PANDAA qDx LASV

Detection of Lassa virus RNA.

Instructions for Use

Overview

This technical user guide is intended for the PANDAA qDx LASV kit, a pathogen detection assay that is uniquely designed to provide accurate and rapid information relevant to the identification and / or management of Lassa fever, caused by infection by Lassa virus. The PANDAA qDx LASV kit is an *in vitro* real-time PCR [qPCR] assay for the amplification and detection of Lassa virus viral RNA.

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

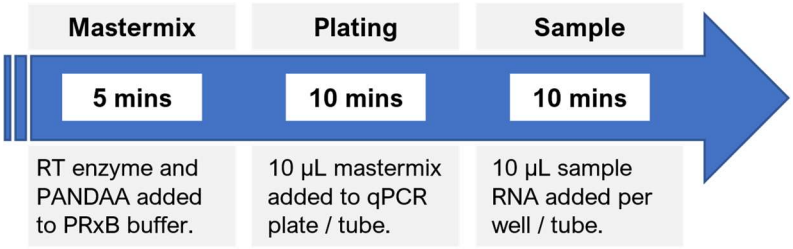
PANDAA Technology

Our revolutionary detection and genotyping technology, PANDAA, uniquely compensates for evolving pathogen diversity, ensuring that PCR diagnostic integrity isn't affected by genomic variation now, or in the future. PANDAA reagents are innovatively designed to mitigate genomic variability by normalizing probe-binding regions. During the initial qPCR 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.

PANDAA qDx LASV

PANDAA qDx LASV 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 Internal Control target is amplified by PANDAA primers and reported using a VIC®-labelled hydrolysis probe. **PANDAA primer and probe designs are proprietary to Aldatu Biosciences.**

The entire PANDAA qDx LASV workflow can be completed in two hours. Assay preparation takes a maximum of 40 minutes with a qPCR run time is 70 minutes. Automated data analysis can be performed in <10 minutes. After adding the RT enzyme and PANDAA to the PRxB mastermix, the user adds 10 µL of the mastermix to the real-time PCR plate / tube. Extracted RNA is added to each of the wells / tubes and **no mixing is necessary.**



Compatible real-time PCR instrumentation

Any qPCR instrument that is capable of detecting FAM and VIC fluorophores, with or without the passive reference dye, ROX, such as those listed in Appendix B.

Contents and Storage

Reagents

The kit includes all amplification reagents for up to 96 reactions, and includes one (1) each of the positive control, negative control, and internal control RNA.

Contents	Cap color	Top Label	Quantity	Volume
PRxB Buffer	Clear	PRxB	2	500 µL
Lassa Virus PANDAA	White	PAN LASV	2	35 µL
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

The PANDAA qDx LASV kit is shipped on dry ice and the kit components will arrive frozen. All components should be stored between -25°C and -15°C upon arrival. RT Enzyme will not freeze solidly even when stored at -25°C between -15°C as it contains glycerol.

A unique feature of the PANDAA assays is that, *after thawing*, all components [excluding the RT enzyme] can be stored between 2°C and 8°C for up to 7 days. More than two freeze-thaw cycles of the reagents should be avoided to ensure assay performance.

Contents	Stability at 2°C to 8°C
PRxB Buffer	7 days
Lassa Virus PANDAA	
Internal Control	
Positive Control	
Negative Control	
RT Enzyme	*Should not be stored at 2°C to 8°C*

All reagents, other than the 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.

RT Enzyme

A specially formulated hot-start reverse transcriptase provided at an optimized concentration for PANDAA qDx.

Lassa Virus PANDAA

Includes uniquely designed PANDAA primers and probes for the amplification and detection of two distinct targets. **PANDAA primer and probe designs are proprietary to Aldatu Biosciences.**

1. LASV [FAM™] – primers and probes which target to the L segment of the Lassa virus genome.
2. Internal Control [VIC®] – primers and probes which detect the internal control RNA, which serves as a process control for both RNA extraction and PCR amplification.

Extraction and Amplification Controls

The **Internal Control** (IC) is an exogenous, non-competitive control that detects MS2 phage-specific RNA, which is either spiked into the lysis buffer prior to extraction, or added directly to the real-time PCR reaction mix. The **Positive Control** is synthetic RNA covering the assay target regions in the Lassa virus genome of the *Nig-08-A18* strain, are provided at 50 copies / µL to yield 500 copies / reaction when using 10 µL RNA / reaction. The **Negative Control** comprises human genomic DNA only.

Sample Preparation for PANDAA qDx LASV

The starting material for the PANDAA qDx LASV kit is extracted viral RNA. If samples have already been extracted then proceed to *PANDAA qDx LASV Kit Step-by-Step Instructions* on page 8.

Nucleic Acid Extraction

Compatible Instrumentation and Reagents

Many commercially-available nucleic acid extraction systems are compatible with real-time PCR applications and are thus suitable for sample preparation prior to PANDAA qDx LASV testing. For any nucleic acid extraction kit or workflow used, manufacturer's instructions for purification of viral RNA should be followed. Protocols that purify total nucleic acid (DNA and RNA) can be used.

Sample Volume

The input volume used for sample extraction will vary by platform but should range from **100µL to 1,000µL** and should be maximized for optimal test sensitivity.

Required Elution Volume

The elution volume used in the sample extraction protocol will vary by platform, however, it should be optimized by minimizing elution volume while ensuring high elution efficiency. Typical recommended elution volumes range from **50 µL to 100 µL**. Each PANDAA qDx LASV reaction requires **10µL** of eluted RNA. Viral RNA extractions should be completed immediately before proceeding into the PANDAA qDx LASV real-time PCR setup. However, extracted nucleic acid may be stored at between 2°C and 8°C for up to 6 hours prior.

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 **1µL of IC** to the sample extraction lysis buffer e.g., if processing 48 samples then add 48 µL of IC to the lysis buffer.

PANDAA qDx LASV Kit Step-by-Step Instructions

A. Before Beginning

In a dedicated lab area for setting up PCR reaction mixes

Remove **RT enzyme** from freezer and place directly on ice. Remove remaining 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.** *Place all tubes on ice immediately after thawing.*

B. PANDAA qDx LASV Reaction Mix Setup [48 Reactions]

1. Briefly **spin the reagent tubes** to collect drops that may have formed on the interior sides of the tubes.
2. Add **30µL of the Lassa Virus PANDAA** directly to the **PRxB tube.**
3. Before removing RT enzyme, pipette up and down 2-3 times to mix. Be careful to only submerge the tip of the pipette to limit excess RT on the exterior of the tip. Add **3µL of the mixed RT enzyme** directly to the **PRxB tube.**
4. If Internal Control (IC) RNA was not added to the lysis buffer prior to sample extraction, it should be added to the PRxB Buffer bottle here. For 48 samples, add **3µL of IC RNA to the PRxB tube.**
5. Gently vortex the **PRxB** (now containing the RT enzyme and PANDAA) to mix. Spin to collect any droplets and keep on ice.

Fewer than 48 Reactions: The complete LASV PANDAA mastermix—containing PRxB, RT enzyme, PANDAA and Internal Control—is **stable for up to 48 hours when stored at 2°C to 8°C.**

If you are require fewer than 48 reactions, you may prepare the complete mastermix for 48 samples and store any residual mastermix.

24 Reaction Modification: Remove **250µL PRxB Buffer** and transfer to a clean tube. Add **15µL LASV PANDAA** and **1.5µL RT Enzyme**. Follow the storage instructions on page 5 for the remaining reagents.

C. Real-time PCR Plate Setup

1. Transfer 10µL of complete LASV PANDAA master mix (contained in the PRxB tube) into each required well of an optical 96-well reaction plate / optical reaction tube suitable for use with your real-time PCR instrument.
2. Add 10µL of eluted sample RNA or 10 µL positive / negative control to each well / tube containing LASV PANDAA master mix. **You do not *need* to pipette up and down to mix.**
3. Seal the 96-well reaction plate with optical adhesive film or reaction tubes. Briefly spin the 96-well plate before placing in the real-time PCR instrument.

D. Real-time PCR Protocol

1. Transfer the sealed plate / tubes to the real-time PCR instrument.
2. Use the qPCR template provided by Aldatu Biosciences, which can be downloaded at www.aldatu.bio/qpcr-templates. Alternatively, you can use the run parameters given on page 10.
3. Total run time should be approximately 60 to 80 minutes.

E. Manual Real-time PCR Settings

Use the following parameters and proceed to run the PANDAA qPCR. Contact Technical Support (support@aldatubio.com) with questions about your real-time PCR instrument requirements and configuration.

Real-Time PCR instrument settings

Setting	Value
Reaction Volume	20µL
Ramp Rate	Fast, if compatible with your real-time PCR instrument. Otherwise use standard ramp rates.
Passive Reference [if applicable]	ROX

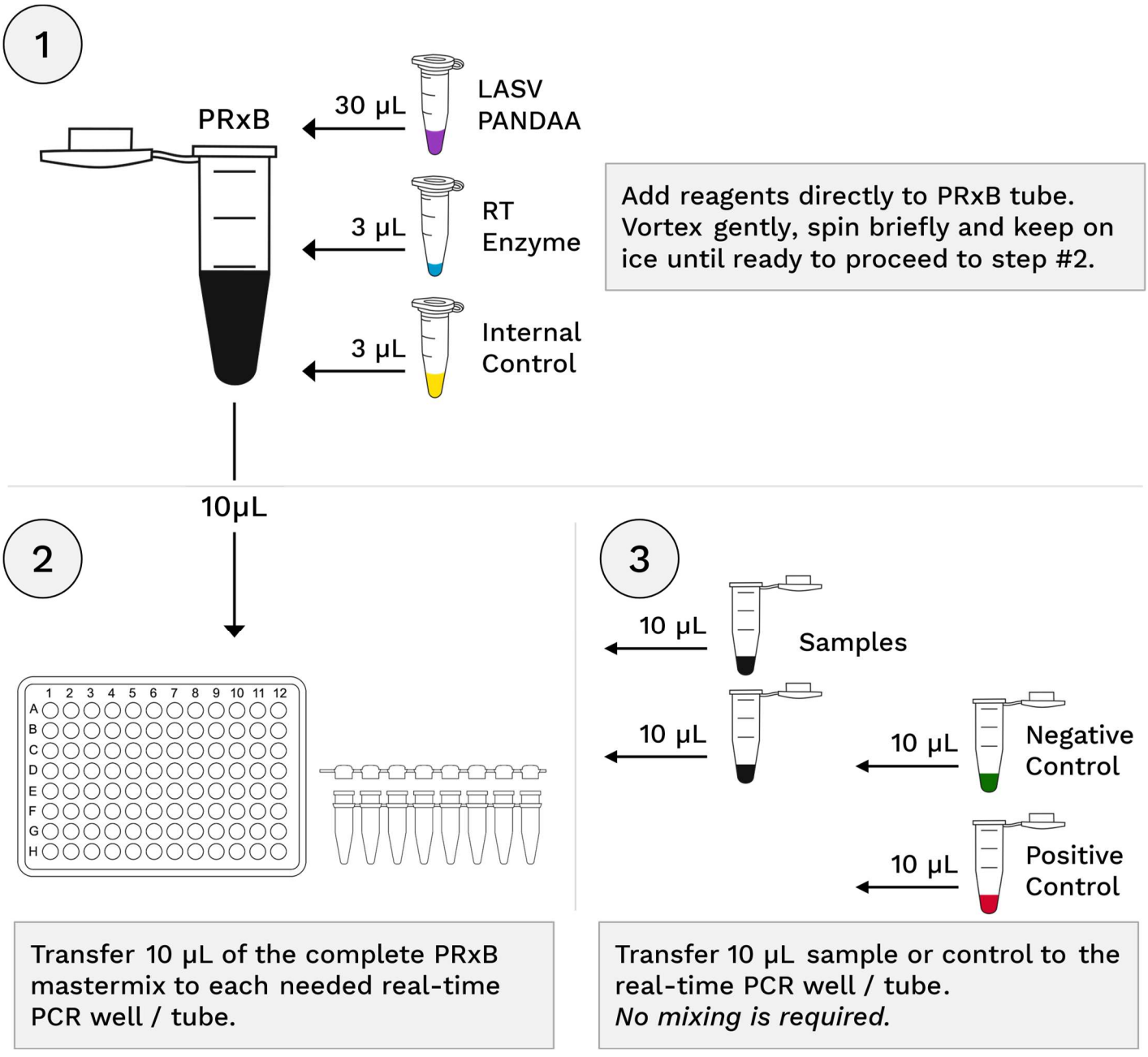
Target Name	Reporter	Quencher
PANDAA qDx LASV	FAM	None
Internal Control	VIC	None

Cycling conditions

During the 60°C anneal and extension phase of the Amplification and Detection step, acquire fluorescence data in the FAM and VIC channels.

Step	Temperature	Time	Cycles
Reverse transcription	50°C	15 minutes	1
Enzyme activation	95°C	2 minutes	1
PANDAA Adaptation	90°C 55°C 60°C	3 seconds 30 seconds 30 seconds	10
Amplification and Detection	90°C 60°C	3 seconds 60 seconds	30

PANDAA qDx LASV Kit Quick Guide



Add reagents directly to PRxB tube. Vortex gently, spin briefly and keep on ice until ready to proceed to step #2.

Transfer 10 µL of the complete PRxB mastermix to each needed real-time PCR well / tube.

Transfer 10 µL sample or control to the real-time PCR well / tube.
No mixing is required.

Data Analysis

Real-Time PCR Analysis Parameters

The qPCR template provided by Aldatu Biosciences will contain the thresholds required to determine the Ct value for the PANDAA qDx LASV and Internal Control [IC] targets. For additional information or consultation, please contact Technical Support (support@aldatubio.com).

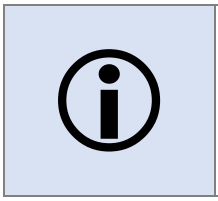
Results Interpretation

A Ct value less than 30 cycles for the LASV [FAM] target indicates that Lassa virus RNA is present in the sample. If the LASV target is greater than or equal to 30 cycles, or not detected, *and* the Internal Control Ct value is less than 30 cycles then Lassa virus RNA is *not* present. If both the LASV *and* Internal control Ct is greater than or equal to 30 cycles, or not detected, then there has been a reagent or extraction failure and the sample should be repeated.

LASV [FAM]	IC [VIC]	Call	Results Interpretation
+	±*	Positive	Lassa virus RNA detected.
-	+	Negative	Lassa virus RNA *not* detected.
-	-	Invalid Result	Reagent or extraction failure. Sample should be retested. If the result is still invalid, a new specimen should be obtained.

* Internal Control Interpretation

PANDAA qDx LASV contains an exogenous, non-competitive extraction control (Internal Control) that detects MS2 phage-specific RNA, which is either spiked into the lysis buffer prior to extraction, or added directly to the real-time PCR reaction mix. Internal Control (IC) is for quality control purposes and detection is not required to call a positive result for Lassa virus. A high Lassa virus viral load may lead to the biased consumption of reaction amplification components leading to a delayed or absent Internal Control signal.



For detailed information or consultation on real-time PCR instrument programming for PANDAA qDx™ LASV, contact Technical Support (support@aldatubio.com).

Positive and Negative Controls

These 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 in order to pass QC; the LASV target must not be detected in the negative control.

Control	Target		Call	Results Interpretation
POS	LASV IC	+ +	PASS	Positive control *passed* QC as the LASV Ct is *below* the cut-off.
POS	LASV IC	- ±	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.
NEG	LASV IC	- +	PASS	Negative control *passed* QC as the LASV Ct is *above* the cut-off.
NEG	LASV IC	+ ±	FAIL	Negative control *failed* QC as the LASV Ct is *below* the cut-off. This may indicate an error during setup or cross-contamination.

Procedural Limitations

The PANDAA qDx LASV kit is released for Research Use Only (RUO) by lab personnel who have been trained in the procedures of the PANDAA qDx LASV assay and in the operation of the systems on site used for nucleic acid extraction and real-time PCR analysis. Good laboratory practices and careful adherence to the procedures specified in the Instructions for Use document are necessary to avoid contamination of reagents.

Negative results do not preclude presence of Lassa virus RNA. False negative or invalid results may occur due to interference. The Internal Control is included in PANDAA qDx LASV to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification.

Detection of Lassa virus RNA may be affected by sample collection methods, patient factors (e.g., presence of symptoms), and/or stage of infection and results should be interpreted by a trained professional in conjunction with the patient's history and clinical signs and symptoms, and epidemiological risk factors. Reliable results depend on proper sample collection, storage and handling procedures. False-negative results may arise from degradation of the viral RNA during shipping/storage.

General Guidelines

Shipping, Storage and Handling

The PANDAA qDx LASV kits are shipped on dry ice. Upon arrival store the kit components in a -25°C and -15°C freezer. RT Enzyme will not freeze solidly even when stored at -25°C and -15°C as it contains glycerol.

Always ensure that reagents have been fully thawed and mixed before use. RT enzyme and RNA control samples should remain on ice while handling.

qPCR Best Practices

Since qPCR 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 briefly spun/centrifuged before use.
- Enzymes (RT Enzyme and PRxB 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

Individuals should be trained according to relevant regulatory and institutional requirements before working with potentially biohazardous materials. One must wear appropriate Personal Protective Equipment and conduct all procedures at an equipped facility with proper safety equipment.

Appendix A: Materials Required but Not Included

All Workflows

The following are required for any nucleic acid extraction and real-time PCR instrument workflow used with the PANDAA qDx LASV test kits. (MLS = Major Laboratory Supplier.)

Components, Materials, or Reagents	Source
Calibrated Pipettes capable of delivering 1 µL to 1000 µL	MLS
Filter Pipette Tips	MLS
Low-bind RNase-free microcentrifuge tubes (0.5 mL to 2.0 mL)	MLS
Plate Spinner	MLS
Benchtop Microcentrifuge	MLS
Vortex	MLS
Cold block or Ice	MLS
Laboratory Freezer, -10°C to -30°C	MLS

Appendix B: Compatible Real-time PCR Instruments

- ABI / Thermo Fisher: 7500, 7700*, 7900*, QuantStudio 3, QuantStudio 5, QuantStudio 6, QuantStudio 7 Pro, QuantStudio 12K Flex, StepOne Plus*, and Viia 7.
- Roche: LightCycler
- Qiagen: Rotor-Gene Q5/6 Plex
- Bio Molecular Systems: MIC

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.

Customer and Technical Support

Email: support@aldatubio.com

Address: Aldatu Biosciences, 313 Pleasant Street, Watertown, MA 02472, United States of America.

Disclaimers

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

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Patents

The PANDAA technology is covered by US Patent No. 10,100,349 and European Patent Application No. 3052656 owned by the President and Fellows of Harvard College and exclusively licensed to Aldatu Biosciences, Inc.

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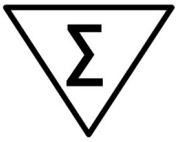
Explanation of Symbols

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 qDx™ LASV kit
	Manufacturer

ALDATU BIOSCIENCES

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