Instructions for Use

RealStar®
Lassa Virus RT-PCR Kit 2.0

03/2019 EN

RealStar®

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Lassa Virus RT-PCR Kit 2.0

For use with

Mx 3005P™ QPCR System (Stratagene)

VERSANT® kPCR Molecular System AD (Siemens Healthcare)

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® Lassa Virus RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of Lassa virus (LASV) specific RNA in human EDTA plasma as an aid for diagnosis in individuals with signs and symptoms of Lassa virus infection in conjunction with epidemiological risk factors.

The RealStar® Lassa Virus RT-PCR Kit 2.0 is intended to be used by qualified personnel in appropriately equipped laboratories following the guidelines on laboratory biosafety.

2. Kit Components

The RealStar® Lassa Virus RT-PCR Kit 2.0 contains 2 different RT-PCR assays with 48 reactions each. It contains two different Positive Controls: one for the GPC gene specific amplification and detection system and one for the L gene specific amplification and detection system.

Lid Color	Component	Number of Vials	Volume [µl/Vial]
Blue	Master A GPC Gene	4	60
Purple	Master B GPC Gene	4	180
Lightblue	Master A L Gene	4	60
Lightpurple	Master B L Gene	4	180
Green	Internal Control	1	1000
Red	Positive Control GPC Gene	1	250
Orange	Positive Control L Gene	1	250
White	Water (PCR grade)	1	500

3. Storage

- The RealStar® Lassa Virus RT-PCR Kit 2.0 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 (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

Lassa virus (LASV), a member of the family *Arenaviridae*, is an enveloped single-stranded RNA virus. LASV is endemic in West Africa, primarily in Benin, Sierra Leone, Guinea, Liberia, and Nigeria (1). Natural host of LASV is *Mastomys natalensis*, a small rodent endemic to sub-Saharan Africa. The rodents live closely to humans and frequently enter houses on the search for food. The virus can be transmitted through direct contact or contaminated food (2). Human-to-human transmission is possible within the community as well as in the health care setting, via aerosols, contact with contaminated body fluids or re-use of contaminated medical equipment (3).

Approximately 80% of all LASV infections remain clinically inconspicuous, 1% of infections result in deaths (3). LASV can cause Lassa hemorrhagic fever (LHF) with high fatality rates, reaching 15-20% among hospitalized patients (4). Fatality rates for pregnant women are even higher, especially in the third trimester of pregnancy (5). LHF is associated with nosocomial outbreaks.

There are no vaccines for LASV available, yet. For the treatment of LHF the anti-viral drug Ribavirin is administered parenterally. The treatment efficacy is high, if it is commenced within six days after the onset of symptoms, but declines rapidly with the delay of administration (6). Supportive therapies include oxygenation, treatment of secondary infections, appropriate fluid and electrolyte balance, and transfusions if indicated.

LASV can be detected early after infection (7) and during the course of the disease, while serological assays detect the virus not until the onset of symptoms (8). Detection of the viral RNA using conventional RT-PCR followed by gel-detection is currently the gold-standard method for LHF diagnosis. Cell culture requires a

BSL-4 containment and can therefore be performed in highly specialized laboratories only (8). Multiple enzyme-linked immunosorbent assay (ELISA) are available, although serological methods are not useful for the identification of acute LHF cases. In addition, patients with severe LHF do not always develop antibodies (8, 9). Molecular diagnostic tools using the real-time RT-PCR technique are ideally suited for the molecular diagnosis of viral infections as they are fast, sensitive and may be performed on inactivated specimens (10, 11), but there is no commercial real-time RT-PCR assay for the detection of LASV available, yet. The considerable sequence diversity between LASV strains is challenging for the development of molecular diagnostic tools. The likelihood of false-negative results can be decreased by using multiple genomic sequences as target regions, e.g. the L gene and the GPC gene.

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[7] Demby AH, Chamberlain J, Brown DW, Clegg CS (1994), Early diagnosis of Lassa fever by reverse transcription-PCR, J Clin Microbiol, 32(12):2898-903.

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[10] Nikisins S, Rieger T, Patel P, Muller R, Gunther S, Niedrig M. (2015), International external quality assessment study for molecular detection of Lassa virus. PLoS Negl Trop Dis 9:e0003793.

[11] Zheng Pang, Aqian Li, Jiandong Li, Jing Qu, Chengcheng He, Shuo Zhang, Chuan Li, Quanfu Zhang, Mifang Liang,and Dexin Li (2014), Comprehensive Multiplex One-Step Real-Time TaqMan qRT-PCR Assays for Detection and Quantification of Hemorrhagic Fever Viruses, PLoS One, 9(4): e95635.

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® Lassa Virus RT-PCR Kit 2.0 is an *in vitro* diagnostic test, based on real-time PCR technology, for the qualitative detection of Lassa virus (LASV) specific RNA in human EDTA plasma as an aid for diagnosis in individuals with signs and symptoms of Lassa virus infection in conjunction with epidemiological risk factors.

The RealStar® Lassa Virus RT-PCR Kit 2.0 is intended to be used by qualified personnel in appropriately equipped laboratories following the guidelines on laboratory biosafety.

The RealStar® Lassa Virus RT-PCR Kit 2.0 consists of two assays, one targeting the LASV GPC gene and another targeting the LASV L gene. Both assays include a heterologous amplification system (Internal Control) to identify possible PCR inhibition and to confirm the integrity of the reagents.

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.

Master Mix GPC Gene: The probe specific for the LASV GPC gene is labelled with the fluorophore FAM™. The probe specific for the Internal Control (IC) is labelled with the fluorophore JOE™.

Master Mix L Gene: The probe specific for the LASV L gene is 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 LASV specific RNA (either the target GPC gene or the target L gene) and the Internal Control in corresponding detector channels of the real-time PCR instrument.

The test for both assays 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® Lassa Virus RT-PCR Kit 2.0 consists of:

- Master A GPC Gene
- Master B GPC Gene
- Master A L Gene
- Master B L Gene

- Internal Control
- Positive Control GPC Gene
- Positive Control L Gene
- Water (PCR grade)

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

The Master A and Master B GPC Gene set contains all components to allow PCR mediated amplification and detection of Lassa virus GPC gene specific RNA and Internal Control in one reaction setup.

The Master A and Master B L Gene set contains all components to allow PCR mediated amplification and detection of Lassa virus L gene specific RNA and Internal Control in one reaction setup.

6.1 Real-Time PCR Instruments

The RealStar® Lassa Virus RT-PCR Kit 2.0 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)
- 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
    - 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.
- Clinical specimens should always be treated as infectious and/or biohazardous in accordance with safe laboratory procedures. Refer to the CDC guideline "Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals" (The guidelines from the CDC's, Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals, May 2005. *https://www.cdc.gov/hai/pdfs/bbp/vhfinterimguidance05_19_05.pdf*).
- 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. Refer also to the guidelines from the CDC's, Interim Guidance for Managing Patients with Suspected Viral Haemorrhagic Fever in U.S. Hospitals, May 2005. *https://www.cdc.gov/hai/pdfs/bbp/vhfinterimguidance05_19_05.pdf*.

8. Procedure

8.1 Sample Preparation

The following specimen type is validated for use with the RealStar® Lassa Virus RT-PCR Kit 2.0:

- Human EDTA plasma.

For guidance with respect to sample processing refer to "National Guidelines on Prevention and Control of Viral Haemorrhagic Fevers" (National Guidelines on Prevention and Control of Viral Haemorrhagic Fevers, Nigeria Centre for Disease Control (NCDC), April 2017. *https://ncdc.gov.ng/themes/common/docs/protocols/18_1501495944.pdf).*

Extracted RNA is the starting material for the RealStar® Lassa Virus RT-PCR Kit 2.0.

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® Lassa Virus RT-PCR Kit 2.0 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® Lassa Virus RT-PCR Kit 2.0 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 each Master Mix (Master Mix GPC Gene and Master Mix L Gene) 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 each Master Mix (Master Mix GPC Gene and Master Mix L Gene) 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 GPC Gene or the Master Mix L Gene 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 Control (Positive Control GPC Gene for the Master Mix GPC and Positive Control L Gene for Master Mix L Gene) and at least one Negative Control is used per Master Mix and run.

- Thoroughly mix the samples and controls with the Master Mix GPC Gene and the Master Mix L Gene 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® Lassa Virus RT-PCR Kit 2.0 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	None

9.2 Fluorescence Detectors (Dyes)

► Define the fluorescence detectors (dyes):

Target	Master Mix	Detector Name	Reporter	Quencher
LASV GPC Gene specific RNA	GPC Gene	LASV	FAM™	(None)
LASV L Gene specific RNA	L Gene	LASV	FAM™	(None)
Internal Control (IC)	GPC Gene and L Gene	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

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® Lassa Virus RT-PCR Kit 2.0 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 (GPC Gene)	+	+/-*
Positive Control (L Gene)	+	+/-*
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		Detection Channel		Result Interpretation
GPC gene specific assay		L gene specific assay		
FAM™	JOE™	FAM™	JOE™	
+	+*	+**	+*	LASV specific RNA detected.
+	+*	-	+/- ***	LASV specific RNA detected.
-	+/-***	+**	+*	LASV specific RNA detected.
-	+	-	+	No LASV specific RNA detected. Sample does not contain detectable amounts of LASV specific RNA.
-	+	-	-	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.
-	-	-	+	RT-PCR inhibition or reagent failure. Repeat testing from original sample or collect and test a new sample.
-	-	-	-	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 LASV RNA load in the sample can lead to a reduced or absent Internal Control signal.

** Samples which are highly positive for LCMV (lymphocytic choriomeningitis virus) may be tested false positive for the LASV L gene specific RNA.

*** If a signal is observed in the FAM™ detection channel for one of the two assays (either the GPC gene or the L gene assay) the sample can be considered LASV RNA positive. It is irrelevant, if the assay not showing a signal in the FAM™ detection channel shows a signal in the JOE™ channel (IC) or not.

11. Performance Evaluation

Performance evaluation of the RealStar® Lassa Virus RT-PCR Kit 2.0 was done using specific *in vitro* transcript for L gene and GPC gene, respectively.

11.1 Analytical Sensitivity

The analytical sensitivity of the RealStar® Lassa Virus RT-PCR Kit 2.0 is defined as the concentration (copies/µl of the eluate) of LASV specific RNA molecules that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of Lassa virus L gene and GPC specific *in vitro* transcripts.

Table 1: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *LASV L gene* specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.620	24	24	100
10.000	24	24	100
3.162	23	23	100
1.000	24	22	91.67
0.316	24	9	37.5
0.032	48	4	8.33
0.010	48	3	6.25
0.003	48	1	2.08

Table 2: PCR results used for the calculation of the analytical sensitivity with respect to the detection of *LASV GPC gene* specific RNA

Input Conc. [copies/µl]	Number of Replicates	Number of Positives	Hit Rate [%]
31.620	24	24	100
10.000	24	24	100
3.162	24	24	100
1.000	24	24	100
0.316	24	13	54.17
0.100	24	5	20.83
0.032	24	1	4.17
0.010	24	0	0
0.003	24	0	0

The analytical sensitivity of the RealStar® Lassa Virus RT-PCR Kit 2.0 was determined by Probit analysis:

- For the detection of LASV L gene specific RNA, the analytical sensitivity is 3.14 copies/µl [95% confidence interval (CI): 1.67 - 7.60 copies/µl]
- For the detection of LASV GPC gene specific RNA, the analytical sensitivity is 1.00 copies/µl [95% confidence interval (CI): 0.64 - 2.12 copies/µl]

11.2 Analytical Specificity

The analytical specificity of the RealStar® Lassa Virus RT-PCR Kit 2.0 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 LASV genotypes will be detected.

The analytical specificity with respect to the cross reacticity of the RealStar® Lassa Virus RT-PCR Kit 2.0 was evaluated by testing a panel of genomic RNA/DNA extracted from pathogens related to Lassa virus, pathogens likely to be present

in the same sample matrix or pathogens causing similar symptoms as an infection with Lassa virus.

The RealStar® Lassa Virus RT-PCR Kit 2.0 did not cross-react with any of the following pathogens:

- Crimean-Congo hemorrhagic fever virus
- Chikungunya virus
- Dengue virus 1
- Dengue virus 2
- Dengue virus 3
- Dengue virus 4
- Ebola virus
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Human immunodeficiency virus 1
- Marburg virus
- Rift Valley fever virus
- West Nile virus
- Yellow fever virus
- Zika virus
- *Plasmodium falciparum*

The analytical specificity with respect to the reactivity of the RealStar® Lassa Virus RT-PCR Kit 2.0 was evaluated by a panel of genomic RNA extracted from different Lassa virus lineages (strains).

The RealStar® Lassa virus RT-PCR Kit 2.0 is able to detect RNA of the following lineages tested for different strains:

- Lineage II (Nig08-04; Nig08-A37)
- Lineage III (Nig08-A18; Nig-CSF; Nig-SL-NL)
- Lineage IV (Lib05-4094; BA366; Josiah)
- Lineage V (AV)

11.3 Precision

Precision of the RealStar® Lassa Virus RT-PCR Kit 2.0 was determined for the GPC gene specific assay and the L Gene specific assay, respectively, 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 3: Precision data for the detection of LASV L gene specific RNA

LASV L gene	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	31.51	0.16	0.51
Inter-Assay Variability	30.98	0.16	0.53
Inter-Lot Variability	31.32	0.24	0.76
Total Variability	31.16	0.30	0.97

Table 4: Precision data for the detection of LASV GPC gene specific RNA

LASV GPC gene	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	31.65	0.22	0.70
Inter-Assay Variability	32.14	0.18	0.57
Inter-Lot Variability	31.96	0.37	1.16
Total Variability	31.98	0.31	0.96

Table 5: Precision data for the detection of the Internal Control using Master Mix L gene

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	32.46	0.16	0.50
Inter-Assay Variability	32.24	0.27	0.83
Inter-Lot Variability	32.51	0.13	0.41
Total Variability	32.34	0.27	0.83

Table 6: Precision data for the detection of the Internal Control using Master Mix GPC Gene

Internal Control	Average Threshold Cycle (C_t)	Standard Deviation	Coefficient of Variation [%]
Intra-Assay Variability	30.72	0.22	0.71
Inter-Assay Variability	30.67	0.17	0.55
Inter-Lot Variability	30.65	0.17	0.54
Total Variability	30.68	0.18	0.59

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. Refer to "Interim Guidance by the WHO (Interim Guidance, World HEalth Organization, 2014, *https://www.who.int/emergencies/diseases/lassa-fever/collection-of-blood-samples-*

for-lassa.pdf?ua=1) and the "National Guidelines on Prevention and Control of Viral Haemorrhagic Fevers" (Nigeria Centre for Disease Control (NCDC), April 2017. *https://ncdc.gov.ng/themes/common/docs/protocols/18_1501495944.pdf*).

- 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 LASV 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® Lassa Virus RT-PCR Kit 2.0 need to be interpreted in consideration of all clinical and laboratory findings.

13. Quality Control

In accordance with the altona Diagnostics GmbH ISO EN 13485-certified Quality Management System, each lot of RealStar® Lassa Virus RT-PCR Kit 2.0 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); 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® Lassa Virus RT-PCR Kit 2.0 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.

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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
Σ	Contains sufficient for “n” tests/reactions (rxns)
	Temperature limit
	Use-by date
	Manufacturer
!	Caution
i	Note
	Version

Notes:

always a drop ahead.

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