ALDATU BIOSCIENCES

REF 2021096

PANDAA® Lassa Virus

Detection of Lassa virus RNA

Instructions for Use

PANDAA® Lassa Virus is for Research Use Only (RUO)

NOT FOR RESALE

RUO For research use only

REF 2021096

−15 ˚C to −25 ˚C

Contents sufficient for 96 reactions

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Overview

Intended Use (RUO)

This technical user guide is for PANDAA® Lassa Virus, a Research Use Only (RUO) real-time RT-PCR assay for the amplification and qualitative detection of Lassa virus RNA. PANDAA Lassa Virus is uniquely designed to provide accurate and rapid information for the identification of Lassa virus and PANDAA VHF Interpretation Software may be used to support analysis and interpretation of results. Use the most current version of this instructions for use available at www.aldatu.bio/downloads.

This kit is for Research Use Only and is not intended for use in diagnostic procedures.

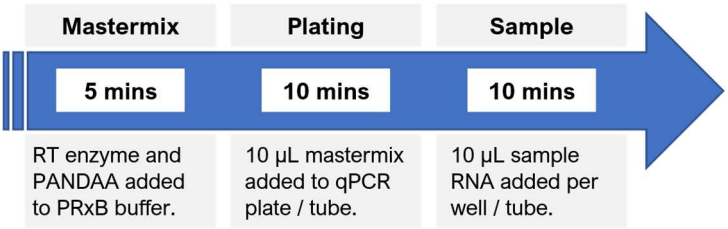
PANDAA Lassa Virus Test Principle

PANDAA Lassa Virus assay is comprised of single reaction mix containing reagents to amplify and detect all known lineages of Lassa virus. The Lassa virus target, located in the L segment, is amplified by PANDAA primers and detected by a FAM-labelled hydrolysis probe.

An exogenous, non-competitive internal control is included to monitor for successful nucleic acid extraction and for the presence of RT-PCR inhibitors. A sample adequacy control (SAC) detects endogenous human RNase P in samples derived from human biological material to control for sample quality and to ensure adequate addition of sample to the reaction.

The entire PANDAA Lassa Virus workflow can be completed in two hours.

Assay preparation takes ~30 minutes with a PCR run time ~70 minutes. Prepare the PANDAA mastermix by adding Stop-Start RT enzyme and PANDAA to the PRxB buffer. Dispense 10 µL of mastermix into each PCR well/tube, then add sample RNA to each well/tube. **No mixing is necessary.**



Compatible Real-Time PCR Instruments

Any real-time PCR instrument that can detect fluorophores in the green, yellow, and red channels, such as those listed in Appendix B.

PANDAA Technology

Aldatu Biosciences' detection and genotyping PANDAA technology uniquely compensates for evolving pathogen diversity, ensuring that PCR diagnostic integrity isn't affected by genomic variation now, or in the future. PANDAA is designed to mitigate genomic variability by normalizing probe-binding regions. During the initial real-time PCR cycles, the target genome is adapted through site-directed mutagenesis to replace any polymorphisms that could cause false negative results.
Read the methods publication here: https://www.nature.com/articles/s42003-021-01751-9.

Contents and Storage

Reagents

The kit includes all amplification reagents for 96 reactions and includes one (1) each of the Positive Control, Negative Control, and Internal Control RNA. **Refer to Appendix A for a list of materials required but not included.**

Contents	Cap color	Top Label	Quantity	Volume
PRxB II Buffer	Clear	PRxB	2	500 µL
Lassa Virus PANDAA	White	PAN LASV	2	35 µL
Stop-Start RT Enzyme	Blue	RT	1	8 µL
Internal Control RNA	Yellow	INT CTRL	1	120 µL
Positive Control	Red	POS	1	100 µL
Negative Control	Green	NEG	1	100 µL

Storage and Stability

PANDAA Lassa Virus is shipped on dry ice, and kit components arrive frozen. Store all components at −15 °C to −25 °C upon receipt, protected from light. Stop-Start RT enzyme contains glycerol and may remain liquid at −15 °C to −25 °C. Keep reagents in the original packaging when not in use and return them promptly to recommended storage conditions. For receiving inspection and handling, see *General Guidelines → Shipping, Storage and Handling* (page 22).

A unique feature of PANDAA assays is that, *after thawing*, all components except Stop-Start RT enzyme may be stored at 2 °C to 8 °C for up to 7 days, protected from light. To maintain assay performance, avoid more than two freeze-thaw cycles for any reagent.

Contents	Stability at 2 °C to 8 °C
PRxB II Buffer	**7 days**
Lassa Virus PANDAA	
Internal Control RNA	
Positive Control	
Negative Control	
Stop-Start RT Enzyme	***Should only be stored at −15 °C to −25 °C***

NOTE: All reagents, *other than the Stop-Start RT enzyme*, may be stored at 2 °C to 8 °C for up to one week after thawing.

Reagents Description

PANDAA Reaction Buffer (PRxB)

A complete custom assay buffer that is compatible with high, low and no ROX real-time PCR machines. Contact Technical Support (support@aldatubio.com) with questions about your real-time PCR instrument requirements and configuration.

Stop-Start RT Enzyme

A specially formulated hot-start reverse transcriptase optimized for PANDAA.

Lassa Virus PANDAA

Includes uniquely designed PANDAA primers and probes for the amplification and detection of three distinct targets:

- **Lassa Virus (LASV):** targeted to the L segment of the Lassa virus genome.
- **Internal Control (IC):** detects the internal control RNA, which serves as a process control for both RNA extraction and PCR amplification.
- **Sample Adequacy Control (SAC):** detects human RNase P in samples derived from human biological material to control for the quantity, quality and adequacy of the specimen.

Extraction and Amplification Controls

- **Internal Control** (IC) is an exogenous, non-competitive control that detects MS2 phage-specific RNA, which should be either spiked into the lysis buffer prior to extraction or added directly to the real-time PCR reaction mix.
- **Positive Control** is a synthetic, non-infectious RNA covering the assay target regions in the Lassa virus genome of the *Nig-08-A18* isolate, provided at 150 copies/µL to yield 1,500 copies/reaction when using 10 µL control per reaction. It is prepared in a background of human genomic DNA and Internal Control RNA.
- **Negative Control** comprises ~0.1 ng/µL human genomic DNA, which is ~30 copies/µL to yield ~300 copies/reaction when using 10 µL control per reaction, and includes Internal Control RNA.

Sample Preparation for PANDAA Lassa Virus

The starting material for the PANDAA Lassa Virus kit is isolated viral RNA. If samples have already been extracted then proceed to *PANDAA Lassa Virus Kit Step-by-Step Instructions* on page 9.

Nucleic Acid Extraction

Compatible Extraction Reagents

Many commercially available nucleic acid extraction systems are compatible with real-time PCR and are suitable for sample preparation prior to PANDAA Lassa Virus testing, including systems that purify total nucleic acid (DNA and RNA). Follow the extraction kit manufacturer's instructions for the nucleic acid extraction kit or workflow used to viral RNA.

Sample Volume

The minimum sample input volume for nucleic acid extraction is 100 µL. Sample input volume may vary by platform and should be maximized to optimize test sensitivity. Refer to *Analytical Sensitivity - System-Level Limit of Detection (IU/mL)* (page 18) for PANDAA Lassa Virus performance with EDTA plasma.

Recommended specimen type	Human plasma collected in EDTA tubes
Minimum sample volume	100 µL

Required Elution Volume

Elution volume may vary by nucleic acid extraction protocol and should be optimized to maximize RNA recovery while minimizing dilution. Typical elution volumes range from **50 µL to 100 µL**. Each PANDAA Lassa Virus reaction requires **10 µL eluted RNA**.

Extracted RNA Handling

Use extracted RNA as soon as possible after extraction. If testing is delayed, extracted RNA may be stored at 2 – 8 °C for up to 6 hours. Extraction eluants and storage conditions can affect RNA stability, which may reduce detection in low-copy-number samples.

Provided Controls

Internal Control for RNA Extraction

The Internal Control (IC) RNA provided can be added to the sample extraction kit lysis buffer to serve as a full process control and verify successful RNA extraction as well as downstream amplification/detection. For each sample being processed, add **1 µL IC** to the sample extraction lysis buffer e.g., if processing 48 samples then add 48 µL IC to the lysis buffer.

PANDAA Lassa Virus Kit Step-by-Step Instructions

A. Before Beginning

PANDAA Lassa Virus is a highly sensitive molecular assay. To prevent false-positive results, follow a strict unidirectional workflow. Always use separate, dedicated lab areas and equipment for reagent preparation and for sample/control addition.

CAUTION

In a dedicated lab area for setting up PCR reaction mixes

Remove reagents from storage and place at room temperature to thaw. Estimated thawing times are 10 minutes for **PRxB buffer** and 5 minutes for **PANDAA tubes**. Remove **Stop-Start RT enzyme (RT)** from the freezer and place it directly on ice. *Tubes should be placed on ice after thawing.*

B. PANDAA Lassa Virus Mastermix Setup (48 Reactions)

1. **Prepare reagents:** Spin the reagent tubes briefly to collect drops on the interior sides of the tubes. Gently vortex the reagent tubes and spin briefly again.
2. **Add reagents to PRxB tube:**
   a. Add 30 µL Lassa Virus PANDAA directly to the PRxB tube.
   b. Add 3 µL Stop-Start RT enzyme directly to the PRxB tube.
      - Submerge only the tip of the pipette to ensure that no excess RT droplets are transferred to the exterior of the tip.
   c. If Internal Control (IC) was not added to the lysis buffer prior to sample extraction, it should be added to the PRxB tube here.
      - For 48 samples, add 3 µL Internal Control (IC) to the PRxB tube if the IC was not added to the lysis buffer prior to sample extraction.
3. **Mix reagents:** Gently vortex the PRxB tube containing the Stop-Start RT enzyme, PANDAA, and Internal Control (if not added to the sample extraction lysis buffer). Spin to collect any droplets.

NOTE: 24 reactions modification

Remove 250 µL PRxB buffer and transfer to a clean tube. Add 15 µL Lassa Virus PANDAA and 1.5 µL Stop-Start RT Enzyme. Add 1.5 µL IC RNA only if the IC was not added to the lysis buffer.

Follow the storage instructions on page 6 for the remaining reagents.

NOTE: Fewer than 48 reactions

If you require fewer than 48 reactions, prepare mastermix for 48 samples and store any unused volume. The complete PANDAA Lassa Virus mastermix (PRxB, Stop-Start RT enzyme, and PANDAA (with or without Internal Control) is **stable for up to 48 hours when stored at 2–8 °C**.

C. Real-time PCR Plate Setup

1. **Dispense mastermix:** Add 10 µL of the complete mastermix from the PRxB tube into each required well of an optical 96-well reaction plate or to each optical reaction tube compatible with your real-time PCR instrument.

CAUTION

Perform the remaining steps in a pre-amplification area designated for RNA handling in your laboratory

2. **Add sample or controls:** Add 10 µL sample RNA, Positive Control, or Negative Control into the top of the mastermix in each well / tube. Do *not* pipette up and down to mix.
3. **Seal and spin (plate only):** Seal the plate with optical adhesive film or cap the reaction tubes. If using a 96-well plate, spin the plate briefly before loading into the real-time PCR instrument.

D. Real-time PCR Protocol

1. Proceed immediately to loading the plate / tubes into the real-time PCR instrument.
2. Use the real-time PCR template provided by Aldatu Biosciences, which can be downloaded from www.aldatu.bio/qpcr-templates. Total run time should be 60 to 80 minutes.

E. Manual Real-time PCR Settings

Use the real-time PCR run template provided by Aldatu Biosciences, available at www.aldatu.bio/qpcr-templates. **Aldatu Biosciences cannot accept any responsibility for inaccurate data that has been generated through incorrect manual programming of the real-time PCR instrument.**

Use the following parameters and proceed to run the PANDAA real-time PCR. **Refer to Appendix B (page 25) for detection channel settings for compatible real-time PCR instruments.** For assistance with instrument configuration, contact Aldatu Biosciences Technical Support (support@aldatubio.com).

Real-Time PCR Instrument Settings

Setting	Value
Reaction volume	20 µL
Ramp rate	Fast, if compatible with your real-time PCR instrument. Otherwise use standard ramp rates. Refer to page 20 for ramp temperatures.
Passive reference (if applicable)	ROX

Target Name	Ex / Em (nm)	Channel	Reporter
PANDAA Lassa Virus (LASV)	495 / 520	Blue/Green*	Refer to Appendix B (page 25) for instrument-specific reporter / dye settings.
Internal Control (IC)	650 / 670	Red	
Sample Adequacy Control (SAC)	554 / 576	Yellow	

*Channel name may differ between instruments.

Cycling Conditions

During the 60 °C anneal and extension phase of the *Amplification and Detection* step, acquire fluorescence data in the green, yellow, and red channels.

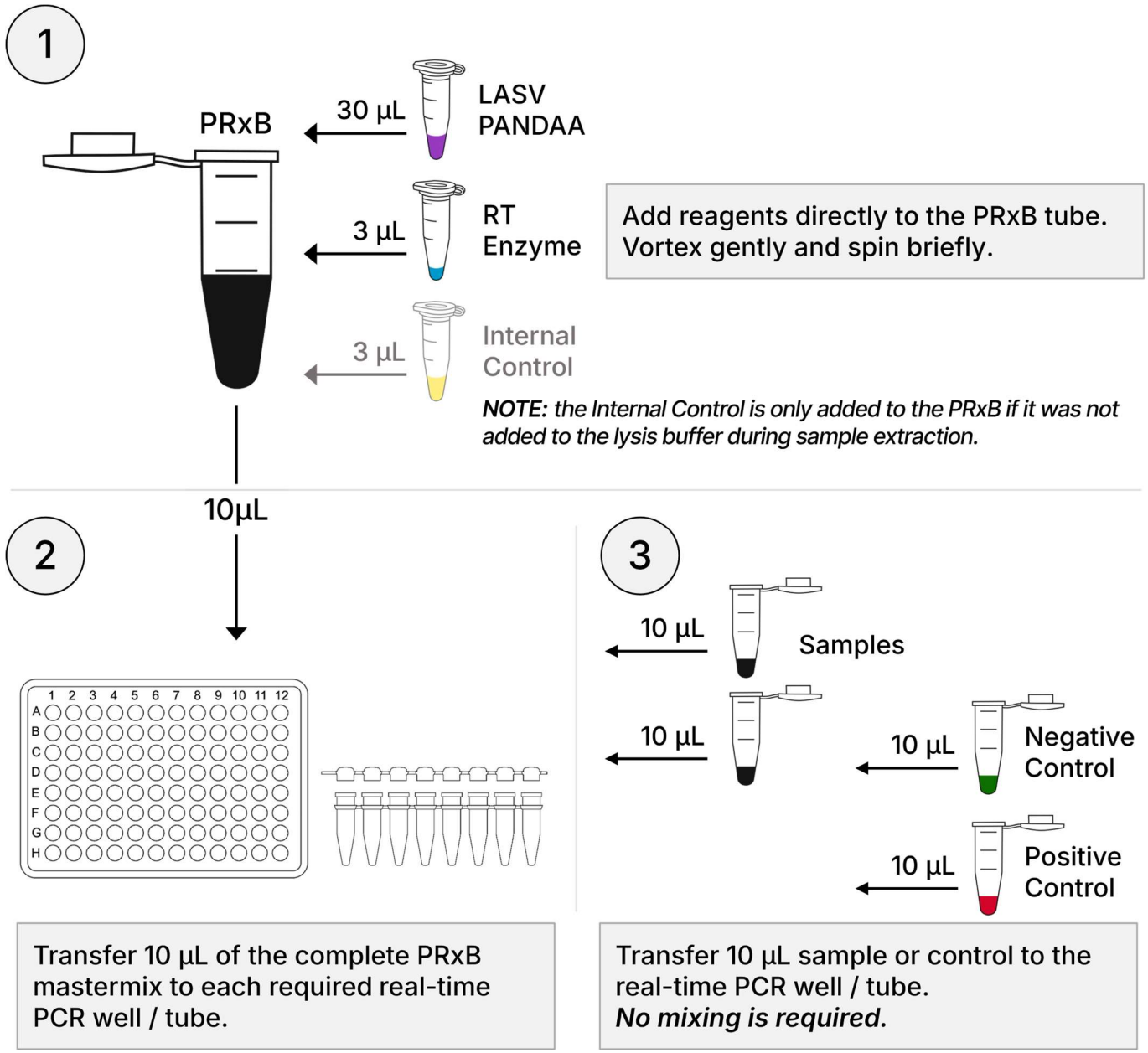
Step	Temperature	Time	Cycles
Reverse transcription	50 °C	15 minutes	1
Enzyme activation	95 °C	2 minutes	1
PANDAA adaptation	95 °C 55 °C 60 °C	1 second 30 seconds 30 seconds	10
Amplification and detection	95 °C 60 °C	1 second (*acquire*) 60 seconds	30

PANDAA LASV Qualification Panel

NOTE: PANDAA LASV Qualification Panel

For first-time instrument use, perform a preliminary run with the PANDAA LASV Qualification Panel (catalog #2121096), **provided free with any Aldatu assay** order. The panel contains ten synthetic, non-infectious RNA samples: nine positive controls covering seven LASV isolates and one negative control (LCMV). Contact support@aldatubio.com for details or visit www.aldatu.bio/lasv-panel.

PANDAA Lassa Virus Kit Quick Guide



Data Analysis and Interpretation of Results

Real-Time PCR Analysis Parameters

Refer to Appendix B (page 25) for instrument-specific analysis settings, including threshold settings for determination of Ct values for the PANDAA Lassa Virus, Internal Control, and Sample Adequacy Control targets. For technical assistance, contact Aldatu Biosciences at support@aldatubio.com.

Threshold Settings

Adjust the LASV threshold following the instrument-specific analysis settings in Appendix B (page 25). Use the instrument's automatic threshold for IC and SAC (recommended). If set manually, place the IC and SAC thresholds in the exponential (log-linear) region above baseline and below plateau; apply the same setting to all samples and controls.

Fluorescence Cutoff

Minor non-specific increases in fluorescence due to probe hydrolysis, dye crosstalk, or signal drift can produce spurious Ct values in the absence of genuine target amplification. The fluorescence cutoff distinguishes these artefactual signals from true positive results.

At the end of the PCR run, **a fluorescence cutoff of 5% should be applied to confirm negative results**. A well is called negative if its baseline-subtracted endpoint fluorescence (ΔRn on Applied Biosystems and Thermo Fisher instruments; baseline-subtracted endpoint RFU on Bio-Rad CFX instruments; fluorescence change on Qiagen Rotor-Gene and Mic instruments) is less than 5% of the highest baseline-subtracted endpoint fluorescence detected on the plate. The highest value on the plate is typically the on-plate Positive Control. Refer to Appendix B (page 25) for instrument-specific instructions. **The fluorescence cutoff is determined automatically when using the *PANDAA VHF Interpretation Software*.**

PANDAA VHF Interpretation Software (Recommended)

PANDAA VHF Interpretation Software is a free, automated tool for reviewing and interpreting Aldatu PANDAA real-time PCR run files for viral hemorrhagic fever assays. The software can import data directly from instrument files generated by supported real-time PCR instruments, apply standardized analysis thresholds, evaluate control validity, and generate summary reports, **typically in a few seconds after importing a run file**. Refer to the *PANDAA VHF Interpretation Software* instructions for use for installation, supported instruments, and detailed operating instructions.

Download the *PANDAA VHF Interpretation Software* from www.aldatu.bio/vhf-software.

Key Capabilities

- Automatically analyzes results directly from instrument data files from multiple real-time PCR instrument platforms.
- Applies standardized analysis criteria (Ct and fluorescence cutoff thresholds).
- Validates quality controls with a structured flag framework.
- Generates formatted reports (PDF and Excel) for documentation and review.
- Provides "Detected/Not Detected" interpretations with appropriate validity flags.

Supported Instruments and File Types

The software can currently accept the analyzed instrument run file directly from multiple instruments and accepts results files from the following real-time PCR instruments in the formats below.

Instrument	Supported File Formats
QuantStudio 5	.eds, .xlsx
QuantStudio 7 Pro	.eds, .xlsx
7500 / 7500 Fast	.eds, .xls
Rotor-Gene Q	.rex, .csv
Mic	.micrun, .xlsx
CFX96	.pcrd, .xlsx
LightCycler 96	.lc96, .txt
LightCycler 480 II	.ixo, .txt

Positive and Negative Controls Interpretation

These PCR controls must be included in every run for the results to be valid. The Positive Control must have a Ct < 30 cycles for the LASV target to pass quality control; the LASV target must not be detected in the Negative Control.

Control	Target		Call	Results Interpretation
POS	LASV	+	PASS	Positive Control *passed* QC as the LASV Ct is *below* the cut-off.
	IC	+		
	SAC	+		
POS	LASV	-	FAIL	Positive Control *failed* QC as the LASV Ct is *above* the cut-off. This may indicate an error during setup, inefficient sample extraction or amplification reagent issues.
	IC	+		
	SAC	+		
NEG	LASV	-	PASS	Negative Control *passed* QC as the LASV Ct is *above* the cut-off.
	IC	+		
	SAC	+		
NEG	LASV	+	FAIL	Negative Control *failed* QC as the LASV Ct is *below* the cut-off. This may indicate an error during setup or cross-contamination.
	IC	+		
	SAC	+		

Results Interpretation

A Ct value < 30 cycles for the PANDAA Lassa Virus (LASV) target indicates that Lassa virus RNA is present in the sample. If the LASV target is ≥ 30 cycles, or not detected, *and* the Internal Control Ct value is < 30 cycles then Lassa virus RNA is *not* present.

If both the LASV *and* Internal control Ct is ≥ 30 cycles, or not detected, then there has been a reagent or extraction failure and the sample should be repeated.

LASV	IC	SAC	Call	Results Interpretation / Recommended Action
+	±*	±*	Positive	Lassa virus RNA detected. Detection of IC or SAC is not required for a positive result.
-	+	+	Negative	Lassa virus RNA not detected; successful RNA extraction and sample adequacy verified.
-	+	-	Invalid Result	SAC not detected; inadequate human biological material, inappropriate specimen collection, or compromised specimen integrity. Repeat extraction and/or obtain a new specimen.
-	-	+	Invalid Result	Internal control not detected; indicates a potential reagent or extraction failure. Repeat extraction and/or obtain a new specimen.
-	+	N/A†	Negative	Lassa virus RNA not detected; successful RNA extraction verified. Sample adequacy control is not applicable for non-human biological specimens.
-	-	N/A†	Invalid Result	Internal control not detected; indicates potential reagent or extraction failure. Repeat extraction and/or obtain a new specimen.

±*: Detection of Internal Control (IC) and Sample Adequacy Control (SAC) is not required to interpret a positive result for LASV, as high viral load can suppress these signals.

N/A†: SAC is not applicable when testing samples that are not derived from human biological material. In these cases, only the Internal Control is required for result interpretation.

* Internal Control and Sample Adequacy Control Interpretation

PANDAA Lassa Virus contains an exogenous, non-competitive extraction control (Internal Control) that is either spiked into the lysis buffer prior to extraction *or* added directly to the PRxB buffer. Additionally, PANDAA Lassa Virus contains amplification and detection reagents for the Sample Adequacy Control (SAC), which detects human nucleic acid. A high Lassa virus viral load may lead to the biased consumption of reaction amplification components and cause a delayed or absent Internal Control signal and/or Sample Adequacy Control signal. Therefore, detection of the Internal Control or Sample Adequacy Control is not required to call a positive result for Lassa virus.

Performance Characteristics

Analytical Reactivity (Inclusivity)

Analytical inclusivity (reactivity) was evaluated using 16 synthetic Lassa virus (LASV) templates. The inclusivity panel comprises sequence-verified RNA templates representing all known Lassa virus lineages selected to cover geographic and genetic diversity. Each template was tested at 500 copies/reaction with 12 replicates per template (3 runs × 4 replicates). LASV RNA copy numbers were assigned by RT-qPCR using dPCR-traceable calibration.

The PANDAA Lassa Virus assay detected 16/16 of isolates (100% detection) across valid replicates. Between isolate performance was consistent, determined by the ΔCt relative to the LASV prototype isolate, Josiah, with a pooled ΔCt range of 1.8 cycles (max−min of mean Ct values across templates). Internal Control (IC) and Sample Adequacy Control (SAC) were detected in 100% of positive control, negative control, and inclusivity panel replicates.

Isolate	Lineage	Accession	Mean Ct ± SD	ΔCt
LASV, AV	V	AY179171	20.5 ± 0.3	-0.1
LASV, G1200-LIB-2010	IV	KM821797	20.5 ± 0.1	-0.2
LASV, IRR_010	III	MK107886	21.7 ± 0.2	1.0
LASV, IRR_016	III	MK107845	21.0 ± 0.1	0.3
LASV, IRR_060	II	MK117909	20.9 ± 0.1	0.2
LASV, ISTH_0779	II	MH053556	21.3 ± 0.2	0.7
LASV, ISTH2376-NIG-2012	II	KM821997	21.6 ± 0.1	0.9
LASV, Josiah	IV	NC_004297	20.6 ± 0.1	Ref
LASV, KAK-428	VI	KT992435	22.2 ± 0.1	1.6
LASV, LP/Pinneo	I	KM822127	20.5 ± 0.1	-0.2
LASV, Nig08-04	II	GU481069	21.3 ± 0.1	0.6
LASV, Nig08-A18	III	GU481071	20.5 ± 0.1	-0.2
LASV, Nig08-A37	II	GU481075	20.8 ± 0.1	0.1
LASV, ONM-299	III	KT992433	22.1 ± 0.1	1.5
LASV, Soromba-R	V	KF478762	22.1 ± 0.1	1.5
LASV, Togo/2016/7082	VII	KU961972	21.5 ± 0.1	0.9

Analytical Sensitivity - System-Level Limit of Detection (IU/mL)

The limit of detection (LoD) of PANDAA Lassa Virus was estimated using the First WHO International Standard for Lassa virus (LASV) RNA (NIBSC 21/112; Lineage IV, Josiah).[1] **The 95% limit of detection of the PANDAA Lassa Virus assay as determined by probit is 203 IU/mL** (95% CI: 146 – 283 IU/mL). This is equivalent to 4 IU/reaction, when extracting 100 µL plasma, eluting in 50 µL, and using 10 µL for PANDAA Lassa Virus. The system analytical sensitivity is for the end-to-end workflow (extraction → amplification) using the minimum sample volume of 100 µL plasma.

For the initial estimation, the WHO Standard was spiked into pooled human EDTA plasma at 2,000, 1,000, and 500 IU/mL, or with PBS as the extraction negative control. Nucleic acid was extracted using the QIAamp Viral RNA Mini Kit (Qiagen). For each concentration, 12 independent extractions were performed and 2 replicates per extraction were amplified on a QuantStudio 5 instrument following the Instructions for Use of the PANDAA Lassa Virus assay (*n* = 24 replicates per concentration).

Concentration (IU/mL)	PANDAA Lassa Virus Result	Hit Rate
2,000	24/24	100%
1,000	24/24	100%
500	24/24	100%
0	0/24	0%

For the refined LoD estimation, the WHO Standard was spiked into pooled human EDTA plasma at 500, 250, and 125 IU/mL, or with PBS for the extraction negative control for a total of 36 replicates per concentration.

Concentration (IU/mL)	PANDAA Lassa Virus Result	Hit Rate
500	36/36	100%
250	35/36	97.2%
125	29/36	80.6%
0	0/36	0%

> System sensitivity is reported in International Units per mL (IU/mL), traceable to the WHO International Standard (IS) 21/112. For LASV, reporting sensitivity in copies/mL is not supported by an international standard and may not be comparable between laboratories or assays. WHO International Standards are the highest-order reference materials for biological substances; calibration to the WHO IS and reporting in IU supports inter-laboratory comparability and more robust determination of analytical sensitivity.

[1] Bentley EM, Richardson S, Atkinson E, et al. The development and evaluation of reference materials for Lassa virus molecular diagnostics. Front Virol. 2025;5. doi:10.3389/fviro.2025.1668042

Analytical Sensitivity - Assay-Level Limit of Detection (copies/reaction)

As the WHO International Standard for LASV RNA is assigned in IU/mL and represents a single lineage, the assay-level LoD in copies/reaction was also estimated in copies/reaction using representative LASV templates spanning the breadth of sequence diversity across LASV lineages. The assay-level LoD is a distinct, claimable attribute that complements the system-level LoD in IU/mL verified separately for the full workflow (extraction → amplification). The two units are not interchangeable; together they provide a complete, traceable description of both the end-to-end system sensitivity (IU/mL) and the assay's intrinsic detection threshold across targeted genetic diversity (copies/reaction).

Data from four representative LASV isolates is shown. Synthetic RNA for each isolates was tested at 20, 10, and 5 copies/reaction. Detection was summarized as the proportion of replicates with a Detected result at each input level. **The assay-level LoD95 of the PANDAA Lassa Virus assay as determined by probit is 8.2 copies/reaction** (95% CI: 7.2 – 9.3 copies/reaction).

LASV Isolate	**5 cp/rxn** Detected / N	**Hit rate**	**10 cp/rxn** Detected / N	**Hit rate**	**20 cp/rxn** Detected / N	**Hit rate**	**LoD95 (cp/rxn)**	**95% CI (cp/rxn)**
KAK-428	48/60	80.0%	58/60	96.7%	60/60	100.0%	8.5	6.4 – 11.2
ONM-299	47/60	78.3%	59/60	98.3%	60/60	100.0%	7.8	6.0 – 10.0
Soromba-R	40/60	66.7%	59/60	98.3%	60/60	100.0%	8.2	6.5 – 10.3
Togo	54/60	90.0%	58/60	96.7%	60/60	100.0%	7.3	5.2 – 10.4
Pooled	**189/240**	**79.1%**	**234/240**	**97.9%**	**239/239**	**100.0%**	**8.2**	**7.2 – 9.3**

PANDAA Lassa Virus Compatibility with Major Real-Time PCR Instruments

The study was conducted on five instrument models using the *PANDAA LASV Qualification Panel* (Aldatu Biosciences, catalog # 2121096): QuantStudio 5, 96-well Fast (QS5), QuantStudio 7 Pro, 96-well Fast (QS7), Mic real-time PCR Cycler (Mic), ABI 7500 Fast (7500), and Rotor-Gene Q 5-Plex (RGQ). For the QS5, QS7 and 7500, both fast and standard thermal ramp rates were evaluated.[2] For each instrument model and ramp rate, the study was conducted across two independent runs performed by separate operators. In each run, four independent replicates of each isolate and concentration in the table below was tested and four independent replicates each of the kit Positive Control and Negative Control.

Baseline Ct values for all panel members were established on the QS5 with fast thermal ramp rates. For each comparison, the LASV threshold was adjusted until the mean Ct of the Positive Control (PC) replicates was within ± 0.5 cycles of mean PC Ct from the QS5 baseline. The average ΔCt relative to the QS5 fast for LASV-positive panel members was −0.1 cycles (SD: ± 0.2 cycles; range −0.7 to 0.4).

LASV Isolate	Copies	Ct QS5 Fast	ΔCt QS5 Std	ΔCt QS7 Fast	ΔCt QS7 Std	ΔCt 7500 Fast	ΔCt 7500 Std	ΔCt Mic	ΔCt RGQ
LP/Pinneo	50	24.6	-0.1	0.0	0.0	0.2	0.1	-0.3	-0.4
Josiah	50	24.7	0.0	0.2	0.3	-0.1	0.1	-0.3	-0.3
Soromba-R	50	27.3	0.2	0.3	-0.3	-0.6	-0.4	0.0	0.1
Togo	50	25.8	0.0	0.1	0.3	-0.2	0.0	0.4	0.4
KAK-428	50	26.7	-0.2	0.1	-0.1	-0.4	-0.5	-0.3	-0.4
Nig08-04	50	25.7	-0.2	0.1	0.1	-0.1	-0.4	-0.3	-0.4
LCMV	5×10^3	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Nig08-A18	5×10^4	14.4	-0.1	-0.1	0.0	-0.1	-0.1	-0.2	-0.2
Nig08-A18	5×10^3	18.2	-0.1	0.0	0.0	-0.1	-0.1	-0.2	-0.1
Nig08-A18	50	25.1	-0.3	-0.2	-0.3	-0.4	-0.7	-0.4	-0.4
Pos Ctrl	1,500	19.9	0.0	0.0	0.0	0.0	0.0	-0.2	0.0
Neg Ctrl	N/A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

N.D. not detected

Instrument	Ramp Run Time (average)	Software Used
QS5	Fast: 75 min; Standard: 90 min	QuantStudio Design & Analysis Software version 1.6.1
QS7	Fast: 77 min; Standard: 90 min	Design & Analysis Software. Release Version: 2.8.0
7500	Fast: 78 min; Standard: 98 min	7500/7500 Fast Real-Time PCR System Software v2.3
Mic	Fast: 87 min	micPCR Software v2.12.7
RGQ	Standard: 100 min	Rotor-Gene Q Software version 2.3.5

[2] Fast thermal ramp rates were 4.1 °C/s (heating) and 3.1 °C/s (cooling). Standard thermal ramp rates were 1.6 °C/s (heating and cooling).

Procedural Limitations

The PANDAA Lassa Virus kit is released for Research Use Only (RUO) and is not intended for use in diagnostic procedures. The assay is intended for use by lab personnel trained in the PANDAA Lassa Virus assay procedure and in the operation of nucleic acid extraction and real-time PCR systems used on site. Good laboratory practices and adherence to the Instructions for Use are required to minimize contamination and ensure reliable assay performance .

This assay has been evaluated for use with human plasma and serum specimens. Analytical performance with human plasma and serum does not establish clinical diagnostic performance or clinical utility. Use of specimen types other than human plasma or serum has not been established and may yield inaccurate or uninterpretable results.

A negative result does not exclude the possibility that Lassa virus RNA may be absent from the sample, below the assay detection limit, or affected by specimen quality, handling, storage, transport, extraction efficiency, or amplification interference. The Internal Control and Sample Adequacy Control are included in PANDAA Lassa Virus to help identify specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. These controls do not eliminate the possibility of interference or other causes of invalid or non-representative results.

General Guidelines

Shipping, Storage and Handling

PANDAA Lassa Virus is shipped on dry ice, and kit components arrive frozen. Store all components at **−15 °C to −25 °C** upon receipt, protected from light. **Stop-Start RT enzyme contains glycerol and may remain liquid** at −15 °C to −25 °C. Keep reagents in the original packaging when not in use and return them promptly to recommended storage conditions.

Inspect all kit components upon receipt. Do not use reagents if they appear compromised (e.g., cracked or leaking vials, illegible labels, or evidence of thawing. If a temperature excursion, shipping damage, or a discrepancy between the kit labels and this IFU is suspected, quarantine the kit and contact Technical Support (support@aldatubio.com) before use.

Ensure all reagents are fully thawed and mixed before use. Keep Stop-Start RT enzyme on ice during handling. Perform the PANDAA Lassa Virus assay in environmental conditions at 20 °C to 25 °C and 30% to 70% relative humidity.

Real-time PCR Best Practices

Since real-time PCR is a sensitive technique and the dynamic range of this assay extends to a very low template copy number, it is critical to perform accurate liquid handling to produce reliable results, and precaution must be taken when executing this protocol.

- Handle reagents and RNA templates at separate / dedicated laboratory areas to prevent cross contamination.
- Always ensure reagents and samples are fully thawed, thoroughly mixed and spun/centrifuged briefly before use.
- Enzymes (Stop-Start RT enzyme and PRxB II buffer) and thawed viral RNA should be kept on ice while being used.
- Ensure that a new pipette tip is used for each step in the protocol. Cross contamination between the samples and controls will affect the accuracy of the results.
- Good laboratory practice should be observed at all times to avoid contamination of reagents, samples, consumables, pipettes, and work areas.

Safety

Handle all specimens, extracted nucleic acids, and used consumables as potentially infectious and in accordance with applicable institutional biosafety procedures and local regulations. Wear appropriate personal protective equipment (PPE) (e.g., gloves, lab coat, and eye protection) and perform work in facilities suitable for molecular testing.

To minimize contamination risk, follow good molecular biology practices, including physical separation of pre-amplification and post-amplification areas, use of aerosol-resistant pipette tips, and routine decontamination of work surfaces and equipment. Dispose of waste according to institutional procedures and local regulations.

Chemical hazards associated with extraction reagents are specific to the extraction method used; follow the extraction kit manufacturer's IFU and Safety Data Sheets (SDS).

Appendix A: Materials Required but Not Included

All Workflows

The following materials are required for nucleic acid extraction (if performed by the user) and for real-time PCR setup and run on a compatible instrument.

Category	Components, Materials, or Reagents
Personal protective equipment	• Appropriate PPE (e.g., gloves, lab coat, eye protection) per institutional requirements.
Liquid handling	• Calibrated pipettes capable of dispensing 1 µL to 1000 µL • Aerosol-resistant (filter) pipette tips
Consumables	• RNase-free microcentrifuge tubes (for sample extraction and/or aliquoting) • Real-time PCR instrument consumables appropriate to the platform (e.g., plates or tubes/strips, optical seals/caps)
Mixing and centrifugation	• Vortex • Plate spinner / plate centrifuge (for brief spin-down of PCR plates, if using plate format) • Benchtop microcentrifuge (for brief spin-down of tubes/strips, as applicable)
Temperature control and storage	• Cold block or ice • Refrigerator, 2 °C to 8 °C (for short-term storage, as applicable) • Laboratory freezer, −15 °C to −25 °C (for kit storage)

Appendix B: Real-time PCR Instrument Setup and Analysis

- **ABI / Thermo Fisher:** ABI 7500 / 7500 Fast *, QuantStudio 5 *, QuantStudio 6 Flex/Pro †, QuantStudio 7 Flex/Pro *, QuantStudio 12K Flex †, and ViiA 7. †
- **Qiagen:** Rotor-Gene Q5/6 Plex *
- **Bio Molecular Systems:** Mic *
- **Roche:** LightCycler 96 *, LightCycler 480 II †
- **Bio-Rad:** CFX96 †

* Validated instruments. Refer to PANDAA Lassa Virus Compatibility with Major Real-Time PCR Instruments (page 20).

† Compatible real-time PCR instruments that have not been validated with PANDAA Lassa Virus by Aldatu Biosciences.

CAUTION

Ensure that your real-time PCR instrument has been installed, calibrated, and maintained according to the manufacturer's instructions and recommendations.

Use the real-time PCR run template provided by Aldatu Biosciences, available at www.aldatu.bio/qpcr-templates. If your real-time PCR instrument is not listed then contact Technical Support (support@aldatubio.com) about your real-time PCR instrument requirements and configuration, and to obtain a run template file with the correct cycling conditions.

Instrument Instructions

ABI / Thermo Fisher

Instruments: ABI 7500 / 7500 Fast, QuantStudio 5, QuantStudio 6 Flex/Pro, QuantStudio 7 Flex/Pro, QuantStudio 12K Flex, and ViiA 7. Templates (file extension *.edt*) provided by Aldatu Biosciences can be downloaded from www.aldatu.bio/qpcr-templates.

Target Name	Channel	Reporter	Quencher
PANDAA Lassa Virus (LASV)	Blue	FAM	NFQ-MGB
Internal Control (IC)	Red	Cy5	NFQ-MGB
Sample Adequacy Control (SAC)	Yellow	TAMRA	NFQ-MGB

ABI / Thermo Fisher Data Analysis

Template files provided by Aldatu Biosciences are programmed with the analysis settings below.

Target Name	Threshold	Baseline
PANDAA Lassa Virus (LASV)	0.5	Auto
Internal Control (IC)	0.05	Auto
Sample Adequacy Control (SAC)	0.05	Auto

1. **Adjust threshold (LASV):** Adjust the threshold for the LASV target so that the mean Ct of the Positive Control (PC) replicates is **~20 cycles (target range: 19 – 21 cycles)**.
2. **Analyze:** Ensure that data are reanalyzed after any threshold adjustments.
3. **Save:** Ensure that the analyzed *.eds* file is saved.
4. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.eds* run file or an exported Excel file.

Using the *.eds* File	Using the Exported Excel File
Import the analyzed *.eds* file directly into the *PANDAA VHF Interpretation Software*.	**Export settings:** From the *Export* menu, ensure that, at a minimum, the following content is selected for export. • *Results* • *Amplification Data* • *Multicomponent Data* **Export:** From the *Export* menu, choose **.xls* or **.xlsx* as the *File Type* and then export all data to one file.

CFX96 (Bio-Rad)

Two separate template files are required for the CFX96, and can be downloaded from www.aldatu.bio/qpcr-templates:

1. **Protocol file:** *PANDAA Lassa Virus - Cycling Conditions - CFX96.prcl*
2. **Plate file:** *PANDAA Lassa Virus - Plate Template - CFX96.pltd*

Using the *.prcl* Protocol File and *.pltd* Plate File

1. **Import protocol file:** After starting a new user-defined run, import the *.prcl* template cycling conditions using *Select Existing* under the *Protocol* tab.
2. **Import plate file:** Under the *Plate* tab, import the *.pltd* plate template containing the assay targets using *Select Existing*.

Target Name	Channel	Fluorophore
PANDAA Lassa Virus (LASV)	1	FAM
Internal Control (IC)	4	Cy5
Sample Adequacy Control (SAC)	2	Cal Orange 560

CFX96 Data Analysis

1. **Show amplification cycles:** After the run is completed, select the *Step Number* dropdown in the *Data Analysis* window and select 8 to show the data collected during the amplification cycles.
2. **Adjust settings:** Under the *Settings* menu ensure that the following settings are selected:
    a. *Cq Determination Mode* ► *Single Threshold*
    b. *Baseline Setting* ► *Baseline Subtracted Curve Fit*
    c. *Analysis Mode* ► *Target*
3. **Adjust threshold (LASV):** Adjust the threshold for the LASV target so that the mean Ct of the Positive Control (PC) replicates is **~20 cycles (target range: 19 – 21 cycles**).
4. **Fluorescence cutoff:** Under the *End Point* tab, edit the following settings for the LASV target:
    a. *End Cycles to Average*: 5
    b. *Percent of Range*: 5.0
    c. Record any wells that are not called *(+) Positive* by endpoint RFU.

5. **Analyze and save:** Ensure that data are reanalyzed after any adjustments and save the *.pcrd* file.
6. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.pcrd* run file or an exported Excel file.

Using the .pcrd File	Using the Exported Excel File
Import the analyzed .pcrd file directly into the *PANDAA VHF Interpretation Software*.	**Export settings:** From the *Export* menu, select *Custom Export*. • Choose Excel 2007 (*.xlsx) as the *Export Format* • Select all options for *Sample Description* and *Quantification*. Ensure that *End RFU* is selected as an export column.

LightCycler 96 and LightCycler 480 II (Roche)

Instruments: A template file for the LightCycler 96 or LightCycler 480 II can be downloaded from www.aldatu.bio/qpcr-templates.

Detection format: Hydrolysis probes.

Target Name	Channel	Dye
PANDAA Lassa Virus (LASV)	1	FAM
Internal Control (IC)	4	Cy5
Sample Adequacy Control (SAC)	2	Yellow555

LightCycler 96 Data Analysis

It is not possible to save analysis settings in the *.lc96* template file provided by Aldatu Biosciences.

1. **Adjust threshold (LASV):** Adjust the threshold for the LASV target so that the mean Ct of the Positive Control (PC) replicates is **~20 cycles (target range: 19 – 21 cycles)**.
2. **Adjust thresholds (IC and SAC):** Place the IC and SAC thresholds in the exponential (log-linear) region above baseline and below plateau; apply the same setting to all samples and controls.
3. **Save:** Ensure that the analyzed *.lc96* run file is saved.
4. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.lc96* run file or an exported text file.

Using the *.lc96* File	**Using the Exported Excel File**
Import the analyzed *.lc96* file directly into the *PANDAA VHF Interpretation Software*.	Ensure that the endpoint fluorescence (*EPF*) column is visible in the *Results Table* before exporting. If the EPF column is hidden, right click on the *Results Table* column headings ► *Column Selector* ► *EPF* **Export:** Right click on the *Results Table* ► *Export to File* ► Save as file type *Text files (*.txt)*.

LightCycler 480 II Data Analysis

Color compensation (multiplex): Apply an appropriate Color Compensation (CC) setting/object for multicolor assays before analysis.

LightCycler 480 software reports the crossing point as Cp, which is treated as equivalent to Ct for reporting. To obtain Ct-equivalent values using a threshold-based method, **use Absolute Quantification – Fit Points** (threshold line method).

1. **Select analysis method:** In the analysis modules, select *Absolute Quantification* ► *Fit Points*. (Fit Points uses a threshold line approach.)
    a. Select *Analysis* ► *Abs Quant/2nd Derivative Max*
    b. Under *Method*, select *Fit Points* (threshold line method)
2. **Adjust threshold (LASV):** Adjust the threshold for the LASV target so that the mean Cp (Ct-equivalent) of the Positive Control (PC) replicates is **~20 cycles (target range: 19 – 21 cycles)**.
3. **Save:** Ensure that the analyzed *.ixo* run file is saved (*.ixo* is a supported export format on LC480).
4. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.ixo* run file or an exported text file.

Using the *.ixo* File	**Using the Exported Excel File**
Import the analyzed *.ixo* file directly into the *PANDAA VHF Interpretation Software*.	**Export:** Ensure that the endpoint fluorescence (EPF) column is visible in the Results Table. Right click on the Results Table ►*Export to File* ► Save as file type *Text files (*.txt)*.

Mic (Bio Molecular Systems)

The Mic Real-Time qPCR Cycler (Mic) from Bio Molecular Systems uses either an *Assay* definition file (file extension *.micassay*) containing cycling conditions, assay targets, and analysis settings, or a *Template* definition file (file extension *.mictemplate*), which additionally contains sample information. Templates provided by Aldatu Biosciences can be downloaded from www.aldatu.bio/qpcr-templates.

1. **Assay file:** *PANDAA Lassa Virus.micassay*
2. **Template file:** *PANDAA Lassa Virus.mictemplate*

Target Name	Channel	Reporter Dye
PANDAA Lassa Virus (LASV)	Green	FAM
Internal Control (IC)	Red	Cy5
Sample Adequacy Control (SAC)	Yellow	NED

Storing the Assay and Template Definition Files

The *.micassay* and/or *.mictemplate* must be saved in the local user document folder on your PC e.g., C:\Users*username*\Documents\Bio Molecular Systems\micPCR\Templates. This folder should have been automatically created when installing the micPCR software. If this folder is missing, contact Aldatu Technical Support (support@aldatubio.com).

Using the *.micassay* Assay Definition File

In the micPCR software select *Run* from the *New* menu. In the new run file, click on the + icon next to *Assays* on the left-hand bar. *PANDAA Lassa Virus* can be selected from *My Assays*. If it is not visible then select *Browse*. Locate and select the *PANDAA Lassa Virus.micassay* file, which will now be loaded into your new run.

Using the *.mictemplate* Template Definition File

The *PANDAA Lassa Virus.mictemplate* contains the same cycling conditions, assay targets, and analysis settings as the *.micassay* file. It is also pre-loaded with the Negative Control in sample position 1 and the Positive Control in sample position 48.

Select *Run from Template* from the *New* menu in the micPCR software. Choose *PANDAA Lassa Virus* and click *OK*. If *PANDAA Lassa Virus* is not visible in the list of templates, confirm that it has been stored in the correct folder on your computer. Alternatively, start a new run using the *.micassay* file.

Mic Data Analysis

Template files provided by Aldatu Biosciences are programmed with the analysis settings below.

Target Name	Method	Exclusion	Cutoff	Threshold
PANDAA Lassa Virus (LASV)	Dynamic	Extensive	5%	Auto
Internal Control (IC)	Dynamic	None	N/A	Auto
Sample Adequacy Control (SAC)	Dynamic	None	N/A	Auto

1. **Adjust threshold:** Adjust the threshold for the LASV target so that the mean Ct of the Positive Control (PC) replicates is **~20 cycles (target range: 19 – 21 cycles)**.
2. **Save:** Ensure that the analyzed *.micrun* file is saved.
3. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.micrun* run file or an exported Excel file.

Using the *.micrun* File	Using the Exported Excel File
Import the analyzed *.micrun* file directly into the *PANDAA VHF Interpretation Software*.	**Export:** From the *Save As* menu, select *Excel Workbook* and save the exported *.xlsx* file.

Rotor-Gene Q (Qiagen)

The Rotor-Gene Q uses template files with the file extension *.ret*. Start a new run in the Rotor-Gene Q software by selecting *New* ► *Open a template in another folder*, and select the ***PANDAA Lassa Virus - RGQ Template.ret*** file. The template file provided by Aldatu Biosciences contains the assay cycling conditions, targets, and gain settings and can be downloaded from www.aldatu.bio/qpcr-templates.

Target Name	Channel	Source	Detector
PANDAA Lassa Virus (LASV)	Green	470nm	510nm
Internal Control (IC)	Red	625nm	660nm
Sample Adequacy Control (SAC)	Yellow	530nm	555nm

Gain and Auto-Gain Settings

Auto-gain optimization is performed before the 1st acquisition. The Rotor-Gene Q template provided by Aldatu Biosciences, *PANDAA Lassa Virus - RGQ Template.ret*, is pre-programmed with this setting. *Do not* perform gain optimization at 60 °C at the beginning of the run.

Rotor-Gene Q Data Analysis

It is not possible to save analysis settings in the *.ret* template file provided by Aldatu Biosciences.

1. **Set global analysis settings for all three assay targets:**
   - *Dynamic Tube* should be selected.
   - *Slope Correct* should be selected for consistent results across all runs.
2. **Set normalization options (LASV):** In the analysis window for the LASV target, Outlier Removal should be set at 5% for the PANDAA Lassa Virus (LASV) target.
3. **Adjust threshold (LASV):** Adjust the threshold for the LASV target so that the mean Ct of the Positive Control (PC) replicates is **~18 cycles (target range: 17 – 19 cycles)**.[3]
4. **Adjust thresholds (IC and SAC):** Place the IC and SAC thresholds in the exponential (log-linear) region above baseline and below plateau; apply the same setting to all samples and controls.

[3] The threshold requirements for the Rotor-Gene Q differ to other instruments due to platform-specific differences in fluorescence reporting. Achieving accurate exponential-phase threshold placement requires positioning the threshold such that the mean Ct of the Positive Control replicates is ~18 cycles (target range: 17–19). At higher threshold settings, low-copy reactions near the limit of detection may fail to cross the threshold, producing false negative results.

5. **Save:** Ensure that the analyzed *.rex* file is saved.
6. **Analysis:** Proceed to data analysis with the *PANDAA VHF Interpretation Software* (page 14) using either the *.rex* run file or an exported Excel file.

Using the *.rex* File	**Using the Exported Excel File**
Import the analyzed *.rex* file directly into the *PANDAA VHF Interpretation Software*.	**Export:** *Select File* ►*Save As* ► *Excel Analysis Sheet* and save the *.csv* file.

Customer and Technical Support

Email: support@aldatubio.com

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Watertown, MA 02472
USA

Disclaimers

This kit is for Research Use Only and is not intended for use in diagnostic procedures.

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Patents

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Copyright

Explanation of Symbols

RUO	For Research Use Only
REF	Catalog number
LOT	Batch number
	Storage temperature range
	Use-by date
	Contents sufficient for n reactions
	Consult Instructions for Use
Cont.	Contents of the PANDAA® Lassa Virus kit
	Manufacturer

ALDATU
BIOSCIENCES

al • da • tu [ɒl - də - tu]: **to become something different**