WHO Emergency Use Assessment and Listing for Zika IVDs
PUBLIC REPORT

Product: RealStar® Zika Virus RT-PCR Kit 1.0
EUAL Number: EAZ 0003-002-00

Emergency Use Assessment and Listing of In Vitro Diagnostics Procedure

WHO has developed an Emergency Use Assessment and Listing (EUAL) procedure to expedite the availability of in vitro diagnostics (IVDs) needed in public health emergency situations. This EUAL procedure will generate WHO recommendations in order to provide advice to procurement agencies and Member States on the acceptability of a specific IVD in the context of a public health emergency, based on a minimum set of available quality, safety, and performance data and an agreed plan for further evaluation.

The EUAL procedure is comprised of three components that aim to assess the safety, quality and performance of the IVD:

- a review of the manufacturer's quality management system documentation;
- a review of the documentary evidence of safety and performance; and
- an independent performance evaluation

RealStar® Zika Virus RT-PCR Kit 1.0 with product code **REF – 591013** manufactured by **altona Diagnostics GmbH,** Mörkenstraße 12, 22767 Hamburg, Germany, CE marked regulatory version, was listed as eligible for WHO procurement on 05 August 2016.

The RealStar® Zika Virus RT-PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the qualitative detection of Zika virus specific RNA in human serum or urine.

The assay 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 test is based on real-time RT-PCR technology, utilizing a 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 fluorescent reporter and quencher dyes. Probes specific for Zika virus RNA are labelled with the fluorophore FAM. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE. Using probes linked to

distinguishable dyes enables the parallel detection of Zika virus specific RNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test consists of three processes in a single tube assay:
• Reverse transcription of target RNA and Internal Control RNA to cDNA
• PCR amplification of target cDNA and Internal Control cDNA
• Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

Content of the kit

Component	Master A	Master B	Internal Control	Positive Control	PCR grade Water
Number of Vials	8	8	1	1	1
Volume [µL/Vial]	60	180	1000	250	500
Lid Colour	Blue	Purple	Green	Red	White

Master A and Master B contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection of Zika virus specific RNA and Internal Control in one reaction setup.

Materials required but not provided

Material	**Product name/description**
Instrumentation for amplification The RealStar® Zika Virus RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:	• Mx 3005P™ QPCR System (Stratagene) • VERSANT® kPCR Molecular System AD (Siemens) • ABI Prism® 7500 SDS and 7500 Fast SDS (Applied Biosystems) • LightCycler® 480 Instrument II (Roche) • Rotor-Gene® 6000 (Corbett Research) • Rotor-Gene® Q 5/6 plex Platform (QIAGEN) • CFX96™ Real-Time System and CFX96™ Deep Well Real-Time System (Bio-Rad)
Kits for nucleic acid extraction The following nucleic acid extraction kit is validated:	• QIAamp® Viral RNA Mini Kit (QIAGEN).
Additional materials	• Desktop centrifuge with a rotor for 2 ml reaction tubes • Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates • Vortex mixer • Appropriate 96 well reaction plates or reaction tubes with

	corresponding (optical) closing material • Pipettes (adjustable) • Pipette tips with filters (disposable) • Powder-free gloves (disposable)

Storage: The test kit should be stored at -25°C to -15°C.

Shelf-life upon manufacture: 9 months.

Warnings/limitations: The performance observed using urine as only specimen type was lower than that observed when using paired specimens or serum only specimens. For this reason urine is not recommended as a sole specimen type at this present time.

WHO EUAL Assessment

altona Diagnostics GmbH submitted an expression of interest for WHO emergency quality assessment of **RealStar® Zika Virus RT-PCR Kit 1.0** on 26 February 2016.

Review of quality management documentation
To establish the eligibility for WHO procurement, **altona Diagnostics GmbH** was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation, it was established that sufficient information was provided by **altona Diagnostics GmbH** to fulfil the requirements described in the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO".

Quality management documentation for Emergency Use Assessment and Listing conclusion: **Acceptable.**

Product dossier assessment
altona Diagnostics GmbH submitted documentation in support of safety and performance for **RealStar® Zika Virus RT-PCR Kit 1.0** as per the "Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO"[1]. The information submitted in the product application was reviewed by WHO

[1] Invitation to manufacturers of in vitro diagnostics for Zika virus to submit an application for emergency use assessment and listing by WHO.

staff and external experts (reviewers) appointed by WHO. The findings of the reviews were reported in accordance with "Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting Zika Virus Nucleic Acid or Antigen" (WHO document number WHO PQDx_240 v 2).

Safety and performance documentation for Emergency Use Assessment and Listing conclusion: **Acceptable.**

Laboratory evaluation

Analytical testing

The limit of detection of the assay was evaluated independently by the Bernhard Nocht Institute for Tropical Medicine (BNITM) in Hamburg, Germany which is a WHO Collaborating Centre for Arbovirus and Haemorrhagic Fever Reference and Research.

Testing was conducted with international standard (IS) developed by the Paul-Ehrlich-Institut (PEI), Langen, Germany on behalf of WHO. The standard has been assigned a potency of 7.70 log10 units/mL based on the results of an international collaborative study and was approved by the Expert Committee on Biological Standardization (ECBS) in October 2016.

The study was performed under the BSL-2 conditions. Reference material was reconstituted in 0.5 ml of nuclease-free water as recommended by PEI (WHO Zika Virus Collaborative Study Protocol Q2 2016).

A 1-log10 step dilution series of the candidate IS spanning the claimed LoD of the assay under evaluation were prepared in both urine and serum. Two replicates of each dilution concentration were tested. In a second experiment, a 5 member 0.5-log step dilution series spanning the estimated LoD was tested using 8 replicates at each concentration level.

Testing was performed using 10 µl of eluted RNA on the Roche LightCycler 480 instrument in accordance with manufacturer's recommendation and as per the instrument's user manual.

The analytical sensitivity (95% limit of detection [LoD]) of **RealStar® Zika Virus RT-PCR Kit 1.0** was determined by Probit analysis in both urine and plasma specimens.

Table 1 Dose for centile 95 (95% LoD) and confidence interval (CI)

	Serum	**Urine**

95% LoD (CI) RNA IU/ml of specimen	**127.5** (60.3 to 862.1)	**134.5** (64.8 to 843.0)

Clinical Testing:
Clinical testing was performed at the Institut Pasteur in Cayenne, French Guiana.

Specimens were collected from patients exhibiting symptoms of Zika virus infection between the 1st and the 8th day following the onset of disease. A total of 208 clinical specimens were obtained from 152 patients (including 54 patients with paired (sera and urine) and/or sequential samples). The specimen types collected were: 102 serum, 1 plasma and 105 urine specimens.

All specimens were tested with the **RealStar® Zika Virus RT-PCR Kit 1.0** as well as the real-time RT-PCR as described by Lanciotti et. al (Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, et al. (2008) Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007; Emerg Infect Dis. 2008 Aug; 14(8): 1232–1239), used as a benchmark assay.

Of the 208 samples included in the comparison study 111 tested positive for Zika virus RNA with the benchmark assay, whereas 97 tested negative. Of the 111 positive specimens, 106 tested positive with the **RealStar® Zika Virus RT-PCR Kit 1.0**, whereas five tested negative. Of the 97 specimens tested negative for Zika virus with the benchmark assay, 89 tested negative and eight positive with the **RealStar® Zika Virus RT-PCR Kit 1.0.**

The results are summarized in the following table:
Table 2 Overall performance with serum and urine specimens

RealStar® Zika Virus RT-PCR Kit 1.0	**Benchmark assay**		
	Positive	Negative	Total
Positive	106	8	114
Negative	5	89	94
Total	111	97	208

Overall performance of the RealStar® Zika Virus RT-PCR Kit 1.0:

- Positive Percent Agreement: 95.5% (106/111) - 95% CI (91.6-99.4)
- Negative Percent Agreement: 91.8% (89/97) - 95% CI (86.3-97.2)

Performance in urine specimens:

Benchmark Assay	**RealStar® Zika Virus RT-PCR Kit 1.0**	
	Positve	**Negative**
49 Positive	46	3
56 Negative	6	50
Total (105)	52	53

Positive Percent Agreement: 93.9 % % (46/49) 95% CI (83.13 -98.72)
Negative Percent Agreement: 89.3% (50/56) 95% CI (78.12-95.96)

Performance in serum specimens:

Benchmark Assay	**RealStar® Zika Virus RT-PCR Kit 1.0**	
	Positve	**Negative**
62 Positive	60	2
41 Negative	2	39
Total	62	41

Positive Percent Agreement: 96.8% (60/62) 95% CI (89.0 - 99.1)
Negative Percent Agreement: 95.1% (39/41) 95% CI (83.47 – 99.40).

Cross reactivity:
Forty four Dengue positive serum specimens were used:
Dengue 1 : 9 specimens
Dengue 2 : 14 specimens
Dengue 3 : 1 specimen
Dengue 4 : 20 specimens

All 44 specimens tested negative with the **RealStar® Zika Virus RT-PCR Kit 1.0.**

Fifty five Chikungunya positive serum specimens were also tested. All 55 specimens tested negative with the **RealStar® Zika Virus RT-PCR Kit 1.0.**

Laboratory evaluation for Emergency Use Assessment and Listing conclusion:
Acceptable.

WHO Emergency Use Assessment and Listing Decision

Based on the review of the manufacturer's submitted data, as well as data generated from the limited laboratory evaluation **RealStar® Zika Virus RT-PCR Kit 1.0** is eligible for WHO procurement.

Post market surveillance to monitor the performance of **RealStar® Zika Virus RT-PCR Kit 1.0** is highly recommended.

Scope and duration of procurement eligibility

RealStar® Zika Virus RT-PCR Kit 1.0 with product code **REF – 591013**, manufactured by **altona Diagnostics GmbH** is considered to be eligible for WHO procurement. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement **altona Diagnostics GmbH** must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. **altona Diagnostics GmbH** is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days of receipt. Furthermore, WHO will continue to monitor the performance of the assay in the field.

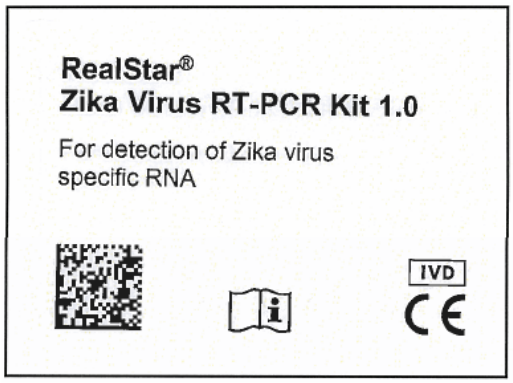
WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality and performance comes to WHO's attention during post-market surveillance activities.

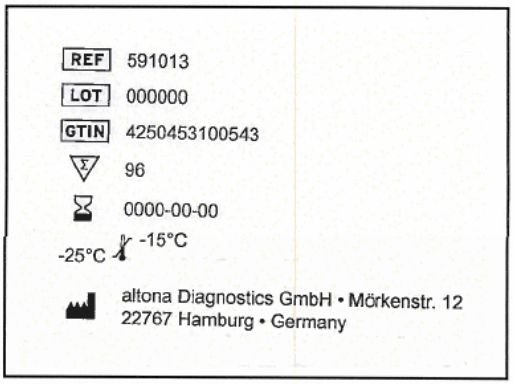
Labelling

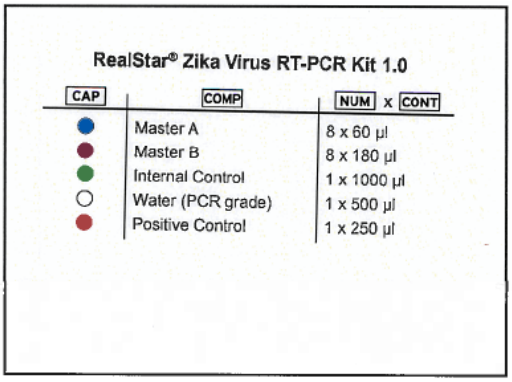
1. **Labels**
2. **Instructions for use**

1. Labels

1.2. Box labels

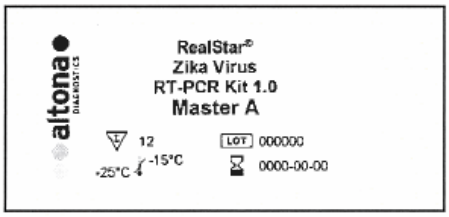




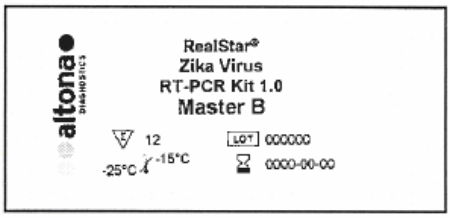


1.2. Tube labels

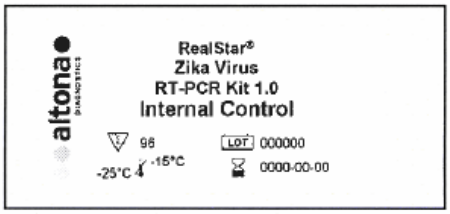
Master A



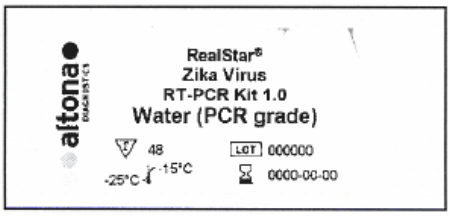
Master B



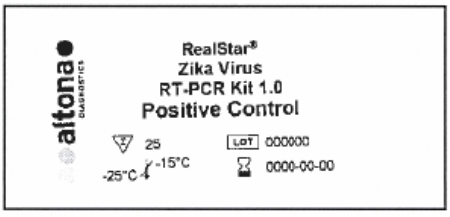
Internal Control



Water (PCR grade)



Positive Control



2. Instructions for use

Version 08/2016

always a drop ahead.

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RealStar®

altona DIAGNOSTICS

Instructions for Use

RealStar®
Zika Virus RT-PCR Kit 1.0

08/2016 EN

RealStar®

Zika Virus RT-PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

Rotor-Gene® 6000 (Corbett Research)

Rotor-Gene® Q5/6 plex Platform (QIAGEN)

CFX96™ Real-Time System (Bio-Rad)

CFX96™ Deep Well Real-Time System (Bio-Rad)

LightCycler® 480 Instrument II (Roche)

CE

IVD

REF 591013

Σ 96

08 2016

altona Diagnostics GmbH • Mörkenstr. 12 • D-22767 Hamburg

MAN-591010-EN-S02

Content

1. Intended Use

The RealStar® Zika Virus RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of Zika virus specific RNA in human serum or urine.

2. Kit Components

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A	8	60
Purple	Master B	8	180
Green	Internal Control	1	1000
Red	Positive Control	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Zika Virus RT-PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored between -25°C and -15°C upon arrival.
- Repeated thawing and freezing of Master reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
- Storage between +2°C and +8°C should not exceed a period of two hours.
- Protect Master A and Master B from light.

4. Material and Devices required but not provided

- Appropriate real-time PCR instrument (see chapter 6.1 Real-Time PCR Instruments)
- Appropriate nucleic acid extraction system or kit
- Desktop centrifuge with a rotor for 2 ml reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Vortex mixer
- Appropriate 96 well reaction plates or reaction tubes with corresponding (optical) closing material
- Pipettes (adjustable)
- Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

NOTE

Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.

NOTE

It is highly recommended to use the 72-well rotor with the appropriate 0.1 ml reaction tubes, if using the Rotor-Gene® 6000 (Corbett Research) or the Rotor-Gene® Q 5/6 plex (QIAGEN).

5. Background Information

Zika virus is an enveloped, single stranded (+) RNA virus of the family *Flaviviridae*. Like many other members of the family it is mainly transmitted by mosquitoes of the genus Aedes. In 1947, the virus was isolated from a rhesus monkey in Uganda for the first time. The first human case of Zika virus infection was discovered in 1968 in Nigeria. Initially, evidence for Zika virus infections has only been found in patients from Africa and South-East Asia. In 2007 the virus caused a large outbreak in Micronesia and other islands in the Pacific Ocean. French Polynesia, Easter Islands and Cook Islands were affected in 2013. Since 2015 the virus is also endemic to South America, namely Brazil, where since then large numbers of suspected cases were recorded. Fever, rash and arthralgia are common symptoms and signs of Zika virus infections which nevertheless usually are mild and self-limiting.

Because Zika, dengue and chikungunya virus are endemic to the same geographical regions and cause similar symptoms, definite identification of the etiological agent is only possible with laboratory testing. Zika virus detection by means of realtime RT-PCR should be done early (up to 10 days) after illness onset. Antibody assays commonly cross-react with closely related flaviviruses and detection of neutralising antibodies by plaque reduction assays is troublesome and can only be done in specialised laboratories.

NOTE	
i	***Due to the relatively fast molecular evolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.***

6. Product Description

The RealStar® Zika Virus RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of Zika virus specific RNA. The assay includes a heterologous amplification system (Internal Control) to identify possible RT-PCR inhibition and to confirm the integrity of the reagents of the kit.

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 fluorescent reporter and quencher dyes.

Probes specific for Zika virus RNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Using probes linked to distinguishable dyes enables the parallel detection of Zika virus specific RNA and the Internal Control in corresponding detector channels of the real-time PCR instrument.

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

- Reverse transcription 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 RealStar® Zika Virus RT-PCR Kit 1.0 consists of:

- Two Master reagents (Master A and Master B)
- Internal Control (IC)
- Positive Control
- PCR grade water

Master A and Master B contain all components (buffer, enzymes, primers, and probes) to allow reverse transcription, PCR mediated amplification and target detection of Zika virus specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® Zika Virus RT-PCR Kit 1.0 was developed and validated to be used with the following real-time PCR instruments:

- Mx 3005P™ QPCR System (Stratagene)
- VERSANT® kPCR Molecular System AD (Siemens)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time System (Bio-Rad)
- CFX96™ Deep Well Real-Time System (Bio-Rad)
- LightCycler® 480 Instrument II (Roche)

7. Warnings and Precautions

Read the Instructions for Use carefully before using the product.

- Before first use check the product and its components for:
  - Integrity
  - Completeness with respect to number, type and filling (see chapter 2. Kit Components)
  - Correct labelling
  - Frozenness upon arrival
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Specimens should always be treated as 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 specimens 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) sample preparation, (ii) reaction setup and (iii) amplification/detection activities. The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- 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 autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Do not use components of the kit that have passed their expiration date.
- Discard sample and assay waste according to your local safety regulations.

8. Procedure

8.1 Sample Preparation

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It has to be ensured that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kit was validated in combination with the RealStar® Zika Virus RT-PCR Kit 1.0 for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
  - Cat. No. 52904 for 50 extractions
  - Cat. No. 52906 for 250 extractions

The extraction of the RNA using the QIAamp® Viral RNA Mini Kit has to be performed following the manufacturer's instructions using 140 µl of specimen as starting material. For elution of the extracted RNA 60 µl AVE buffer should be used.

Alternative nucleic acid extraction systems and kits might also be appropriate.
The suitability of the nucleic acid extraction procedure for use with RealStar® Zika Virus 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, it is highly recommended to perform an additional centrifugation step for 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

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

CAUTION	
	***The use of carrier RNA is crucial for extraction efficiency and stability of the extracted nucleic acid.***

For additional information and technical support regarding pre-treatment and sample preparation please contact our Technical Support (see chapter 14. Technical Assistance).

8.2 Master Mix Setup

All reagents and samples should be thawed completely, mixed (by pipetting or gentle vortexing) and centrifuged briefly before use.

The RealStar® Zika Virus RT-PCR Kit 1.0 contains a heterologous Internal Control (IC), which can either be used as a RT-PCR inhibition control or as a control of the sample preparation procedure (nucleic acid extraction) and as a RT-PCR inhibition control.

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

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Internal Control	1 µl	12 µl
Volume Master Mix	**21 µl**	**252 µl**

► If the IC is used as a control for the sample preparation procedure and as a RT-PCR inhibition control, add the IC during the nucleic acid extraction procedure.

► 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, always and only depends on the elution volume. It represents 10% of the elution volume. E.g. when using the QIAamp® Viral RNA Mini Kit the RNA with an volume of 60 µl AVE buffer, 6 µl of IC per sample must be added to the specimen/lysis buffer mixture.

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

Number of Reactions (rxns)	1	12
Master A	5 µl	60 µl
Master B	15 µl	180 µl
Volume Master Mix	**20 µl**	**240 µl**

CAUTION	
	***If the IC (Internal Control) was added during the sample preparation procedure, the Master Mix for the controls must be prepared including the IC.***

CAUTION	
	***Never add the IC directly to the specimen.***

8.3 Reaction Setup

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

► Add 10 µl of the sample (eluate from the nucleic acid extraction) or 10 µl of the controls (Positive or Negative Control).

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

► Make sure that at least one Positive and one Negative Control is used per run.

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

► Close the 96-well reaction plate with appropriate lids or optical adhesive film and the reaction tubes with appropriate lids.

► Centrifuge the 96-well reaction plate in a centrifuge with a microtiter plate rotor for 30 seconds at approximately 1000 x g (~ 3000 rpm).

9. Programming the Real-Time PCR Instrument

For basic information regarding the setup and programming of the different real-time PCR instruments, please refer to the user manual of the respective instrument. For detailed programming instructions regarding the use of the RealStar® Zika Virus RT-PCR Kit 1.0 on specific real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

9.1 Settings

► Define the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	ROX™

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
Zika virus specific RNA	Zika virus	FAM™	(None)
Internal Control	IC	JOE™	(None)

9.3 Temperature Profile and Dye Acquisition

► Define the temperature profile and dye acquisition:

	Stage	Cycle Repeats	Acquisition	Temperature [°C]	Time [min:sec]
Reverse Transcription	Hold	1	-	55	20:00
Denaturation	Hold	1	-	95	02:00
Amplification	Cycling	45	-	95	00:15
			yes	55	00:45
			-	72	00:15

9.4 Special Remarks on the Setup of Recommended Real-Time PCR Instruments

Please find below special remarks on the setup of LightCycler® 480 Instrument II (Roche), CFX96™ Real-Time PCR Detection System (Bio-Rad), CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad), ABI Prism® 7500 SDS and ABI Prism® 7500 Fast SDS (Applied Biosystems), Rotor-Gene® 6000 (Corbett Research) and Rotor-Gene® Q 5/6 plex Platform (QIAGEN), VERSANT® kPCR Molecular System AD (Siemens Healthcare) and Mx 3005P™ QPCR System.(Stratagene)

LightCycler® 480 Instrument II

1. In the “Experiment settings”, select “Detection Format: Dual Color Hydrolysis Probe / UPL Probe”.

2. Make sure by checking the “Customize” field that the setting shown for the “Filter Combinations” are “FAM™ (465-510)” and “VIC™/HEX/Yellow555 (533-580)”.

ABI Prism® 7500 SDS

Go to “Plate Setup”, “Define Targets and Samples”, “Assign Targets and Samples”:

1. Select the whole plate.

2. Click the assign-boxes for both targets. The targets should appear in the wells in the plate layout.

3. Make sure to choose “none” in the “Select the dye to use as the passive reference” (default setting is “ROX™”).

ABI Prism® 7500 SDS Fast

The same settings for “Plate Setup” as for the ABI Prism® 7500 SDS apply (see above). For the Fast version, go to “Experiment properties”. The ramp speed has to be set to “Standard (~2 hours to complete a run)”. The RealStar® Zika Virus RT-PCR Kit 1.0 is not compatible with the fast cycling conditions and the increased ramp rates.

CFX96™ Real-Time PCR Detection System and CFX96™ Deep Well Real-Time PCR Detection System

Open the “Plate Editor” window and select all wells of the 96 well-plate. Click “Select Fluorophores”. For “Channel 1” check the box behind FAM™ and for “Channel 2” check the box behind VIC™. Assign samples to the wells by selecting the appropriate “Sample Type “and afterwards “Load” FAM™ and VIC™ to the wells. The target name of FAM™ should be set to “Zika virus” and the target name of VIC™ to “Internal Control”.

Rotor-Gene® 6000 and Rotor-Gene® Q 5/6 plex/MDx Platform

Chose the 72-Well-Rotor and the appropriate reaction volume. The Gain optimization should be performed before 1st acquisition.

VERSANT® kPCR Molecular System AD

Under the “Instrument” drop down menu select the “Filter Set Gain Settings”. A pop up window will appear. Set the following configurations: Cy®5 x8, ROX™ x4, JOE™ x8 and FAM™ x8. Close the pop-up window by clicking the OK button.

Mx 3005P™ QPCR System

Under the “Instrument” drop down menu select the “Filter Set Gain Settings”. A pop up window will appear. Set the following configurations: Cy®5 x8, ROX™ x4, JOE™ x8 and FAM™ x8. Close the pop-up window by clicking the OK button.

10. Data Analysis

For basic information regarding data analysis on specific real-time PCR instruments, please refer to the user manual of the respective instrument.

For detailed instructions regarding the analysis of the data generated with the RealStar® Zika Virus RT-PCR Kit 1.0 on different real-time PCR instruments please contact our Technical Support (see chapter 14. Technical Assistance).

10.1 Validity of Diagnostic Test Runs

10.1.1 Valid Diagnostic Test Run

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

Control ID	Detection Channel	
	FAM™	JOE™
Positive Control	+	+
Negative Control	-	+

10.1.2 Invalid Diagnostic Test Run

A 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.

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	Zika virus specific RNA detected.
-	+	No Zika virus specific RNA detected. Sample does not contain detectable amounts of Zika virus specific RNA.
-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.

* Detection of the Internal Control in the JOE™ detection channel is not required for positive results in the FAM™ detection channel. A high Zika virus RNA load in the sample can lead to a reduced or absent Internal Control signal.

11. Performance Evaluation

11.1 Performance Evaluation without Nucleic Acid Extraction

11.1.1 Analytical Sensitivitiy

The analytical sensitivity of the RealStar® Zika Virus RT-PCR Kit 1.0 is defined as the concentration (copies/µl of the eluate) of Zika virus specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified Zika virus RNA.

Table 1: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of Zika virus specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.622	24	24	100
10.000	24	24	100
3.162	24	24	100
1.000	24	24	100
0.500	24	23	96
0.316	24	20	83
0.100	24	10	42
0.050	24	8	33
0.032	24	6	25

The analytical sensitivity of the RealStar® Zika Virus RT-PCR Kit 1.0 was determined by Probit analysis:

- For the detection of Zika virus specific RNA, the analytical sensitivity is 0.61 copies/µl eluate [95% confidence interval (CI): 0.39 - 1.27 copies/µl]

11.1.2 Analytical Specificity

The analytical specificity of the RealStar® Zika Virus 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 all relevant Zika virus genotypes will be detected.

The analytical specificity of the RealStar® Zika Virus RT-PCR Kit 1.0 was evaluated by testing a panel of genomic RNA extracted from non-Zika virus, alphaviruses and other pathogens.

The RealStar ® Zika Virus RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Dengue virus serotype 1
- Dengue virus serotype 2
- Dengue virus serotype 3
- Dengue virus serotype 4
- Japanese encephalitis virus
- Marburg virus (MARV)
- Sudan ebolavirus (SEBOV)
- St. Louis encephalitis virus
- West Nile virus
- Yellow fever virus
- Zaire ebolavirus (ZEBOV)
- Human parvovirus B19
- Chikungunya virus
- Murray Valley encephalitis virus
- *Plasmodium falciparum*

CAUTION	
	***Due to sequence homology between Usutu virus RNA and the target region used for the detection of Zika virus specific RNA, cross-reactivity with Usutu virus RNA cannot be ruled out. Usutu virus is a bird virus rarely infecting humans. It does not cause severe or fatal disease in human patients and an infection usually remains asymptomatic.***

11.1.3 Precision

Precision of the RealStar® Zika Virus RT-PCR Kit 1.0 was determined as intra-assay variability (variability within one experiment), inter-assay variability (variability between different experiments) and inter-lot variability (variability between different production lots). Total variability was calculated by combining the three analyses.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) - values. At least eight replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 2: Precision data for the detection of Zika virus specific RNA

Zika virus	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	31.33	0.07	0.21
Inter-Assay Variability	31.35	0.10	0.32
Inter-Lot Variability	31.42	0.11	0.35
Total Variability	31.39	0.11	0.35

Table 3: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	28.55	0.06	0.22
Inter-Assay Variability	28.44	0.14	0.49
Inter-Lot Variability	28.44	0.17	0.60
Total Variability	28.48	0.15	0.53

11.2 Performance Evaluation including Nucleic Acid Extraction

11.2.1 Serum Samples

Estimation of the Limit of Detection (LoD):

Serial dilutions of Zika virus strain H/PF/2013 (stock concentration TCID50/ml: $10^{6.82}$) obtained from the European Virus Archive (Marseille, France) were prepared. Using qRT-PCR and quantified *in vitro* transcribed RNA the number of Zika virus genome equivalents (geq) per milliliter stock was determined to be 1.26E+09.

For nucleic acid extraction, 126 µl of pooled serum were combined with 560 µl AVL buffer, containing 6 µl of the Internal Control (provided with the RealStar® Zika Virus RT-PCR Kit 1.0) and spiked with 14 µl diluted Zika virus stock. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 60 µl AVE buffer. Each sample was extracted in triplicate and tested with the RealStar® Zika Virus RT-PCR Kit 1.0 on the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad). The lowest concentration at which all three replicates were tested positive was treated as the tentative LoD. The results can be found in Table 4:

Table 4: Determination of the tentative LoD for the RealStar® Zika Virus RT-PCR Kit 1.0

Target	Concentration geq/ml	Call rate	Replicate 1 C_t (FAM™)	Replicate 2 C_t (FAM™)	Replicate 3 C_t (FAM™)
Zika virus (strain H/PF/2013)	2515.71	3/3	33.52	33.76	33.87
	795.61	3/3	35.13	35.36	34.85
	251.62	3/3	36.98	35.92	35.86
	79.57	2/3	42.00	-	36.05
	25.17	1/3	36.74	-	-
	7.96	1/3	-	37.49	-
	2.52	0/3	-	-	-

The RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 3/3 replicates with a concentration of 251.62 geq/ml serum.

Confirmation of the Limit of Detection (LoD):

Based on the tentative LoD, diluted Zika virus stock was spiked into 20 individual serum samples to a final concentration of 251.62 geq/ml. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) as described above. The obtained eluates were tested with the RealStar® Zika Virus RT-PCR Kit 1.0 on the following instruments:

- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- Rotor-Gene® Q (QIAGEN)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)

The results can be found in Tables 5, 6, 7 and 8.

Table 5: LoD confirmation on CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
1	+	36.84	31.55
2	+	38.29	32.16
3	+	36.07	31.71
4	+	35.18	31.32
5	+	36.95	31.60
6	+	36.22	31.71
7	+	35.48	32.06
8	+	35.12	32.04
9	+	36.05	31.49
10	+	36.07	31.89
11	+	35.77	31.69
12	-	-	32.68
13	+	36.51	32.17
14	+	36.21	32.32
15	+	34.96	31.66
16	+	36.08	32.18
17	+	35.97	32.05
18	+	35.47	31.41
19	+	36.07	31.86
20	+	36.15	31.87

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
Statistics	Mean C_t (n=19)	36.08	31.87
	SD	0.76	0.34
	CV %	2.09	1.06
	Result	19/20	

The RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 19/20 replicates at a concentration of 251.62 geq/m.l Therefore, the confirmed LoD is 251.62 geq/ml serum.

Table 6: LoD confirmation on Rotor-Gene® Q (QIAGEN)

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
1	+	30.34	31.55
2	+	30.74	32.16
3	+	30.57	31.71
4	+	30.05	31.32
5	+	30.29	31.60
6	+	30.58	31.71
7	+	30.60	32.06
8	+	30.63	32.04
9	+	30.32	31.49
10	+	30.42	31.89
11	+	30.29	31.69
12	+	31.39	32.68

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
13	+	31.02	32.17
14	+	30.89	32.32
15	+	30.36	31.66
16	+	30.83	32.18
17	+	30.64	32.05
18	+	30.27	31.41
19	+	30.29	31.86
20	+	30.32	31.87
Statistics	Mean C_t (n=20)	35.22	30.54
	SD	0.71	0.32
	CV %	2.01	1.04
	Result	20/20	

At the concentration of 251.62 geq/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/ml serum for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® Q (QIAGEN).

Table 7: LoD confirmation on ABI Prism® 7500 SDS (Applied Biosystems)

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
1	+	36.18	30.20
2	+	35.11	30.67
3	+	35.02	30.38
4	+	35.49	29.88

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
5	+	34.86	30.35
6	+	35.37	30.09
7	+	35.61	30.33
8	+	34.67	30.28
9	+	34.85	30.12
10	+	35.61	29.91
11	+	34.82	30.05
12	+	35.78	31.07
13	+	34.78	30.95
14	+	36.43	30.75
15	+	34.25	30.19
16	+	35.33	30.57
17	+	34.86	30.31
18	+	36.58	30.00
19	+	35.10	30.13
20	+	34.74	30.32
Statistics	Mean C_t (n=20)	35.27	30.32
	SD	0.62	0.33
	CV %	1.74	1.08
	Result	20/20	

At the concentration of 251.62 geq/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/ml serum for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS (Applied Biosystems).

Table 8: LoD confirmation on LightCycler® 480 Instrument II (Roche)

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
1	+	35.5	30.93
2	+	36.93	31.28
3	+	35.84	30.73
4	+	35.49	30.60
5	+	36.89	30.79
6	+	36.48	30.81
7	+	35.88	30.80
8	+	35.37	30.89
9	+	35.46	30.59
10	+	35.85	31.01
11	+	35.90	30.55
12	+	36.33	31.74
13	+	36.30	31.28
14	+	37.59	31.22
15	+	35.74	30.77
16	+	36.18	31.04
17	+	36.48	31.13
18	+	36.00	30.66
19	+	36.48	30.78
20	+	35.82	30.78

Zika virus concentration = 251.62 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
Statistics	Mean C_t (n=20)	36.13	30.92
	SD	0.57	0.29
	CV %	1.57	0.95
	Result	20/20	

At the concentration of 251.62 geq/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 251.62 geq/ml serum for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II (Roche).

11.2.2 Urine Samples

Estimation of the Limit of Detection (LoD):

Serial dilutions of Zika virus strain H/PF/2013 (stock concentration TCID50/ml: $10^{6.82}$) obtained from the European Virus Archive (Marseille, France) were prepared. Using qRT-PCR and quantified in vitro transcribed RNA the number of Zika virus genome equivalents (geq) per milliliter stock was determined to be 1.26E+09.

For nucleic acid extraction, 126 µl of pooled urine were combined with 560 µl AVL buffer, containing 6 µl of the Internal Control (provided with the RealStar® Zika Virus RT-PCR Kit 1.0) and spiked with 14 µl diluted Zika virus stock. The final mix was subjected to the extraction procedure following the manufacturer's instructions for the QIAamp® Viral RNA Mini Kit (QIAGEN). Elution was performed in 60 µl AVE buffer. Each sample was extracted in triplicate and tested with the RealStar® Zika Virus RT-PCR Kit 1.0 on the CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad). The lowest concentration at which all three replicates were tested positive was treated as the tentative LoD. The results can be found in Table 9:

Table 9: Determination of the tentative LoD for the RealStar® Zika Virus RT-PCR Kit 1.0

Target	Concentration geq/ml	Call rate	Replicate 1 C_t (FAM™)	Replicate 2 C_t (FAM™)	Replicate 3 C_t (FAM™)
Zika virus (strain H/PF/2013)	2515.71	3/3	33.15	33.34	33.11
	795.61	3/3	34.10	33.83	35.07
	251.62	3/3	35.54	35.64	35.27
	79.57	2/3	36.78	37.41	36.40
	25.17	1/3	38.38	37.72	-
	7.96	1/3	-	-	38.22
	2.52	0/3	-	-	-

The RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 3/3 replicates with a concentration of 79.57 geq/ml urine.

Confirmation of the Limit of Detection (LoD):

Based on the tentative LoD, diluted Zika virus stock was spiked into 20 individual urine samples to a final concentration of 79.57 geq/ml. Nucleic acids were extracted with the QIAamp® Viral RNA Mini Kit (QIAGEN) as described above. The obtained eluates were tested with the RealStar® Zika Virus RT-PCR Kit 1.0 on the following instruments:

- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)
- Rotor-Gene® Q (QIAGEN)
- ABI Prism® 7500 SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)

The results can be found in Tables 10, 11, 12 and 13.

Table 10: LoD confirmation on CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

Zika virus concentration = 79.57 geq/ml			
Specimen	Pos/Neg	C_t (FAM™)	C_t (VIC™)
1	+	36.72	30.77
2	+	36.34	31.06
3	+	37.21	31.07
4	+	36.41	31.21
5	+	37.89	30.59
6	-	-	31.21
7	+	36.12	31.25
8	+	36.25	30.43
9	+	38.03	30.64
10	+	37.36	31.11
11	+	38.08	31.99
12	+	37.26	30.95
13	+	36.30	31.27
14	+	35.75	31.74
15	+	36.68	31.01
16	+	39.07	31.08
17	+	37.17	31.67
18	+	35.79	31.04
19	+	38.01	31.44
20	+	37.17	31.05

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (VIC™)**
Statistics	Mean C_t (n=19)	37.03	31.08
	SD	0.90	0.27
	CV %	2.42	0.87
	Result	19/20	

The RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the CFX96™ Deep Well Real-Time PCR Detection System detected 19/20 replicates at a concentration of 79.57 geq/ml. Therefore, the confirmed LoD is 79.57 geq/ml urine.

Table 11: LoD confirmation on Rotor-Gene® Q (QIAGEN)

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
1	+	36.45	29.66
2	+	35.47	30.20
3	+	36.03	30.37
4	+	35.70	30.08
5	+	35.97	29.53
6	+	37.38	30.41
7	-	-	30.03
8	+	37.34	30.23
9	+	35.36	29.49
10	+	36.40	30.10
11	+	35.57	29.83
12	+	36.52	29.74

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
13	+	37.59	30.21
14	+	35.49	29.53
15	+	36.12	29.89
16	+	36.53	29.83
17	+	36.76	30.73
18	+	36.55	29.79
19	+	36.19	30.39
20	+	37.39	29.79
Statistics	Mean C_t (n=19)	36.36	29.99
	SD	0.70	0.34
	CV %	1.92	1.13
	Result	19/20	

At the concentration of 79.57 geq/ml 19/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/ml urine for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the Rotor-Gene® Q (QIAGEN).

Table 12: LoD confirmation on ABI Prism® 7500 SDS (Applied Biosystems)

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
1	+	36.11	29.48
2	+	36.63	29.97
3	+	35.76	29.94
4	+	37.29	29.85
5	+	36.54	29.56
6	+	37.37	29.86
7	+	36.36	30.03
8	+	37.27	30.11
9	+	36.05	29.45
10	+	36.65	30.06
11	+	37.33	29.86
12	+	35.88	29.53
13	+	36.09	30.02
14	+	37.58	29.51
15	+	37.36	29.86
16	+	36.07	29.80
17	+	36.50	30.23
18	+	37.36	29.81
19	+	35.86	30.20
20	+	35.74	29.74

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
Statistics	Mean C_t (n=20)	36.58	29.84
	SD	0.65	0.24
	CV %	1.79	0.80
	Result	20/20	

At the concentration of 79.57 geq/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/ml urine for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the ABI Prism® 7500 SDS (Applied Biosystems).

Table 13: LoD confirmation on LightCycler® 480 Instrument II (Roche)

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
1	+	37.29	30.15
2	+	37.03	30.34
3	+	37.67	30.48
4	+	36.83	30.42
5	+	36.58	30.02
6	+	36.90	30.69
7	+	37.50	30.66
8	+	38.63	30.71
9	+	36.74	30.10
10	+	37.95	30.54
11	+	37.13	30.52
12	+	38.12	30.29

Zika virus concentration = 79.57 geq/ml			
Specimen	**Pos/Neg**	**C_t (FAM™)**	**C_t (JOE™)**
13	+	36.53	30.54
14	+	37.41	30.19
15	+	37.16	30.43
16	+	36.70	30.61
17	+	37.79	30.86
18	+	37.28	30.41
19	+	40.00	30.86
20	+	38.42	30.35
Statistics	Mean C_t (n=20)	37.48	30.46
	SD	0.84	0.24
	CV %	2.25	0.78
	Result	20/20	

At the concentration of 79.57 geq/ml 20/20 replicates were detected positive and thereby confirm the LoD to be 79.57 geq/ml urine for the RealStar® Zika Virus RT-PCR Kit 1.0 in conjunction with the QIAamp® Viral RNA Mini Kit manual extraction system and the LightCycler® 480 Instrument II (Roche).

11.3 Clinical Performance Evaluation

To evaluate the clinical performance of the RealStar® Zika Virus RT-PCR Kit 1.0. a total of 208 clinical specimens from 153 patients with signs and symptoms of Zika virus infection (106 female (F) and 47 male (M)) were analyzed retrospectively in a blinded fashion. From the 208 samples tested 103 were serum and 105 were urine specimens.

For RNA extraction the QIAamp® Viral RNA Mini Kit (QIAGEN) was used. 140 µl of each urine or serum sample were combined with 560 µl AVL buffer, containing 6 µl of the Internal Control provided with the RealStar® Zika Virus RT-PCR Kit 1.0. The sample/AVL buffer mix was subjected to the extraction procedure following the manufacturer's instructions. Elution was performed in 60 µl AVE buffer.

Eluates were tested with the RealStar® Zika Virus RT-PCR Kit 1.0. as well as with the real-time RT-PCR as described by Lanciotti et. al (Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, et al. (2008) Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007; Emerg Infect Dis. 2008 Aug; 14(8): 1232–1239).

For the execution of the real-time RT-PCR described by Lanciotti et al. the following oligonucleotides; Table 14 have been used:

Table 14: Oligonucleotides used for real-time RT-PCR described by Lanciotti et al.

Primer/Probe	Sequence 5'-> 3'
Zika virus 1086	CCGCTGCCCAACACAAG
Zika virus 1162c	CCACTAACGTTCTTTTGCAGACAT
Zika virus 1107-FAM	AGCCTACCTTGACAAGCAGTCAGACACTCAA

Both tests, the RealStar® Zika Virus RT-PCR Kit 1.0. as well as the real-time RT-PCR described by Lanciotti et al., have been performed on a CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad). Results generated for the different samples tested are shown in Table 15:

Table 15: Results from testing clinical samples

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
1	F	2	Serum	-	-	29.88	-	-
		2	Urine	-	-	31.57	-	-
2	F	5	Urine	-	-	29.50	-	-
3	F	1	Serum	-	-	30.37	-	-
		1	Urine	-	-	29.49	-	-
4	F	1	Serum	-	-	30.32	-	-
		1	Urine	-	-	30.16	-	-
5	F	1	Serum	-	-	31.04	-	-
		1	Urine	-	-	30.10	-	-
6	F	3	Serum	-	-	30.77	-	-
		3	Urine	-	-	30.14	-	-
7	M	4	Serum	-	-	30.05	-	-
		4	Urine	-	-	31.21	-	-
8	M	0	Serum	-	-	29.61	-	-
		0	Urine	-	-	29.72	-	-
9	F	6	Urine	-	-	29.88	-	-
10	F	4	Serum	-	-	31.88	-	-
		4	Urine	-	-	29.83	-	-
11	F	0	Serum	-	-	31.33	-	-
		0	Urine	-	-	30.35	-	-
12	M	3	Serum	-	-	30.10	-	-
		3	Urine	-	-	30.34	-	-

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
13	F	4	Urine	33.03	+	29.92	33.85	+
14	F	2	Serum	33.53	+	29.84	32.73	+
15	M	5	Serum	-	-	30.53	-	-
		5	Urine	33.76	+	30.02	34.83	+
16	M	2	Serum	-	-	30.45	-	-
		2	Urine	-	-	29.87	-	-
17	F	3	Serum	30.12	+	31.45	29.68	+
18	F	3	Urine	-	-	29.81	-	-
19	F	2	Serum	-	-	29.60	-	-
		2	Urine	33.41	+	30.24	33.95	+
20	F	6	Urine	-	-	29.85	38.64	+
21	M	2	Serum	-	-	31.79	-	-
		2	Urine	-	-	29.96	-	-
22	M	1	Serum	-	-	32.04	-	-
		1	Urine	-	-	29.86	-	-
23	F	2	Serum	-	-	30.90	-	-
24	F	7	Urine	-	-	30.46	-	-
25	F	0	Serum	33.35	+	30.43	33.33	+
		3	Urine	31.93	+	31.23	32.81	+
26	F	3	Serum	-	-	30.81	-	-
		3	Urine	-	-	30.13	-	-
27	F	0	Urine	-	-	29.40	-	-
28	M	5	Serum	-	-	31.43	-	-
		5	Urine	-	-	30.49	-	-
29	F	7	Urine	-	-	30.09	-	-

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
30	M	1	Serum	-	-	32.57	-	-
		1	Urine	-	-	29.97	-	-
31	F	1	Serum	35.91	+	31.19	37.41	+
		1	Urine	30.14	+	30.06	30.05	+
32	M	2	Serum	-	-	30.49	-	-
		2	Urine	-	-	30.29	-	-
33	F	2	Serum	32.15	+	31.35	31.84	+
34	F	0	Urine	35.43	+	29.90	36.43	+
35	M	7	Urine	34.39	+	29.59	35.78	+
36	F	2	Serum	-	-	30.01	37.85	+
37	F	2	Urine	-	-	30.02	-	-
38	M	8	Urine	-	-	35.35	-	-
39	F	6	Serum	30.94	+	30.13	30.34	+
40	M	3	Serum	-	-	29.80	-	-
		3	Urine	-	-	30.76	-	-
41	M	3	Serum	-	-	30.36	-	-
		3	Urine	-	-	30.38	-	-
42	F	0	Serum	30.42	+	31.04	29.72	+
43	M	6	Urine	-	-	30.04	-	-
44	F	1	Serum	35.92	+	30.69	36.27	+
		1	Urine	35.16	+	29.77	38.48	+
45	M	6	Urine	-	-	30.06	-	-
46	M	4	Serum	-	-	30.98	-	-
		4	Urine	-	-	30.11	-	-
47	M	3	Urine	34.23	+	30.42	36.50	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
48	M	2	Urine	26.17	+	29.09	25.65	+
49	M	4	Serum	-	-	30.08	-	-
		4	Urine	-	-	30.23	-	-
50	F	6	Urine	-	-	29.91	-	-
51	F	2	Serum	35.04	+	32.35	34.74	+
52	F	3	Serum	33.48	+	31.29	33.18	+
53	M	5	Serum	-	-	29.69	-	-
		5	Urine	-	-	30.04	-	-
54	F	1	Serum	26.08	+	29.83	25.24	+
55	F	2	Urine	-	-	30.02	-	-
56	F	6	Serum	-	-	31.71	-	-
		6	Urine	-	-	31.11	-	-
57	F	7	Urine	-	-	30.10	-	-
58	F	4	Serum	-	-	32.75	-	-
		4	Urine	-	-	30.20	-	-
59	M	1	Serum	-	-	30.41	-	-
		1	Urine	-	-	29.87	-	-
60	M	0	Serum	-	-	29.62	-	-
		0	Urine	-	-	29.82	-	-
61	F	2	Serum	-	-	30.52	-	-
62	F	7	Urine	-	-	30.06	-	-
63	F	0	Serum	-	-	31.47	-	-
		0	Urine	-	-	29.74	-	-
64	F	7	Urine	28.03	+	30.03	28.68	+
65	F	2	Serum	37.79	+	29.33	38.15	+
		2	Urine	36.07	+	29.47	37.66	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
66	F	2	Serum	33.06	+	31.45	33.21	+
67	F	4	Serum	30.66	+	29.53	30.19	+
68	M	1	Serum	-	-	30.79	-	-
69	M	2	Serum	35.08	+	31.68	35.14	+
70	F	3	Urine	-	-	30.39	39.17	+
71	F	1	Serum	-	-	29.83	-	-
72	M	1	Serum	36.58	+	31.89	-	-
73	F	3	Urine	33.36	+	30.55	32.98	+
74	F	4	Serum	-	-	30.94	-	-
		4	Urine	-	-	30.92	-	-
75	F	3	Serum	35.03	+	29.82	35.30	+
76	M	3	Serum	34.06	+	29.57	34.01	+
77	F	3	Urine	35.02	+	31.02	34.66	+
78	F	6	Serum	32.00	+	30.08	32.03	+
		6	Urine	33.55	+	30.06	35.52	+
79	F	2	Serum	34.99	+	29.65	36.29	+
		2	Urine	26.59	+	29.72	26.94	+
80	M	3	Serum	31.93	+	29.78	31.17	+
		3	Urine	37.78	+	32.00	-	-
81	F	4	Serum	35.07	+	31.86	35.32	+
		4	Urine	34.51	+	30.83	36.42	+
82	F	1	Serum	33.51	+	29.88	33.09	+
83	F	0	Serum	26.83	+	33.66	26.55	+
		8	Urine	19.37	+	-*	17.88	+
84	F	3	Urine	34.83	+	30.38	35.38	+
85	M	5	Urine	33.31	+	30.25	33.31	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
86	M	6	Urine	-	-	30.52	-	-
87	M	3	Serum	35.07	+	29.62	34.18	+
		3	Urine	31.01	+	30.34	30.48	+
88	F	6	Serum	-	-	29.54	-	-
		6	Urine	28.58	+	30.67	28.01	+
89	F	3	Serum	30.87	+	29.74	29.98	+
90	F	2	Serum	28.58	+	29.51	27.77	+
91	F	1	Serum	33.71	+	31.35	33.66	+
92	F	3	Urine	35.60	+	30.03	38.29	+
93	M	3	Serum	36.78	+	29.61	37.92	+
94	M	6	Urine	-	-	30.34	38.81	+
95	F	3	Serum	37.60	+	30.86	37.61	+
96	F	5	Urine	33.77	+	30.35	34.09	+
97	M	3	Serum	28.47	+	29.75	27.18	+
98	F	2	Serum	32.09	+	32.02	31.38	+
		2	Urine	37.64	+	31.16	38.33	+
99	F	1	Urine	33.47	+	30.57	35.44	+
100	F	4	Urine	-	-	30.31	-	-
101	M	7	Urine	-	-	30.30	-	-
102	F	0	Serum	-	-	32.62	-	-
103	F	3	Urine	33.63	+	30.30	33.45	+
104	F	2	Serum	36.05	+	32.30	36.08	+
		2	Urine	37.63	+	30.10	38.13	+
105	F	0	Serum	33.25	+	31.81	33.38	+
106	F	1	Urine	34.63	+	29.89	35.41	+
107	F	2	Serum	37.96	+	32.13	39.51	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
108	F	5	Serum	33.81	+	29.98	33.68	+
		5	Urine	35.89	+	30.31	36.57	+
109	F	1	Serum	-	-	30.38	-	-
110	M	7	Urine	-	-	30.61	-	-
111	F	1	Serum	37.07	+	31.19	38.54	+
112	F	2	Urine	35.76	+	29.94	-	-
113	F	2	Serum	31.13	+	31.50	30.62	+
114	M	1	Urine	-	-	29.83	-	-
115	F	5	Serum	-	-	30.30	36.89	+
		5	Urine	31.12	+	30.35	30.34	+
116	F	2	Serum	35.75	+	30.80	37.02	+
117	F	3	Urine	37.90	+	30.37	-	-
118	F	1	Serum	35.27	+	33.39	35.16	+
119	F	3	Serum	35.41	+	32.99	36.20	+
120	F	3	Urine	36.50	+	29.81	-	-
121	M	6	Urine	28.27	+	30.13	28.31	+
122	M	3	Serum	30.27	+	29.45	29.05	+
123	M	1	Serum	25.15	+	28.89	24.59	+
124	F	3	Urine	34.03	+	30.57	35.76	+
125	F	5	Urine	27.22	+	29.90	27.75	+
126	F	3	Urine	34.14	+	30.02	35.86	+
		1	Urine	37.81	+	30.20	-	-
127	F	2	Serum	27.47	+	29.41	27.38	+
		6	Serum	37.28	+	32.06	37.99	+
		6	Urine	26.93	+	29.28	25.90	+
128	M	2	Serum	29.58	+	29.62	28.76	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
129	F	3	Serum	34.26	+	30.16	33.89	+
		3	Urine	37.72	+	31.16	-	-
130	M	3	Urine	35.43	+	30.31	35.13	+
131	F	0	Serum	-	-	30.37	-	-
		0	Urine	36.28	+	29.79	36.82	+
132	F	3	Serum	30.58	+	29.49	30.10	+
133	F	4	Serum	35.20	+	32.73	35.45	+
134	F	0	Urine	-	-	29.62	-	-
135	M	4	Serum	32.37	+	31.16	32.22	+
		4	Serum	34.19	+	31.47	34.39	+
		4	Urine	33.21	+	30.41	34.16	+
136	F	0	Serum	36.58	+	32.12	37.04	+
137	F	3	Urine	32.11	+	29.89	31.41	+
138	F	2	Serum	34.84	+	30.31	34.56	+
139	M	3	Urine	33.61	+	29.59	33.88	+
140	M	2	Serum	31.01	+	30.44	30.39	+
		2	Urine	33.44	+	30.17	33.48	+
141	F	4	Urine	31.12	+	29.59	30.32	+
142	F	3	Serum	33.65	+	29.54	33.50	+
143	F	0	Serum	29.11	+	30.82	28.39	+
		0	Urine	35.88	+	29.89	37.81	+
144	F	0	Serum	31.06	+	29.53	30.12	+
145	F	4	Serum	33.29	+	32.66	33.17	+
146	F	4	Urine	26.17	+	29.81	26.00	+
147	M	1	Urine	-	-	30.58	-	-
148	M	5	Urine	31.22	+	29.70	30.27	+

Table 15: (continuation)

Patient ID	Gender	Days after Onset of Symptoms	Specimen Type	RealStar® Zika Virus RT-PCR Kit 1.0			Real-time RT-PCR described by Lanciotti et al.	
				C_t (FAM™)	Call	C_t (VIC™)	C_t (FAM™)	Call
149	F	2	Serum	30.07	+	30.23	29.81	+
150	F	0	Serum	35.36	+	30.58	35.23	+
151	F	2	Serum	28.97	+	30.06	28.29	+
152	F	5	Serum	37.16	+	30.29	-	-
		5	Urine	-	-	30.05	-	-
153	F	2	Serum	-	-	30.16	-	-

* Detection of the Internal Control in the VIC™ detection channel is not required for positive results in the FAM™ detection channel. The high Zika virus load in the sample leads to an absent Internal Control signal.

Analysis of results with respect to paired serum/urine samples

Paired urine and serum specimens were collected from 52 patients in the study and were analyzed. A patient was considered infected with Zika virus (i.e. positive infection status) if the serum and/or the urine sample were tested positive for Zika virus specific RNA with the real-time RT-PCR assay described by Lanciotti et al. The patient's infection status was considered negative if both the serum and the urine sample were tested negative with the real-time RT-PCR assay described by Lanciotti et al..

Of the 52 paired samples analyzed 23 showed a positive result for the serum and/or urine specimen (i.e. patient infection status = positive) with the real-time RT-PCR assay described by Lanciotti et al., whereas 29 paired samples were negative for both, the serum and the urine specimen (i.e. patient infection status = negative). All 23 patients with positive infection status were tested positive also with the RealStar® Zika Virus RT-PCR Kit 1.0. in the serum and/or urine sample.

Of the 29 paired samples from patients with negative infection status 28 were tested negative for Zika virus specific RNA in the serum as well as in the urine specimen with the RealStar® Zika Virus RT-PCR Kit 1.0. One patient with negative infection status was tested positive in the serum sample and negative in the urine sample with the RealStar® Zika Virus RT-PCR Kit 1.0.

In conclusion, the positive percent agreement of the results generated with the RealStar® Zika Virus RT-PCR Kit 1.0. with the results from the real-time RT-PCR assay described by Lanciotti et al. is 100.0%. The negative percent agreement between the two assays is 96.6%. The results are summarized in Table 16:

Table 16: Result summary for patient infection status (detection of Zika virus RNA in serum and/or urine from patients with paired serum/urine specimens taken). Total number of paired samples was 52

Results from real-time RT-PCR assay described by Lanciotti et al.	Results of the RealStar® Zika Virus RT-PCR Kit 1.0		
	Positve		Negative
23 Positive	23†		0
29 Negative	1*		28
Total (52 paired samples)	24		28
			95% CI
Positive Percent Agreement	23/23	100.0%	85.7% - 100.0%
Negative Percent Agreement	28/29	96.6%	82.8% - 99.4%

* This patient was positive only in the serum sample and negative in the urine sample with the RealStar® Zika Virus RT-PCR Kit 1.0

† Four of these patients were positive only in the urine sample and negative in the serum sample for both assays. Two patients were positive in the serum sample and negative in the urine sample with the assay described by Lanciotti et al., but positive in the serum and the urine sample with the RealStar® Zika Virus RT-PCR Kit 1.0. One patient was positive in the serum and in the urine sample with the assay described by Lanciotti et al., but positive only in the urine sample and negative in the serum sample with the RealStar® Zika Virus RT-PCR Kit 1.0

Analysis of results with respect to the specimen type serum

Of the 103 serum samples included in the comparison study 62 were tested positive for Zika virus RNA with the real-time RT-PCR assay described by Lanciotti et al., whereas 41 were tested negative. Of the 62 positive serum samples 60 were also tested positive with the RealStar® Zika Virus RT-PCR Kit 1.0., whereas two were tested negative. Of the 41 serum samples tested negative for Zika virus with the real-time RT-PCR assay described by Lanciotti et al. 39 were tested negative and two positive with the RealStar® Zika Virus RT-PCR Kit 1.0.

In conclusion for serum, the positive percent agreement of the results generated with the RealStar® Zika Virus RT-PCR Kit 1.0 with the results from the real-time RT-PCR assay described by Lanciotti et al. is 96.8%. The negative percent agreement between the two assays is 95.1%. The results are summarized in Table 17:

Table 17: Result summary for the detection of Zika virus RNA in serum samples. Total of serum samples was 103

Results from real-time RT-PCR assay described by Lanciotti et al.	Results of the RealStar® Zika Virus RT-PCR Kit 1.0		
	Positve		Negative
62 Positive	60		2
41 Negative	2		39
Total (103 samples)	62		41
			95% CI
Positive Percent Agreement	60/62	96.8%	89.0% - 99.1%
Negative Percent Agreement	39/41	95.1%	83.9% - 98.7%

Analysis of results with respect to the specimen type urine

Of the 105 urine samples included in the comparison study 49 were tested positive for Zika virus RNA with the real-time RT-PCR assay described by Lanciotti et al., whereas 56 were tested negative. Of the 49 positive urine samples 46 were also tested positive with the RealStar® Zika Virus RT-PCR Kit 1.0, whereas three were tested negative. Of the 56 urine samples tested negative for Zika virus with the real-time RT-PCR assay described by Lanciotti et al. 50 were tested negative and six positive with the RealStar® Zika Virus RT-PCR Kit 1.0.

In conclusion for urine, the positive percent agreement of the results generated with the RealStar® Zika Virus RT-PCR Kit 1.0 with the results from the real-time RT-PCR assay described by Lanciotti et al. is 93.9%. The negative percent agreement between the two assays is 89.3%. The results are summarized in Table 18:

Table 18: Result summary for the detection of Zika virus RNA in urine samples. Total number of urine samples was 105

Results from real-time RT-PCR assay described by Lanciotti et al.	Results of the RealStar® Zika Virus RT-PCR Kit 1.0		
	Positve		Negative
49 Positive	46		3
56 Negative	6		50
Total (105 samples)	52		53
			95% CI
Positive Percent Agreement	46/49	93.9%	83.5% - 98.0%
Negative Percent Agreement	50/56	89.3%	78.5% - 95.0%

12. Limitations

- Strict compliance with the instructions for use is required for optimal results.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in *in vitro* diagnostic procedures.
- Good laboratory practice is essential for proper performance of this assay. Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- This assay must not be used on the specimen directly. Appropriate nucleic acid extraction methods have to be conducted prior to using this assay.
- The presence of RT-PCR inhibitors may cause false negative or invalid results.
- Potential mutations within the target regions of the Zika virus genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogen.
- As with any diagnostic test, results of the RealStar® Zika Virus RT-PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.
- Due to sequence homology between Usutu virus RNA and the target region used for the detection of Zika virus specific RNA, cross-reactivity with Usutu virus RNA cannot be ruled out. Usutu virus is a bird virus rarely infecting humans. It does not cause severe or fatal disease in human patients and an infection usually remains asymptomatic.

13. Quality Control

In accordance with the altona Diagnostics GmbH EN ISO 13485-certified Quality Management System, each lot of RealStar® Zika Virus RT-PCR Kit 1.0 is tested against predetermined specifications to ensure consistent product quality.

14. Technical Assistance

For technical advice, please contact our Technical Support:

e-mail: **support@altona-diagnostics.com**
phone: **+49-(0)40-5480676-0**

15. Literature

Versalovic, James, Carroll, Karen C.,Funke, Guido, Jorgensen, James H., Landry, Marie Louise and David W. Warnock (ed). Manual of Clinical Microbiology. 10th Edition. ASM Press, 2011.

Cohen, Jonathan, Powderly, William G, and Steven M Opal. Infectious Diseases, Third Edition. Mosby, 2010.

16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); Mx 3005P™ (Stratagene); VERSANT® (Siemens Healthcare); ABI Prism® (Applied Biosystems); LightCycler® (Roche); Rotor-Gene®, QIAamp®, (QIAGEN); CFX96™ (Bio-Rad); JOE™, FAM™, ROX™ (Life Technologies).

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The RealStar® Zika Virus RT-PCR Kit 1.0 is a CE-marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/EC.

Product not licensed with Health Canada and not FDA cleared or approved.
Not available in all countries.

17. Explanation of Symbols

IVD	*In vitro* diagnostic medical device
LOT	Batch code
CAP	Cap color
REF	Product number
CONT	Content
NUM	Number
COMP	Component
GTIN	Global trade identification number
	Consult instructions for use
	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
	Caution
	Note
	Version

Notes: