altona DIAGNOSTICS

Instructions for Use

RealStar®
Yellow Fever Virus RT-PCR Kit 1.0

09/2021 EN

RealStar®

RealStar®

Yellow Fever Virus RT-PCR Kit 1.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare Diagnostics)

ABI Prism® 7500 SDS (Applied Biosystems)

ABI Prism® 7500 Fast SDS (Applied Biosystems)

LightCycler® 480 Instrument II (Roche)

Rotor-Gene® 6000 (Corbett Research)

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

CFX96™ Real-Time PCR Detection System (Bio-Rad)

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

CE

IVD

REF 671013

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Content

1. Intended Use

The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of yellow fever virus specific RNA.

2. Kit Components

Table 1: 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

* To be used as Negative Control

3. Storage

- The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 is shipped on dry ice. The components of the kit should arrive frozen. If one or more components is not frozen upon receipt, or if tubes have been compromised during shipment, contact altona Diagnostics GmbH for assistance.
- All components should be stored at -25 °C to -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 at +2 °C to +8 °C should not exceed a period of 2 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 (see chapter 8.1 Sample Preparation)
- 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

Yellow fever virus (YFV) is the prototype of the genus *Flavivirus*, which comprises around 70 different arthropod-borne viruses [**1**]. The YFV genome is an 11kb single-stranded positive-sense RNA genome coding for a polyprotein, which is post- and co-translationally processed into three structural proteins and seven non-structural proteins [**2**,**3**]. Yellow fever is endemic in tropical regions of Africa and South America [**1**].

Three forms of yellow fever are distinguished: 1) urban yellow fever in which the virus is spread from person to person by peri-domestic *Aedes aegypti* mosquitoes, 2) intermediate yellow fever caused by the YFV, which is transmitted by semi-domestic mosquitoes to both monkeys and humans, and 3) jungle (sylvan) yellow fever in which YFV is transmitted by tree-hole breeding mosquitoes to non-human primates and sometimes humans [**1**,**2**].

The majority of patients infected with YFV have no or only mild illness. In persons who develop symptoms, the incubation is typically 3–6 days. The initial symptoms include abrupt onset of fever, chills, severe headache, back pain, general body aches, nausea, and vomiting, fatigue, and weakness. After a brief symptome remission which lasts hours to a day, approximately 15 % of infected individuals progress to develop a more severe form of the disease. This severe form is characterized by high fever, jaundice, bleeding, and eventually shock and failure of multiple organs [**4**,**5**].

No specific treatments have been found to benefit patients with yellow fever, only supportive care to treat dehydration, respiratory failure, and fever [**1**,**4**,**6**].

All the current commercially available YF vaccines are live attenuated viral vaccines from the 17D lineage, which elicits a rapid, exceptionally strong, and markedly durable adaptive immune response [**4**,**5**].

The clinical diagnosis of YF is difficult because of symptom similarities with a wide range of diseases, including dengue fever, other hemorrhagic viral diseases, leptospirosis, viral hepatitis, and malaria; hence laboratory confirmation is essential [**2**].

[1] Monath, Thomas P., and Pedro F.c. Vasconcelos. “Yellow fever.” Journal of Clinical Virology, vol. 64, 2015, pp. 160–173., doi:10.1016/j.jcv.2014.08.030.

[2] Domingo, C., et al. “Advanced Yellow Fever Virus Genome Detection in Point-of-Care Facilities and Reference Laboratories.” Journal of Clinical Microbiology, vol. 50, no. 12, Oct. 2012, pp. 4054–4060., doi:10.1128/jcm.01799-12.

[3] Volk, D.e., et al. “Yellow Fever Envelope Protein Domain III NMR Structure (S288-K398).” Oct. 2008, doi:10.2210/pdb2jqm/pdb.

[4] Monath, Thomas P, and Alan D.t Barrett. “Pathogenesis and Pathophysiology of Yellow Fever.” Advances in Virus Research, 2003, pp. 343–395., doi:10.1016/s0065-3527(03)60009-6

[5] Deubel, Vincent, et al. “Molecular detection and characterization of yellow fever virus in blood and liver specimens of a non-Vaccinated fatal human case.” Journal of Medical Virology, vol. 53, no. 3, 1997, pp. 212–217., doi:10.1002/(sici)1096-9071(199711)53:3 212::aid-jmv5] 3.0.co;2-b.

[6] Pan American Health Organization (PAHO)/ World Health Organization (WHO) “Laboratory Diagnosis of Yellow Fever Virus infection” February 2018

NOTE

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® Yellow Fever Virus RT-PCR Kit 1.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of yellow fever virus specific RNA.

This kit was developed for the detection of all described yellow fever virus strains including the vaccine strain 17D.

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 labeled with fluorescent reporter and quencher dyes.

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

Using probes linked to distinguishable dyes enables the parallel detection of YFV 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 labeled probes

The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 consists of:

- Master A
- Master B
- Internal Control
- Positive Control
- Water (PCR grade)*

* To be used as Negative Control

Master A and Master B contain all components (PCR buffer, reverse transcriptase, DNA polymerase, magnesium salt, primers and probes) to allow reverse transcription, PCR mediated amplification and detection of YFV specific RNA and the Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® Yellow Fever 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 Healthcare Diagnostics)
- ABI Prism® 7500 SDS (Applied Biosystems)
- ABI Prism® 7500 Fast SDS (Applied Biosystems)
- LightCycler® 480 Instrument II (Roche)
- Rotor-Gene® 6000 (Corbett Research)
- Rotor-Gene® Q5/6 plex Platform (QIAGEN)
- CFX96™ Real-Time PCR Detection System (Bio-Rad)
- CFX96™ Deep Well Real-Time PCR Detection System (Bio-Rad)

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
  - Being frozen 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 separately 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 of contaminating 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

Extracted RNA is the starting material for the RealStar® Yellow Fever Virus RT-PCR Kit 1.0.

The quality of the extracted RNA has a profound impact on the performance of the entire test system. It is recommended to ensure that the system used for nucleic acid extraction is compatible with real-time PCR technology. The following kits and systems are suitable for nucleic acid extraction:

- QIAamp® Viral RNA Mini Kit (QIAGEN)
- QIAsymphony® (QIAGEN)
- NucliSENS® easyMAG® (bioMérieux)
- MagNA Pure 96 System (Roche)
- m2000sp (Abbott)
- Maxwell® 16 IVD Instrument (Promega)
- VERSANT® kPCR Molecular System SP (Siemens Healthcare)

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the nucleic acid extraction procedure for use with RealStar® Yellow Fever 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 1 min at approximately 17,000 x g (~ 13,000 rpm), using a new collection tube, prior to the elution of the nucleic acid.

Avoid thaw-freeze-cycles where possible.

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® Yellow Fever 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.
- No matter which method/system is used for nucleic acid extraction, the IC **must not** be added directly to the specimen. The IC should always be added to the specimen/lysis buffer mixture. The volume of the IC which has to be added, always and only depends on the elution volume. It represents 10 % of the elution volume. For instance, if the nucleic acid is going to be eluted in 60 µl of elution buffer or water, 6 µl of IC per sample must be added into 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, at least the negative control must include the IC.

CAUTION

No matter which method/system is used for nucleic acid extraction, 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 [Water (PCR grade) included in the RealStar® Yellow Fever Virus RT-PCR Kit 1.0] 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 1,000 x g (~ 3,000 rpm).

After completion of the PCR setup the PCR mix in the sealed 96-well reaction plate/ closed optical reaction tube is stable at +20 °C to +25 °C for max. 30 min.

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® Yellow Fever 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

► Program the instrument with the following settings:

Settings	
Reaction Volume	30 µl
Ramp Rate	Default
Passive Reference	None

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
YFV specific RNA	YFV	FAM™	(None)
Internal Control (IC)	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 Starting the Real-Time PCR

Place the 96-well reaction plate/optical reaction tubes in the real-time PCR instrument and start the real-time PCR run according to the user manual of the respective instrument.

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® Yellow Fever 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 (qualitative)

A **qualitative** diagnostic test run is **valid**, if the following control conditions are met:

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

* The presence or absence of a signal in the JOE™ channel is not relevant for the validity of the test run.

10.1.2 Invalid Diagnostic Test Run (qualitative)

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

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

10.2 Interpretation of Results

10.2.1 Qualitative Analysis

Detection Channel		Result Interpretation
FAM™	JOE™	
+	+*	YFV specific RNA detected.
-	+	No YFV specific RNA detected. Sample does not contain detectable amounts of YFV 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 YFV RNA load in the sample can lead to a reduced or absent Internal Control signal.

11. Performance Evaluation

Performance evaluation of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 was done using a yellow fever virus specific *in vitro* transcript.

11.1 Analytical Sensitivity

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

Table 2: RT-PCR results used for the calculation of the analytical sensitivity with respect to the detection of YFV specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.600	24	24	100
10.000	24	24	100
3.160	24	24	100
1.000	24	24	100
0.316	24	21	87.5
0.100	24	9	37.5
0.032	24	4	16.7
0.010	24	2	8.3
0.003	24	0	0

The analytical sensitivity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 was determined by probit analysis:

- For the detection of YFV specific RNA, the analytical sensitivity is 0.69 copies/µl [95 % confidence interval (CI): 0.41 - 1.56 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® Yellow Fever 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 YFV genotypes will be detected.

11.2.1 Cross-Reactivity

The analytical specificity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 with respect to cross-reactivity with other pathogens than YFV was evaluated by testing a panel of genomic RNA/DNA extracted from viruses related to YFV and other pathogens causing similar symptoms. The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Chikungunya virus
- Crimean-Congo hemorrhagic fever virus
- Dengue virus serotype 1
- Dengue virus serotype 4
- Ebola virus
- Hepatitis C virus
- Japanese encephalitis virus
- Lassa virus
- Marburg virus
- Murray Valley encephalitis virus
- *Plasmodium falciparum*
- West Nile virus
- Zika virus

In addition, the analytical specificity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 was evaluated for the WHO by the US Centers for Disease Control and Prevention, Division of Vector-Borne Diseases (Fort Collins, Colorado, USA). The US CDC is a WHO Collaborating Centre for Arthropod-Borne Viruses Reference and Research. The evaluation was conducted according to the “WHO Protocol for the Laboratory Evaluation of Yellow Fever Nucleic Acid Assays”. The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 did not cross-react with any of the following pathogens:

- Chikungunya virus
- Dengue virus serotypes 1-4
- Ebola virus
- HIV
- Influenza A (H1N1)
- Japanese encephalitis virus
- Lassa virus
- Marburg virus
- Measles virus
- Powassan virus
- West Nile virus
- Zika virus

11.2.2 Inclusivity

Inclusivity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 was evaluated for WHO by the US Centers for Disease Control and Prevention, Division of Vector-Borne Diseases (Fort Collins, Colorado, USA). The US CDC is a WHO Collaborating Centre for Arthropod-Borne Viruses Reference and Research. The laboratory evaluation was conducted according to the "WHO Protocol for the Laboratory Evaluation of Yellow Fever Nucleic Acid Assays". All YFV strains tested (for details refer to Table 3) were detected by the RealStar® Yellow Fever Virus RT-PCR Kit 1.0.

Table 3: Strains tested by CDC/WHO for inclusivity

YFV Strain	Location	Year
17D-204 Vaccine	N/A	N/A
Asibi	Ghana	1927
14FA	Angola	1971
614819	Panama	1974
BA-55	Nigeria	1986
BC-7914	Kenya	1993
FMD-1240	Peru	2007
CAREC M2-09	Trinidad	2009
InHRR 10a-10	Venezuela	2010

11.3 Precision

Precision of the RealStar® Yellow Fever 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 3 analyses.

The variability data are expressed in terms of standard deviation and coefficient of variation based on threshold cycle (C_t) values. At least 6 replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

Table 4: Precision data for the detection of YFV specific RNA (conc. approx. 50 x LoD)

YFV	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	32.72	0.14	0.41
Inter-Assay Variability	32.39	0.22	0.68
Inter-Lot Variability	32.46	0.29	0.91
Total Variability	32.50	0.25	0.77

Table 5: Precision data for the detection of YFV specific RNA (conc. approx. 3 x LoD)

YFV	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	36.25	0.58	1.60
Inter-Assay Variability	36.19	0.33	0.91
Inter-Lot Variability	36.16	0.42	1.16
Total Variability	36.21	0.41	1.14

Table 6: Precision data for the detection of the Internal Control

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	29.44	0.07	0.23
Inter-Assay Variability	29.66	0.30	1.02
Inter-Lot Variability	29.40	0.07	0.23
Total Variability	29.58	0.27	0.91

11.4 Diagnostic Evaluation

Nucleic acids extracted from serum samples from 30 patients with confirmed YFV infection were tested in parallel with the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 and the in-house YFV real-time RT-PCR assay (based on Domingo et al., 2012) by the Instituto Oswaldo Cruz – Fiocruz, Brazil. In addition, 15 individual serum samples from persons not infected with YFV were tested.

From 30 confirmed YFV positive samples all 30 were tested positive for YFV RNA with the reference (i.e., the in-house YFV real-time RT-PCR assay based on Domingo et al., 2012) and the RealStar® Yellow Fever Virus RT-PCR Kit 1.0.

Out of the 15 YFV negative samples all 15 samples were tested negative for YFV RNA with the reference. Using the RealStar® Yellow Fever Virus RT-PCR Kit 1.0, 14 samples were tested negative for YFV RNA and one sample was tested positive.

Table 7: Results of the evaluation of the diagnostic sensitivity and specificity for YFV in serum samples

		YFV real-time RT-PCR assay (based on Domingo et al., 2012)	
		+	-
RealStar® Yellow Fever Virus RT-PCR Kit 1.0	+	30	1
	-	0	14

In conclusion, in relation to the results generated with the in-house YFV real-time RT-PCR assay (based on Domingo et al., 2012) the diagnostic sensitivity and specificity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 for the detection of YFV is 100 % and 93.3 %, respectively.

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 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 (e.g. heparin) may cause false negative or invalid results.
- Potential mutations within the target regions of the YFV genome covered by the primers and/or probes used in the kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

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

14. Technical Assistance

For customer support, 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); ABI Prism® (Applied Biosystems); NucliSENS®, easyMAG® (bioMérieux); CFX96™ (Bio-Rad); JOE™ (Life Technologies); Maxwell® (Promega); Rotor-Gene®, QIAamp®, QIAsymphony® (QIAGEN); LightCycler® (Roche); VERSANT® (Siemens Healthcare); Mx 3005P™ (Stratagene); FAM™ (Thermo Fisher Scientific).

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The RealStar® Yellow Fever 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

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

Notes:

always a drop ahead.

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