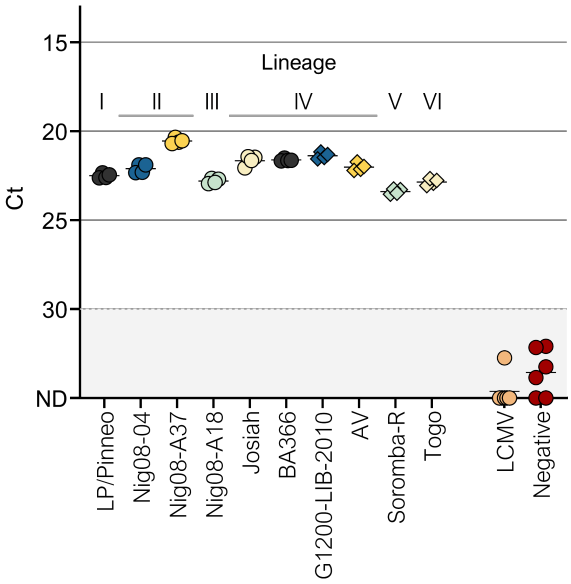
PANDAA LASV Performance Summary

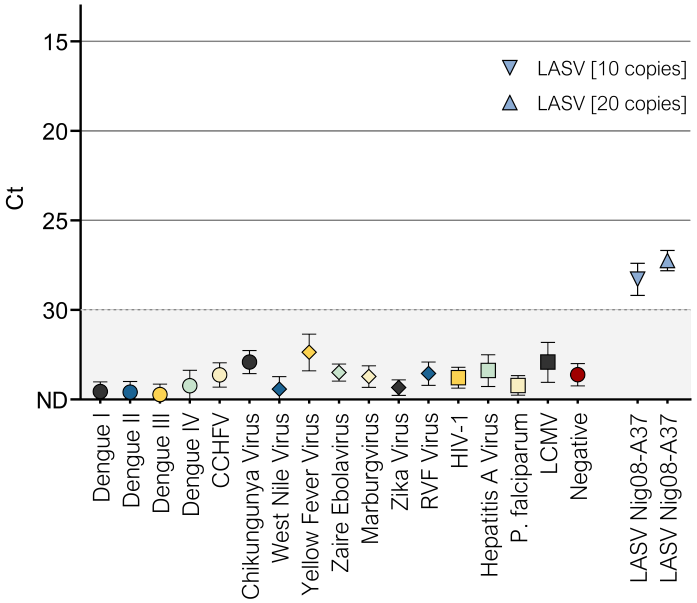
PANDAA LASV: the first diagnostic for the universal detection of all Lassa virus lineages.

Adaptive PCR by our PANDAA technology ensures the enduring integrity of molecular diagnostics as assay redesign is not required to detect new viral variants. PANDAA incorporates predicted viral genetic diversity into the assay design to proactively account for genomic variation that may arise in future viral variants. Through a process called adaptation, PANDAA uniquely corrects for sequence diversity, which is a common cause of assay failure and false negatives results when using conventional real-time PCR.[1]

Detection of all LASV lineages by PANDAA.

As the first universal, pan-lineage real-time PCR assay for the detection of Lassa virus (LASV), PANDAA LASV captures all LASV lineages with high sensitivity and specificity. **(Left)** Regardless of the lineage—and therefore the sequence diversity in the primer- and probe-binding sites—PANDAA LASV limit of detection was 10 RNA copies / reaction. A closely-related arenavirus, LCMV, at 5,000 copies / reaction does not cross the Ct cut-off of 30 cycles. **(Right)** PANDAA specificity was evaluated using a panel of full genomic RNA / DNA (BEI Resources) at ≥5x10^5 copies / reaction, a 50,000-fold excess of the assay LOD. None of the specificity panel members crossed the Ct cut-off of 30 cycles, and 10 copies / reaction of the LASV strain Nig08-A37 was clearly distinguishable. Data represent mean Ct values and 95% CI.





Clinical performance of PANDAA LASV.

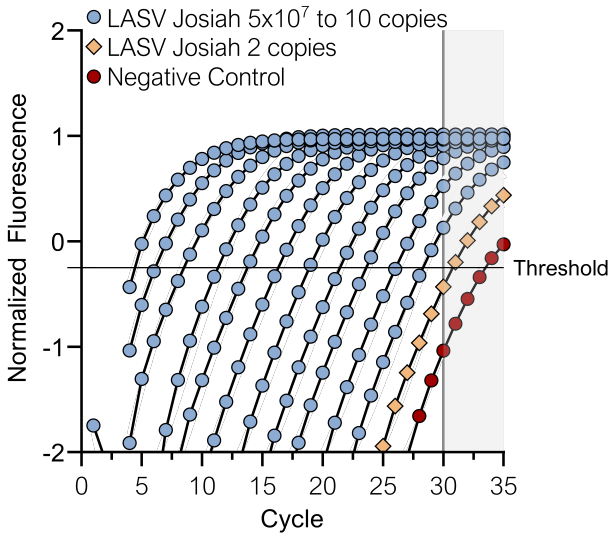
Evaluations were performed at national labs at two sites in Nigeria, in Bauchi state (predominantly LASV lineage I), and in Ondo state (LASV lineage II). Samples were first tested using the *RealStar® Lassa Virus RT-PCR* kit (altona Diagnostics GmbH). All retrospective samples were collected between January and April 2023, and all prospectively collected samples in June and July 2023. The retrospective study was designed to use more positive than negative samples as prospectively collected samples from June and July were expected to have a low positivity rate. A second phase of the study is underway to use 100 samples from the FIND biobank at FMC Owo to generate a dataset with 400 samples. Similar evaluations are intended for Sierra Leone and Liberia (LASV lineage IV).

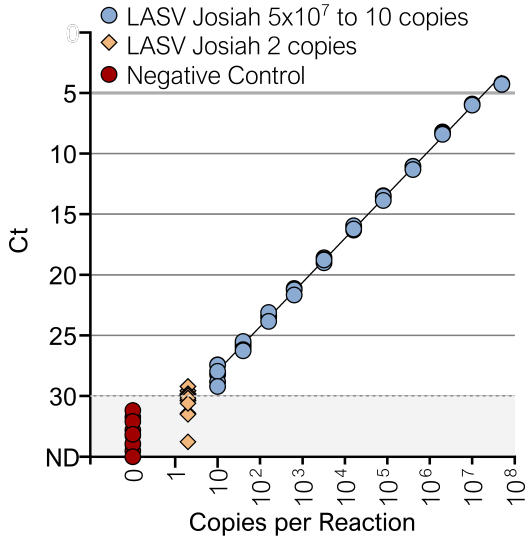
Site	Samples	Positive	Negative	Sensitivity	Specificity	Accuracy
Abubakar Tafawa Balewa University Teaching Hospital, Bauchi	Retrospective Prospective	60 9	40 41	97.1% * (89.9%–99.6%)	100% (95.5%–100%)	98.7% (95.3%–99.8%)
Federal Medical Centre (FMC) Owo, Ondo	Retrospective Prospective	64 7	33 38	100% (94.9%–100%)	100% (94.9%–100%)	100% (97.4%–100%)
Combined [n=292]	**Total**	**140**	**152**	**98.6%** (94.9%–99.8%)	**100%** (97.6%–100%)	**99.3%** (97.5%–99.9%)

* Two samples were negative by PANDAA and were positive for only one of the two gene targets (L gene) by the *RealStar® Lassa Virus RT-PCR* kit with a Ct of 37.7 and 38.9 cycles. Data are given with 95% confidence intervals.

PANDAA LASV has a limit of detection down to 10 RNA copies / reaction.

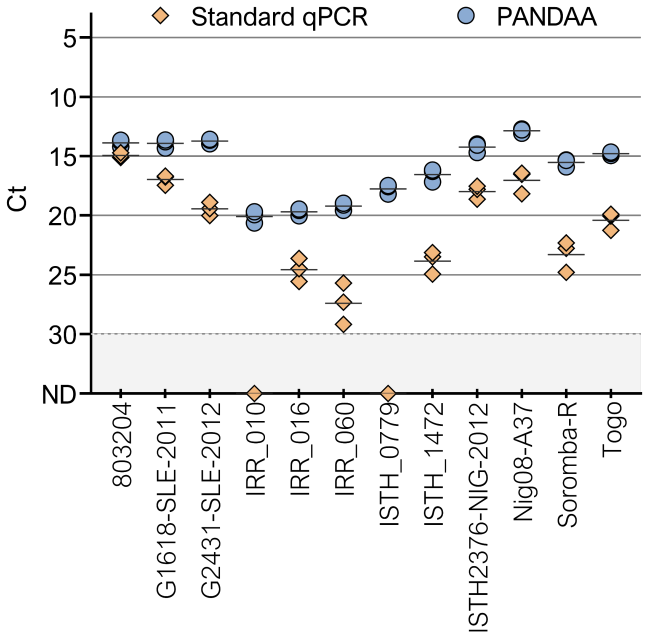
The limit of detection for LASV reference strain Josiah using serial dilutions from $5x10^7$ to 2 RNA copies / reaction with a Ct cut-off of 30 cycles to make a positive call. Although 2 copies / reaction are detected (orange), they cross cut-off at a Ct >30. The human genomic DNA negative control (red) can be differentiated from the 10 and 2 copies / reaction. **(Left)** the amplification curves with the threshold and Ct cut-off; **(Right)** the linear relationship between input copy number and Ct.





PANDAA improves sensitivity and rescues LASV detection compared to conventional real-time PCR.

As proof-of-concept, PANDAA was compared to conventional real-time PCR, and was shown to increase sensitivity by an average of 50-fold for the most divergent LASV isolates. Regardless of the LASV isolate used, PANDAA returns comparable Ct values. However, with conventional real-time PCR, the sensitivity of LASV detection is highly variable, and for two LASV strains results in a false negative result, which were rescued by PANDAA. Data shown use 10^4 copies / reaction.



LASV Isolate	Conventional	PANDAA	ΔCt	Increase
803204	15.0	13.8	-1.2	2.2 x
G1618-SLE-2011	16.7	13.8	-3.0	6.8 x
G2431-SLE-2012	19.4	13.6	-5.8	40.5 x
IRR_010	ND	19.9	*Detection Rescued*	
IRR_016	24.5	19.6	-4.9	23.5 x
IRR_060	27.3	19.1	-8.2	192.6 x
ISTH_0779	ND	17.6	*Detection Rescued*	
ISTH_1472	23.5	16.3	-7.2	101.1 x
ISTH2376-NIG-2012	17.8	14.1	-3.7	10.9 x
Nig08-A37	16.5	12.8	-3.8	11.1 x
Soromba-R	22.7	15.3	-7.4	116.5 x
Togo	20.0	14.7	-5.3	29.5 x

1. MacLeod IJ, Rowley CF, Essex M. PANDAA intentionally violates conventional qPCR design to enable durable, mismatch-agnostic detection of highly polymorphic pathogens. Communications Biology. 2021 Feb 18;4(1):1–13. https://www.nature.com/articles/s42003-021-01751-9

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