitivity of the TaqPath™ Zika Virus Kit (ZIKV) to heat inactivated Zika virus was assessed by determining the lowest number of Genomic Copies (GC) of heat inactivated virus that could be detected in ≥ 95% of samples tested. LOD testing consisted of 2 phases.

Phase 1: A broad range of titration from 100 GC/mL to 5000 GC/mL using a sample matrix (serum or urine) to identify the preliminary LOD.

Phase 2: A two part confirmation test:

i. Phase 2 test 1: Multiple heat inactivated viral titrations in a sample matrix to confirm the LOD from Phase 1.
ii. Phase 2 test 2: A confirmation of Phase 2 test 1 results by repeating Phase 2 test 1.

A summary of the LOD study confirmation (Phase 2 test 2) using heat inactivated virus is shown in Table 6.

Table 6. LOD - Heat Inactivated Virus in Serum and Urine

Virus	Matrix	Target Concentration (GC/mL)	Call Rate	Preliminary LOD (GC/mL)
ZIKV Puerto Rico 2015	Serum	375	20/20	200
		250	20/20	
		200	20/20	
		125	17/20	
		100	19/20	
		75	14/20	
	Urine	250	20/20	70
		100	20/20	
		75	19/20	
		70	19/20	
		65	18/20	
		60	17/20	

Note: A pool of negative serum or urine matrix was used for preparing contrived samples.

LOD- Live Zika Virus (Strain: PRVABC59)

The sensitivity of the TaqPath™ Zika Virus Kit (ZIKV) with live Zika virus was assessed by determining the lowest number of Genomic Copies (GC) of live Zika virus that could be detected in ≥ 95% of samples tested.

Live ZIKV Puerto Rico 2015 viral stock was diluted in human serum and urine samples. Nucleic acid extraction was performed using the MagMAX™ Pathogen RNA/DNA Kit and the KingFisher™ Flex Purification System. Twenty (20) replicates of each dilution were tested with the TaqPath™ Zika Virus Kit (ZIKV). The lowest concentration at which 19 of 20 replicates tested positive (i.e., Ct ≤38.00) was

considered the LOD of live Zika virus. A summary of the LOD test results using live virus are shown in Table 7.

Table 7. LOD- Live Virus in Serum and Urine

Virus	Matrix	Target Concentration (GC/mL)	Call Rate	Preliminary LOD (GC/mL)
ZIKV Puerto Rico 2015	Serum	400	20/20	50
		200	20/20	
		100	20/20	
		50	20/20	
		25	15/20	
	Urine	70	20/20	35
		35	19/20	
		17.5	16/20	
		8.8	11/20	
		4.4	5/20	

LOD testing using live Zika virus showed that the TaqPath™ Zika Virus Kit (ZIKV) had higher sensitivity to live Zika virus when compared to heat inactivated Zika virus. This is due to potentially less stable viral RNA in the sample as a result of the viral heat inactivation process. Results of the live and heat inactivated LOD studies are shown in Table 8 below.

Table 8. LOD Results Summary

	Live Zika Virus	Heat Inactivated Zika Virus
Serum	50 GC/mL	200 GC/mL
Urine	35 GC/mL	70 GC/mL

iii. FDA Sensitivity Study

An analytical sensitivity study was performed on the TaqPath™ Zika Virus Kit (ZIKV) using reference materials (S1 and S2) and a protocol provided by the FDA. The protocol included an LOD range finding and confirmatory LOD study. The results of the study are shown in Table 9.

Table 9. FDA Reference Material LOD Results

FDA Reference Material	Specimen Type	Confirmed LOD in RNA NAAT Detectable Units/mL
S1	Serum	3330
S1	Urine	3330
S2	Serum	500
S2	Urine	500

Analytical Specificity- Interfering Substances

Hematin and leukocytes were evaluated as interfering substances for both serum and urine. Immunoglobulin G (IgG) and urea were evaluated as serum-specific and urine-specific interfering substances, respectively. Heat inactivated ZIKV Puerto Rico 2015 viral stock was spiked in human serum to 600 GC/mL (3X LOD of heat inactivated virus in serum) or in urine to 210 GE/mL (3X LOD of heat inactivated virus in urine) in the presence of various concentrations of the interfering substances listed above. Three replicates of each condition were tested with the TaqPath™ Zika Virus Kit (ZIKV). The highest concentration of interfering substance at which all three replicates tested positive (*i.e.*, Ct ≤38.00) was considered the tolerance level. Test results for serum and urine are shown in Table 10.

Table 10. Interfering Substances

Virus	Concentration	Matrix	Interfering Substance	Call Rate	Average Ct ± SD
ZIKV Puerto Rico 2015	600 GC/mL	Serum	None	3/3	32.66 ± 0.40
			Hematin (40 µM)	3/3	34.12 ± 0.82
			Leukocytes ($3x10^5$ cells/mL)	3/3	32.95 ± 0.32
			IgG (4.1 mg/reaction)	3/3	33.58 ± 0.31
	210 GC/mL	Urine	None	3/3	33.91 ± 1.90
			Hematin (40 µM)	3/3	34.24 ± 0.56
			Leukocytes ($3x10^5$ cells/mL)	3/3	34.9 ± 1.42
			Urea (500 mM)	3/3	32.8 ± 0.68

Analytical Specificity- Cross-reactivity

Cross-reactivity of the TaqPath™ Zika Virus Kit (ZIKV) was evaluated by wet-testing related flaviviruses and synthetic RNA. Synthetic RNA targets consisted of a series of partial transcripts covering multiple regions of the respective viral genome. Positive detection was defined as Ct values ≤38.00. Testing was performed in triplicate. In addition, cross-reactivity was evaluated by performing in silico analyses. Results of the wet-tests and in silico analyses are found in Table 10 and Table 11.

Table 10. Cross-reactivity Results (wet-test)

Target	Sample Type	Target Concentration	Result
Zika	Inactivated virus	$3.6x10^6$	Positive
Chikungunya	Inactivated virus	$5.2x10^6$	Negative
Dengue serotype 1	Inactivated virus	$2.9x10^6$	Negative
Dengue serotype 2	Inactivated virus	$5.8x10^6$	Negative
Dengue serotype 3	Inactivated virus	$2.4x10^6$	Negative
Dengue serotype 4	Inactivated virus	$4.8x10^6$	Negative
St Louis	Synthetic RNA	$3.7x10^6$	Negative
West Nile	Synthetic RNA	$3.6x10^6$	Negative

Table 11. Cross-reactivity Results (in silico)

		Homology			
Virus	**TaxID**	**Zika Fwd**	**Zika Probe**	**Zika Rev**	**Cross Reactivity (Yes/No)**
Leptospira	171	67%	61%	50%	No
Rickettsia	780	50%	56%	50%	No
Trypanosoma cruzi	5693	72%	72%	65%	No
Plasmodium sp	5820	56%	61%	55%	No
Plasmodium falciparum	5833	61%	56%	55%	No
Schistosoma	6181	NA	67%	NA	No
Varicella Zoster Virus	10335	50%	NA	45%	No
Cytomegalovirus	10358	72%	56%	NA	No
Epstein Barr Virus	10376	56%	56%	50%	No
Hepatitis B virus	10404	56%	56%	NA	No
Adenovirus 7	10519	50%	50%	45%	No
Parvovirus (B19)	10798	NA	NA	45%	No
Barmah Forest Virus	11020	NA	NA	NA	No
Eastern Equine encep. Virus	11021	50%	NA	NA	No
O'nyong-nyong Virus	11027	NA	NA	NA	No
Ross River Virus	11029	NA	NA	50%	No
Western Equine encep. Virus	11039	NA	NA	NA	No
Rubella Virus	11041	78%	NA	50%	No
Dengue virus 1	11053	50%	NA	50%	No
Dengue virus 2	11060	50%	50%	45%	No
Dengue virus 3	11069	56%	50%	75%	No
Dengue virus 4	11070	72%	72%	75%	No
Japanese encephalitis	11071	61%	67%	50%	No
St Louis encephalitis	11080	61%	NA	60%	No
West Nile Virus	11082	56%	67%	65%	No
Hepatitis C Virus	11102	72%	61%	NA	No
Measles Virus	11234	50%	72%	45%	No
HIV	11676	NA	NA	NA	No
Enterovirus	12059	67%	56%	60%	No
Group A Streptococcus	36470	NA	NA	NA	No
Chikungunya Virus	37124	NA	NA	NA	No
Yellow Fever	40005	78%	72%	45%	No
Mayaro Virus	59301	NA	NA	NA	No
Spondweni Virus	64318	NA	NA	NA	No
Borrelia burgdorferi	64895	56%	61%	55%	No
Adenovirus B	108098	50%	61%	45%	No
Adenovirus C	129951	50%	50%	NA	No
Adenovirus D	130310	NA	NA	NA	No
Adenovirus B1	565302	NA	NA	NA	No

Clinical Evaluation

Clinical evaluation consisted of testing a series of blinded positive and negative serum and urine specimens. The specimens were tested after being blinded.

The study consisted of:

- Sixty-seven (67) serum samples determined to be positive for Zika virus using an EUA comparator assay. The serum samples were obtained from Boca Biolistics.
- Fifty-five (55) urine samples determined to be positive for Zika virus using an EUA comparator assay. The urine samples were obtained from Boca Biolistics.
- Twenty-five (25) contrived serum samples spiked with live Zika virus at a concentration equal to 1x LOD and 5x LOD.
- Twenty-five (25) contrived urine samples spiked with live Zika virus at a concentration equal to 1x LOD and 5x LOD.
- Seventy-six (76) Zika negative serum samples. Samples were determined to be negative Zika virus using an EUA comparator assay.
- Seventy-four (74) Zika negative urine samples. Samples were determined to be negative for Zika virus using an EUA comparator assay.

Testing was performed using one QuantStudio™ Dx Real-Time PCR Instrument (software version 1.0.1).

TABLE 11: Clinical Validation Summary (Serum)

Specimen Category: Serum	Number of Samples Tested	TaqPath Zika Virus Kit	
		Positive	Negative
Natural Zika negative serum obtained from Primex Clinical Laboratories Inc. in Van Nuys, CA confirmed Zika negative using an EUA comparator assay *	76	0/76	76/76
Natural Zika positive serum obtained from Boca Biolistics, LLC in Pompano Beach, FL confirmed Zika positive using an EUA comparator assay*	67	61/67	6/67**
Contrived Zika Positive 1x LOD (live virus)	25	25/25	0/25
Contrived Zika Positive 5x LOD (live virus)	25	25/25	0/25
Percent Positive Agreement; 95% CI		94.9% (111/117); 90.8% - 98.9%	
Percent Negative Agreement; 95% CI		100% (76/76); Lower 95% CI = 95.3%	

*EUA comparator assay was a Transcription-Mediated Amplification IVD assay authorized by FDA for detection of ZIKV RNA in serum and plasma with analytical sensitivity in the range 100-150 RNA Units/mL in plasma.
**Upon retest 1/6 of these negatives turned positive, suggesting the specimen had Zika virus levels close to the LoD of the TaqPath Zika Virus Kit

TABLE 12: Clinical Validation Summary (Urine)

Specimen Category: Urine	Number of Samples Tested	TaqPath Zika Virus Kit	
		Positive	Negative
Natural Zika negative urine obtained from Primex Clinical Laboratories Inc. in Van Nuys, CA confirmed Zika negative using an EUA comparator assay*	74*	0/74	74/74
Natural Zika positive urine obtained from Boca Biolistics, LLC in Pompano Beach, FL confirmed Zika positive using an EUA comparator assay*	55	45/55	10/55**
Contrived Zika Positive 1x LOD (live virus)	25	24/25	1/25
Contrived Zika Positive 5x LOD (live virus)	25	24/25	1/25
Percent Positive Agreement; 95% CI		88.6%(93/105); 82.4% - 94.8%	
Percent Negative Agreement; 95% CI		100% (74/74); Lower 95% CI = 95.1%	

*EUA Comparator assay was an Transcription-Mediated Amplification IVD assay authorized by FDA for detection of ZIKV RNA in processed urine with analytical sensitivity in the range 150-300 RNA Units/mL.
**Upon retest 4/10 of these negatives turned positive suggesting the specimen tested had Zika virus levels close to the LoD of the TaqPath Zika Virus Kit

14. Technical Assistance

For customer support, please contact Thermo Fisher Scientific Technical Support:
Phone: (800) 955-6288
e-mail: techsupport@lifetech.com

TaqPath™ Zika Virus Kit (ZIKV)

Lyophilized reagents for real-time RT-PCR detection of Zika virus RNA (0.1-mL block)

Catalog Number A31745

Pub. No. MAN0016037 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Intended Use

The TaqPath™ Zika Virus Kit (ZIKV) is a real-time RT-PCR test intended for the qualitative detection of RNA from the Zika virus in serum and urine (collected alongside a patient-matched serum specimen) from individuals meeting Centers for Disease Control and Prevention (CDC) Zika virus clinical criteria (e.g., clinical signs and symptoms associated with Zika virus infection) and/or CDC Zika virus epidemiological criteria (e.g., history of residence in or travel to a geographic region with active Zika transmission at the time of travel, or other epidemiologic criteria for which Zika virus testing may be indicated), by laboratories in the United States that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of Zika virus RNA, which is generally detectable in serum and/or urine during the acute phase of infection and up to 14 days following onset of symptoms, if present. Positive results are indicative of current infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude Zika virus infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The TaqPath™ Zika Virus Kit (ZIKV) is intended for use by trained clinical laboratory personnel who have received specific training on the use of the TaqPath™ Zika Virus Kit (ZIKV) on the Applied Biosystems® QuantStudio Dx Real-time PCR instrument and software system. The assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

Product Description

The TaqPath™ Zika Virus Kit (ZIKV) is a lyophilized real-time reverse transcription polymerase chain reaction assay for qualitative detection of RNA from the Zika virus in serum or urine (collected alongside a patient-matched serum specimen). Each assay consists of two primers and one dye labeled probe. RNA is purified using the MagMAX™ Pathogen RNA/DNA Kit.

For in vitro Diagnostic Use
For Prescription Use Only
For Use Under an Emergency Use Authorization (EUA) Only

Shipment and Storage

Table 1 TaqPath™ Zika Virus Kit (ZIKV) (Cat. No. A31745; 96 reactions)

Contents	Amount	Storage
Lyophilized Assay Fast, 0.1 ml tube	12x8 tube strips	• 18-28°C for up to 1 year(1) • 2-8°C for long-term storage • Protect from moisture
MicroAmp™ Optical 8-Cap Strips	12x8 cap strips	Room Temperature

(1)Product is shipped at ambient temperature.

Required materials (not supplied)

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Real-Time PCR Instrument:

• QuantStudio™ Dx Real-Time PCR Instrument with 96-well fast block (Thermo Fisher Scientific; Catalog#: 4480299)

ZIKV Positive Control:

• Zika Virus Culture Fluid (ZeptoMetrics Corporation; Catalog#: 0810525CF) ***or*** ZIKV Inactivated Virus Suspension, Puerto Rico 2015 isolate (Vircell S.L.; Catalog#: MC018)

Automated Nucleic Acid Extraction System and Materials:

• KingFisher™ Flex Purification System, with 96 Deep-well Head (Thermo Fisher Scientific; Catalog#: 5400630)
• MagMAX™ Express-96 Deep Well Plates (Thermo Fisher Scientific; Catalog#: 4388476)
• MagMAX™ Express-96 Standard Plates (Thermo Fisher Scientific; Catalog#: 4388475)
• MagMAX™ Express-96 Deep Well Tip Combs (Thermo Fisher Scientific; Catalog#: 4388487)

Nucleic Acid Extraction Kit:

• MagMAX™ Pathogen RNA/DNA Kit (Thermo Fisher Scientific; Catalog#: 4462359)

General Laboratory Equipment:

• Microcentrifuge, capable of 16,000 × g (Eppendorf, Part no. 5415D; or equivalent)
• Microplate Shaker (Thermo Fisher Scientific, Catalog#: 88880023; or equivalent
• Centrifuge with a rotor for microtiter plates
• Vortex mixer
• Single- and multichannel pipettes

General Laboratory Materials and Consumables:

• 100% ethanol, ACS reagent grade or equivalent
• 100% isopropanol, ACS reagent grade or equivalent
• Nuclease-free water
• 1.5 mL microcentrifuge tubes
• 2 mL microcentrifuge tubes
• Pipette tips with filters

ThermoFisher SCIENTIFIC

Warnings and Precautions

The TaqPath™ Zika Virus Kit (ZIKV) workflow should be performed by qualified and trained staff to avoid the risk of erroneous results. Separate areas for the preparation of patient samples and controls to prevent false positive results. Samples and reagents must be handled under a laminar airflow hood or biological safety cabinet.

- The assay is for in vitro diagnostic use under the FDA Emergency Use Authorization Only.
- Urine and serum specimens should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.
- Follow necessary precautions when handling specimens. Use personal protective equipment (PPE) consistent with current guidelines for the handling of potentially infectious samples.
- Always use pipet tips with aerosol barriers. Tips used must be sterile and free from DNases and RNases.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.
- Modifications to assay reagents, assay protocol, or instrumentation are not permitted, and are in violation of the product Emergency Use Authorization.
- Do not use the kit after the indicated expiry date.
- Dispose of waste in compliance with the local, state, and federal regulations.

Samples and Controls

Patient serum and urine samples must be collected according to appropriate laboratory guidelines. RNA from patient samples is purified using the MagMAXTM Pathogen RNA/DNA Kit.

Positive and negative test controls must be examined prior to the interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Cutoff values for valid test results are as follows:
- A positive Zika virus control Ct value is ≤ 38.00
- PPIA endogenous control Ct value is ≤ 38.00
- Nuclease-Free Water (negative control) Ct value is > 38.00

Instructions for Use

Prepare Positive Zika Control & Extract RNA using the MagMAX Pathogen RNA/DNA Kit

- Prepare the ZIKV Positive Control. In the case of the ZIKV Inactivated Virus Suspension (Vircell S.L.; Catalog#: MC018) dilute to 1 × 10^6 copies/mL using the Carrier RNA as a diluent (Carrier RNA is included in the MagMAX™ Pathogen RNA/ DNA Kit)
- Prior to initiating the procedure, label MagMAX™ Express-96 Deep Well Plates as "sample plate" (one plate), "wash 1" (two plates), and "wash 2" (two plates).
- Label MagMAX™ Express- 96 Standard Well Plates as "elution plate" (one plate) and "tip comb" (one plate).
- Mark the wells on all plates that will receive patient samples, and positive and negative control samples.

Prepare Wash Solutions

1. Prepare Wash Solution 1 by adding 125 mL of 100% isopropanol to the Wash Solution 1 Concentrate bottle.
2. Prepare Wash Solution 2 by adding 232mL 100% ethanol to the Wash Solution 2 Concentrate bottle.
3. Mix both wash solutions by inverting the bottles 3-4 times. Store both solutions at room temperature for up to 6 weeks.

Prepare Lysis/Binding Solution

Prepare the required amount of Lysis/Binding Solution on each day of use.

Table 1. Lysis/Binding Solution Preparation

Number of Samples	Lysis/Binding Solution (mL)	Carrier RNA (mL)	100% Isopropanol (mL)
1	0.35	0.0023	0.35
2	0.7	0.0046	0.70
3	1.05	0.0069	1.05
4	1.4	0.0092	1.40
5	1.75	0.0115	1.75
6	2.1	0.0138	2.10
7	2.45	0.0161	2.45
8	2.8	0.0184	2.80
9	3.15	0.0207	3.15
10	3.5	0.0230	3.50

4. Determine the volume (in mL) of Lysis/Binding Solution Concentrate, carrier RNA, and 100% isopropanol to mix, based on the total number of patient samples to be processed, plus one negative and one positive control sample. Representative volumes are shown in Table 1.
5. Combine the components then mix by vortexing. Store the Lysis/Binding Solution at room temperature for up to 8 hours.

Prepare Bead Mix

6. Determine the volume of Nucleic Acid Binding Beads and Lysis Enhancer to mix based on the total number of samples. Representative volumes are shown in Table 2.
7. Mix the Nucleic Acid Binding Beads by vortexing.
8. On ice, combine the required volumes of Nucleic Acid Binding Beads and Lysis Enhancer, then mix by vortexing. Store the Bead Mix on ice for up to 4 hours.

Table 2. Bead Mix Preparation

Number of Samples	Nucleic Acid Binding Beads (µL)	Lysis
1	1	10
2	2	20
3	3	30
4	4	40
5	5	50
6	6	60
7	7	70
8	8	80
9	9	90
10	100	100

Prepare the Processing Plate

9. Add 300 µL of Wash Solution 1 to each well in the two deep-well plates labeled as "wash 1".
10. Add 450 µL of Wash Solution 2 to each well in the two deep-well plates labeled as "wash 2".
11. Add 50 µL of Elution Buffer to the standard-well plate labeled as "elution plate".

12. To the deep-well plate labeled as "sample plate" add the following in the indicated order:

1) 700 µL of Lysis/Binding Solution.
2) 20 µL Bead Mix.
3) 10 µL of the ZIKV positive control to the appropriate wells.
4) 300 µL of nuclease-free water to the positive control wells and negative control wells.
5) 300 µL of sample to the appropriate wells.
6) Seal and cover the plate.

Process Samples KingFisher™ Flex Purification System

13. Power on the KingFisher Flex Purification System and select the. "MagMAX_Pathogen_High_Volume_1" protocol.
14. Start the "MagMAX_Pathogen_High_Volume_1" protocol.
15. Load the plates onto the processor as directed.
16. Store the elution plate on ice for immediate use in RT-qPCR or seal the plate and store at -20°C for up to 1 month, or at -80°C for long-term storage.

TaqPath™ Zika Virus Kit (ZIKV) RT-qPCR

1. If necessary, thaw the elution plate on ice.
2. Remove the cap of the 8-well strip. Do not reuse the cap on the strip.
3. Per RNA sample, add 25µL to the corresponding well of the assay strip. Apply a new cap to seal tubes.
4. Mix by flicking the strip several times or by vortexing. Briefly centrifuge the assay strip after mixing.
5. Place the assay strip onto the QuantStudio™ Dx Real-Time PCR Instrument. Run the assay using the following cycle parameters:

Table 3. PCR Cycling Parameters

Steps	Thermal Cycling Conditions	
Reverse Transcription	HOLD	50°C for 10 min
Activation	HOLD	95°C for 2 min
PCR Amplification	40 cycles	95°C for 3 sec
		60°C for 30 sec

Analyze the Data

6. Analyze data using the automatic baseline and threshold settings in the QuantStudio™ Dx Real-Time PCR Instrument software.
7. Determine the Ct (cycle threshold) and standard deviation (if needed) for each assay.
8. Review Ct data for the positive and negative control samples, and the PPIA control assay. Export run data as an Excel file.

Validity of Diagnostic Runs

For a diagnostic test run to be considered valid, the results for positive and negative controls listed in Table 4 must be observed.
In the case of an invalid diagnostic run, all associated patient samples are considered invalid. Fresh patient samples and controls must be tested.

Table 4. Required Diagnostic Run Control Results

Sample ID	Detection Channels	
	VIC™ (PPIA)	FAM™ (ZIKV)
Negative Control	NEGATIVE	NEGATIVE
Zika Positive Control	NEGATIVE	POSITIVE
*NEGATIVE defined as Ct >38.00 *POSITIVE defined as Ct ≤38.00		

See the "Interpretation of Results" section on the next page to assess test results.

Interpretation of Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. Table 5 correlates the TaqPath™ Zika Virus Kit (ZIKV) with the result interpretation.

Note: Positive results are indicative of current infection.

Table 5. Result Interpretation

TaqPath Result (+/-)	Zika Positive Control	PPIA Control	Negative Control (Water)	Interpretation of Results
+	+	+	-	Zika RNA is detected in sample
+	+	-	-	Zika RNA is detected in sample
-	+	+	-	**Zika RNA is not detected in sample
+	+	+	+	*Invalid- Repeat testing is required
+	-	+	+	*Invalid- Repeat testing is required
+	+	-	+	*Invalid- Repeat testing is required
+	-	-	+	*Invalid- Repeat testing is required
+	-	+	-	*Invalid- Repeat testing is required
+	-	-	-	*Invalid- Repeat testing is required
-	-	-	-	*Invalid- Repeat testing is required
-	-	+	-	*Invalid- Repeat testing is required
-	+	-	-	*Invalid- Repeat testing is required
-	-	-	+	*Invalid- Repeat testing is required
-	+	-	+	*Invalid- Repeat testing is required
-	-	+	+	*Invalid- Repeat testing is required
-	+	+	+	*Invalid- Repeat testing is required

Note: positive detection signal defined as Ct ≤38.00; negative detection signal defined as Ct >38.00. Detection of the PPIA control is not required for positive results in the FAM™ detection channel. For example, high viral detection signals can reduce or eliminate detection signals in other channels.

*Invalid results may be due to: operator error, instrument failure, PCR inhibitors, or reagent failure. If repeat testing is invalid, then all associated patient samples are considered invalid. Fresh patient samples and controls must be tested.

**A patient-matched serum specimen is currently required for serological follow up testing of negative RT-PCR results per the CDC testing algorithm (found at http://www.cdc.gov/zika/index.html).

If the repeat testing is valid, continue to evaluate patient samples as stated in the table.

Assay Limitations

- The use of this assay as an *In vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

- TaqPath™ Zika Virus Kit (ZIKV) was established for human urine and serum samples only. Other specimen types have not been evaluated and should not be tested with this assay.

- Human urine and serum samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

- False-negative results may arise from:
    - o Improper sample collection
    - o Degradation of the viral RNA during shipping/storage
    - o Specimen collection after nucleic acid can no longer be found in the specimen matrix
    - o Using unauthorized extraction or assay reagents
    - o The presence of RT-PCR inhibitors
    - o Mutation in the Zika virus
    - o Failure to follow instructions for use

- False-positive results may arise from:
    - o Cross contamination during specimen handling or preparation
    - o Cross contamination between patient samples
    - o Specimen mix-up
    - o RNA contamination during product handling

- The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. TaqPath™ Zika Virus Kit (ZIKV) cannot rule out diseases caused by other bacterial or viral pathogens.
- Negative results do not preclude infection with Zika virus, and should not be the sole basis of a patient management decision.
- A patient matched serum specimen is required for serological follow up testing of all negative RT-PCR results, per the CDC testing algorithm. (Found at http://www.cdc.gov/zika/index.html).
- Laboratories are required to report all positive results to the appropriate public health authorities.

Performance Characteristics

The analytical performance of the TaqPath™ Zika Virus Kit (ZIKV) was evaluated by determining limit of detection (LOD) using live and heat inactivated Zika virus, characterizing the impact of interfering substances and cross-reactivity. Clinical evaluation was determined using live and heat inactivated Zika virus.

Information and and study results of analytical validation and clinical evaluation can be found at:

https://www.thermofisher.com/us/en/home/life-science/pcr/real-time-pcr/real-time-pcr-research-areas/arbovirus-zika-detection-real-time-pcr.html

Conditions of Authorization for Laboratories

The TaqPath™ Zika Virus Kit (ZIKV) Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/MedicalDevices/Safety/EmergencySituations/ucm161496.htm. However, to assist clinical laboratories running the TaqPath™ Zika Virus Kit (ZIKV), the relevant Conditions of Authorization are listed below.

- Authorized laboratories will include with reports of the results of the TaqPath™ Zika Virus Kit (ZIKV) the authorized Fact Sheet for Healthcare Providers and the authorized Fact Sheet for Patients. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) using the KingFisher™ Flex Purification System (KingFisher) and MagMAX™ Pathogen RNA/DNA Kit or with other authorized extraction methods.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) on the Applied Biosystems® QuantStudio Dx Real-time PCR instrument, or other authorized instruments.

- Authorized laboratories will perform the TaqPath™ Zika Virus Kit (ZIKV) on human serum, or urine (collected with a patient-matched serum specimen), or other authorized specimen types.

- Authorized laboratories will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will collect information on the performance of the test and report to DMD/OIR/CDRH (via email CDRH-EUA-Reporting@fda.hhs.gov) and Thermo Fisher any suspected occurrence of false positive or false negative results of which they become aware.

- All laboratory personnel using the test should be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.

- Thermo Fisher, its authorized distributor(s), and authorized laboratories, will ensure that any records associated with this EUA are maintained until notified by FDA. Such records will be made available to FDA for inspection upon request.

Manufacturer's address: Life Technologies Corporation | 6055 Sunol Blvd | Pleasanton, CA 94566

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0016037

Revision	Date	Description
A.0	28 July 2017	New document.