WHO Public Report

Product: RealStar Yellow Fever Virus RT PCR Kit 1.0

On 03 May 2021, RealStar® Yellow Fever Virus RT-PCR Kit 1.0 with product code 671013, manufactured by altona Diagnostics GmbH, was accepted for WHO listing.

**Intended use:**
According to the claim of intended use from altona Diagnostics GmbH, “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.”

**Assay principle:**
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 Yellow Fever Virus (YFV) RNA are labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

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® Yellow Fever 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 (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 Internal Control in one reaction setup.

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)

Test kit contents:

<table>
<thead>
<tr>
<th>Component</th>
<th>96 tests (product code 671013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>altona Diagnostics GmbH RealStar® Yellow Fever Virus RT PCR Kit 1.0</td>
<td></td>
</tr>
<tr>
<td>Master A</td>
<td>8 vials, each 60 uL/Vial</td>
</tr>
<tr>
<td>Master B</td>
<td>8 vials, each 180 uL/Vial</td>
</tr>
<tr>
<td>Internal Control</td>
<td>1 vial, each 1000 uL/Vial</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 vial, each 250 uL/Vial</td>
</tr>
<tr>
<td>Water (PCR grade)</td>
<td>1 vial, each 500 uL/Vial</td>
</tr>
</tbody>
</table>

Items 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)

Storage:

The RealStar® Yellow Fever Virus RT-PCR Kit 1.0 has a shelf life of 12 months when used and stored as intended, according to the instructions for use. It is shipped on dry ice. The components of the kit should arrive frozen. If one or more components are not frozen upon receipt, or of the 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.

Shelf-life upon manufacture:
12 months.

Warnings/limitations:

Refer to the latest version of instructions for use.

Product dossier assessment

In accordance with the WHO procedure for evaluation of YFV nucleic acid tests, altona Diagnostics GmbH submitted technical documentation in support of the safety and performance of the assay. Notwithstanding, certain aspects of the technical documentation were considered lacking during technical review of this documentation. The following commitments must be fulfilled for this product to remain listed on the WHO website:

Commitments for prequalification:
1. To provide a revised IFU by 31 July 2021.
2. To provide and implement a post market performance plan agreed by WHO to provide further evidence of clinical performance.
3. To agree to assist WHO in the assignment of a cut-off value based on data generated from YFV endemic area for serum specimens. This information and certain other instructional information are to be added to an application note, made available to all users.

Manufacturing site inspection

A desk review of the quality management system documentation including specific manufacturing documentation of altona Diagnostics GmbH, located at Mörkenstraße 12, 22767 Hamburg, Germany, was undertaken by WHO. There was sufficient information to establish the overall suitability and good standing of the manufacturer’s quality system in accordance with WHO requirements.

As a commitment to listing, altona Diagnostics GmbH has committed to fulfilling the following requirements by 31 July 2021:
1. The manufacturer is required to provide a written commitment that the
procedure RS YFV 1.0.pdf and other relevant/related procedures will be reviewed and amended as to address the deficiencies noted, namely:

- Retesting should only be allowed following a thorough investigation, and only in the case of a proven laboratory error.
- The description of the retesting process was lacking, hence it appeared as if a failed result could simply be replaced with a subsequent pass result.

2. The manufacturer has provided a written commitment that they will update their Design Control procedures to include the reference to WHO requirements.

3. The manufacturer has provided a written commitment that they will provide the full list of their critical suppliers of biological/biotech materials/semi-finished products, and if any suppliers were originally accidentally omitted, the manufacturer is required to forward the evidence of the QMS certification for the initially accidentally omitted suppliers, if any.

4. The manufacturer indicated that they propose to implement “Train the Trainer “ program to enable technical demonstrations and support in remote areas, instead of the technical demonstrations otherwise routinely provided at the HQ in Hamburg, however details were not provided. The manufacturer has agreed to work with WHO to create a suitable training plan.

5. The manufacturer has provided a written commitment that they will provide any customer feedback related to any version of the product included in this submission (and already supplied, either commercially or for research) to WHO.

**Product performance evaluation**

altona Diagnostics GmbH RealStar® Yellow Fever Virus RT PCR Kit 1.0 was evaluated for WHO by the US Centres for Disease Control and Prevention (CDC) in Q4 2020. The US CDC is a WHO Collaborating Centre for 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”. The following results were obtained and are provided in contrast with data submitted by the manufacturer of the assay in technical documentation submitted to WHO. The findings support the listing of this product.
<table>
<thead>
<tr>
<th>Performance characteristic (Note CoV = Coefficient of Variation CI = Confidence Interval)</th>
<th>CDC/WHO Independent Assessment</th>
<th>Altona Manufacturer’s Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intra-assay precision</strong></td>
<td>YF17D-204 CoV (0.17-0.59)%</td>
<td></td>
</tr>
</tbody>
</table>
Panama 1974 CoV (0.41-2.5)%;  
Asibi CoV (0.57-3.1)% | ssRNA YFV_ASIBI_IVT CoV (0.23-1.6)% |
| **Inter-assay precision** | YF17D-204 CoV (0.29-1.1)% |  
Panama 1974 CoV (1.2-3.5)%  
Asibi CoV (1.4-6.1)% | ssRNA YFV_ASIBI_IVT CoV (0.68-1.02)% |
| **Inter-lot precision** | YFD17D-204 CoV (0.29–1.42)% |  
Panama 1974 CoV (0.65–1.89)%  
Asibi CoV (1.26–3.37)% | ssRNA YFV_ASIBI_IVT CoV (0.23-1.1)% |
| **Inter-method precision** | BioRad CFX96 and ABI Prism 7500 Fast SDS  
Ct values generated on the BioRad CFX96 and the ABI Prism 7500 Fast SDS differed by less than 1 Ct.  
CoVs of Ct values were lower for the CFX96 (range, 0.045 to 1.26%) compared to the ABI7500 (range, 0.169 to 3.189%) | ssRNA YFV_ASIBI_IVT CoV (0.23-1.6)% |
| **Limit of Detection 95% LOD** | YF17D-204  
1,245 c/ml  
95% CI (497-1,640 c/ml) | ssRNA YFV_ASIBI_IVT CoV (0.23-1.6)%  
690 c/ml;  
95% CI (410-1,560 c/ml) |
| **Inclusivity** | All strains detected  
See table 1 below | No studies performed |
| **Analytical Specificity: Cross Reactivity** | Negative for the following viruses: Zika, West Nile, dengue serotypes 1-4, Powassan, Japanese encephalitis, chikungunya, Ebola, Marburg, Lassa, measles, | Negative for the following viruses CHIKV, CCHFV. Dengue 1 virus, Dengue 4 virus, Ebola virus, HCV, Japanese Encephalitis virus, Marburg virus, Murray Valley |
HIV, and Influenza A H1N1 viruses  
encephalitis virus, Lassa virus, WNV, Zika virus and parasite: P falciparum,

Analytical Specificity: Interference  
No negative influence of interfering specimens  
See table 2 below  
No negative influence of interfering specimens  
See table 2 below

Clinical Sensitivity  
100% with 95% CI (73.5-100)%  
100% with 95% CI (88.4-100)%

Clinical Specificity Serum  
100% with 95% CI (92.1-100)%  
93.3% with 95% CI (68.1-99.8)%

Clinical Specificity Plasma  
100% with 95% CI (88.4-100)%

Table 1 Strains tested by CDC/WHO for inclusivity

<table>
<thead>
<tr>
<th>Yellow fever Strain</th>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>17D-204 Vaccine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asibi Ghana</td>
<td></td>
<td>1927</td>
</tr>
<tr>
<td>614819 Panama</td>
<td></td>
<td>1974</td>
</tr>
<tr>
<td>BA-55 Nigeria</td>
<td></td>
<td>1986</td>
</tr>
<tr>
<td>BC-7914 Kenya</td>
<td></td>
<td>1993</td>
</tr>
<tr>
<td>14FA Angola</td>
<td></td>
<td>1971</td>
</tr>
<tr>
<td>CAREC M2-09 Trinidad</td>
<td></td>
<td>2009</td>
</tr>
<tr>
<td>FMD-1240 Peru</td>
<td></td>
<td>2007</td>
</tr>
<tr>
<td>InHRR 10a-10</td>
<td></td>
<td>2010</td>
</tr>
</tbody>
</table>

Table 2 Analytical Specificity/Interfering substances

<table>
<thead>
<tr>
<th>Substance</th>
<th>CDC/WHO Evaluation Concentration</th>
<th>CDC/WHO Result</th>
<th>Altona Evaluation Concentration</th>
<th>Altona Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>5.6 mM/L</td>
<td>No interference</td>
<td>1 g/dL</td>
<td>No interference</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>257 uM/L</td>
<td>No interference</td>
<td>30 mg/dL</td>
<td>No interference</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>200 g/L</td>
<td>No interference</td>
<td>2 g/dL</td>
<td>No interference</td>
</tr>
<tr>
<td>Malaria</td>
<td>No interference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human genomic DNA</td>
<td>Not tested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>Not tested</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Not tested</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Labelling

1. Labels
2. Instructions for use
1. Labels

Tube Labels

Master A

Master B

Internal Control

Water (PCR grade)

Positive Control

Box Labels

RealStar® Yellow Fever Virus RT-PCR Kit 1.0
For the detection of yellow fever virus specific RNA
2. Instructions for Use
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)
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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>
<thead>
<tr>
<th>Lid Color</th>
<th>Component</th>
<th>Number of Vials</th>
<th>Volume [µl/Vial]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>Master A</td>
<td>8</td>
<td>60</td>
</tr>
<tr>
<td>Purple</td>
<td>Master B</td>
<td>8</td>
<td>180</td>
</tr>
<tr>
<td>Green</td>
<td>Internal Control</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>Red</td>
<td>Positive Control</td>
<td>1</td>
<td>250</td>
</tr>
<tr>
<td>White</td>
<td>Water (PCR grade)</td>
<td>1</td>
<td>500</td>
</tr>
</tbody>
</table>

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 are not frozen upon receipt, or if the 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 (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.

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 symptom 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].
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 labelled with fluorescent reporter and quencher dyes.

Probes specific for YFV 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 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 labelled probes

The RealStar® Yellow Fever 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 (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 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 3005™ 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

- 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

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 has to be ensured 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:
RealStar® Yellow Fever Virus RT-PCR Kit 1.0

- 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 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.*

*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:

<table>
<thead>
<tr>
<th>Number of Reactions (rxns)</th>
<th>1</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Master A</td>
<td>5 µl</td>
<td>60 µl</td>
</tr>
<tr>
<td>Master B</td>
<td>15 µl</td>
<td>180 µl</td>
</tr>
<tr>
<td>Internal Control</td>
<td>1 µl</td>
<td>12 µl</td>
</tr>
<tr>
<td><strong>Volume Master Mix</strong></td>
<td><strong>21 µl</strong></td>
<td><strong>252 µl</strong></td>
</tr>
</tbody>
</table>

► 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:
### 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).

<table>
<thead>
<tr>
<th>Reaction Setup</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Master Mix</td>
<td>20 µl</td>
</tr>
<tr>
<td>Sample or Control</td>
<td>10 µl</td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>30 µl</strong></td>
</tr>
</tbody>
</table>

- 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® 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

Define the following settings:

<table>
<thead>
<tr>
<th>Settings</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction Volume</td>
<td>30 µl</td>
</tr>
<tr>
<td>Ramp Rate</td>
<td>Default</td>
</tr>
<tr>
<td>Passive Reference</td>
<td>None</td>
</tr>
</tbody>
</table>

9.2 Fluorescence Detectors (Dyes)

Define the fluorescence detectors (dyes):

<table>
<thead>
<tr>
<th>Target</th>
<th>Detector Name</th>
<th>Reporter</th>
<th>Quencher</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFV specific RNA</td>
<td>YFV</td>
<td>FAM™</td>
<td>(None)</td>
</tr>
<tr>
<td>Internal Control (IC)</td>
<td>IC</td>
<td>JOE™</td>
<td>(None)</td>
</tr>
</tbody>
</table>
9.3 Temperature Profile and Dye Acquisition

Define the temperature profile and dye acquisition:

<table>
<thead>
<tr>
<th>Stage</th>
<th>Cycle Repeats</th>
<th>Acquisition</th>
<th>Temperature [°C]</th>
<th>Time [min:sec]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>Hold</td>
<td>1</td>
<td>-</td>
<td>55</td>
</tr>
<tr>
<td>Denaturation</td>
<td>Hold</td>
<td>1</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td>Amplification</td>
<td>Cycling</td>
<td>45</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>yes</td>
<td>55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td>72</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Control ID</th>
<th>Detection Channel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>+/-*</td>
</tr>
<tr>
<td>Negative Control</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Detection Channel</th>
<th>Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAM™</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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

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.

The analytical specificity of the RealStar® Yellow Fever Virus RT-PCR Kit 1.0 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:

- Crimean-Congo Hemorrhagic Fever virus
- Chikungunya 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
- West Nile virus
- Zika virus
- *Plasmodium falciparum*

### 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 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 six replicates per sample were analysed for intra-assay variability, inter-assay and inter-lot variability.

**Table 2:** Precision data for the detection of YFV specific RNA

<table>
<thead>
<tr>
<th>YFV</th>
<th>Average Threshold Cycle (C(_t))</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay Variability</td>
<td>32.72</td>
<td>0.14</td>
<td>0.41</td>
</tr>
<tr>
<td>Inter-Assay Variability</td>
<td>32.39</td>
<td>0.22</td>
<td>0.68</td>
</tr>
<tr>
<td>Inter-Lot Variability</td>
<td>32.46</td>
<td>0.29</td>
<td>0.91</td>
</tr>
<tr>
<td>Total Variability</td>
<td>32.50</td>
<td>0.25</td>
<td>0.77</td>
</tr>
</tbody>
</table>
Table 3: Precision data for the detection of the Internal Control

<table>
<thead>
<tr>
<th>Internal Control</th>
<th>Average Threshold Cycle (Ct)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intra-Assay Variability</td>
<td>29.44</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>Inter-Assay Variability</td>
<td>39.66</td>
<td>0.30</td>
<td>1.02</td>
</tr>
<tr>
<td>Inter-Lot Variability</td>
<td>29.40</td>
<td>0.07</td>
<td>0.23</td>
</tr>
<tr>
<td>Total Variability</td>
<td>29.58</td>
<td>0.27</td>
<td>0.91</td>
</tr>
</tbody>
</table>

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


16. Trademarks and Disclaimers

RealStar® (altona Diagnostics); ABI Prism® (Applied Biosystems); ATCC® (American Type Culture Collection); CFX96™ (Bio-Rad); Cy® (GE Healthcare); FAM™, JOE™, ROX™ (Life Technologies); LightCycler® (Roche); SmartCycler® (Cepheid); Maxwell® (Promega); Mx 3005P™ (Stratagene); NucliSENS®, easyMag® (bioMérieux); Rotor-Gene®, QIAamp®, MinElute®, QIASymphony® (QIAGEN); VERSANT® (Siemens Healthcare).

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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.

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# 17. Explanation of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVD</td>
<td><em>In vitro</em> diagnostic medical device</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch code</td>
</tr>
<tr>
<td>CAP</td>
<td>Cap color</td>
</tr>
<tr>
<td>REF</td>
<td>Product number</td>
</tr>
<tr>
<td>CONT</td>
<td>Content</td>
</tr>
<tr>
<td>NUM</td>
<td>Number</td>
</tr>
<tr>
<td>COMP</td>
<td>Component</td>
</tr>
<tr>
<td>GTIN</td>
<td>Global trade identification number</td>
</tr>
<tr>
<td>📖</td>
<td>Consult instructions for use</td>
</tr>
<tr>
<td>📸</td>
<td>Contains sufficient for “n” tests/reactions (rxns)</td>
</tr>
<tr>
<td>🟢</td>
<td>Temperature limit</td>
</tr>
<tr>
<td>🕒</td>
<td>Use-by date</td>
</tr>
<tr>
<td>🏫</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>🔴</td>
<td>Caution</td>
</tr>
<tr>
<td>📅</td>
<td>Note</td>
</tr>
<tr>
<td>🕒</td>
<td>Version</td>
</tr>
</tbody>
</table>
Notes:
always a drop ahead.