ReLASV® Pan-Lassa Antigen ELISA Kit

(LASV Nucleoprotein Antigen Detection)

For Research Use Only

Not for use in diagnostic procedures.
The performance characteristics of this product have not been established.

INSTRUCTIONS FOR USE

PRINCIPLE OF THE TEST

Lassa fever (LF) is a severe, often fatal, febrile illness endemic to West Africa caused by Lassa virus (LASV; family Arenavirdae)[1, 2]. LASV encodes four major proteins, including the envelope glycoproteins (GP1 and GP2), the structural protein Z and the nucleoprotein (NP). Advanced protein chemistry techniques have been used to develop non-infectious, recombinant LASV NP antigen. Using the NP antigen, specific rabbit polyclonal antibodies have been created to detect the presence of LASV NP antigen in plasma or serum of suspected Lassa fever patients[3, 4]. The ELISA kit utilizes a mixture of LASV NP-specific antibodies raised against the three most prevalent lineages of LASV (lineage II, III in Nigeria, lineage IV in Sierra Leone, Guinea, Liberia, and Mali) to provide Pan-Lassa cross-reactivity to the ReLASV® Pan-Lassa Antigen ELISA Kit.

The assay is performed as a direct ELISA to detect LASV NP antigen. Diluted samples, reference, and controls are incubated in microwells coated with purified LASV NP specific polyclonal antibody. Incubation allows the NP antigen present in the samples to react with the immobilized antibody. After the removal of unbound serum or plasma proteins by washing, purified LASV NP specific polyclonal antibody conjugated to horseradish peroxidase (HRP) is added to form complexes with the bound antigen. Following another washing step, the bound anti-LASV NP HRP conjugate is assayed by the addition of TMB substrate. Color develops in the wells at an intensity proportional to the concentration of LASV NP antigen. Optical Density (O.D.) results are obtained by reading the absorbance (A450nm minus A620nm) using an ELISA plate reader. NP antigen concentration can be estimated from the reference curve prepared from the NP Antigen Reference provided in the kit. It is recommended that the user establish a cut-off for the study population using normal (non-febrile) control samples.

REAGENTS

Store at 2–8°C. Do Not Freeze. Each ReLASV® Pan-Lassa Antigen ELISA Kit contains the following reagents:

Component	1-plate kit	10-plate kit
Anti-NP Coated Microwell Plate (resealable bag with desiccant)	One 12x8 plate	Ten 12x8 plate
Sample Diluent 1 (Yellow)	2 bottles (60 mL)	2 bottles (250 mL)
NP Antigen Reference (NP antigen diluted in human plasma), Lyophilized	2 vials (0.25 mL)	20 vials (0.25 mL)
Negative Control (human plasma), Lyophilized	2 vials (0.25 mL)	20 vials (0.25 mL)
Anti-NP HRP Conjugate Solution (Orange)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Substrate (TMB and H_2O_2); ready to use (Amber Bottle)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Stopping Solution (2% methanesulfonic acid) (Red Cap)	1 bottle (15 mL)	1 bottle (120 mL)
ELISA Wash Concentrate (33X PBS-Tween)	1 bottle (60 mL)	2 bottles (120 mL)

WARNINGS AND PRECAUTIONS

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Lassa Virus is classified as NIAID Category A agent. Handling of infectious blood and serum requires advanced biocontainment (BSL-4) facilities. Use of this product in BSL -1, -2 or -3 facilities is not recommended. If advanced biocontainment facilities are not available the use of all possible universal precautions is highly recommended including face shields, masks or respiratory equipment, disposable gowning and gloves. Decontamination equipment and solutions should be readily available. Biohazardous wastes should be autoclaved and/or incinerated.

1. Human source material used to prepare the Reference and Controls included in this kit have been tested and shown to be negative for antibodies to HBsAg, HCV, and HIV 1 & 2 by FDA required tests. However, all human blood derivatives, including patient samples, should be handled as potentially infectious material.
2. Do not pipette by mouth.
3. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
4. When testing in facilities with limited biocontainment equipment, wear disposable gloves while handling samples and kit reagents and wash hands thoroughly afterwards.
5. When testing in facilities with limited biocontainment equipment, wear disposable face shields, masks and gowning while handling samples and kit reagents and dispose in biohazard waste containers after use.
6. When testing in facilities with limited biocontainment equipment, wear rubber boots while handling samples and kit reagents and decontaminate with bleach solution after use.
7. The Substrate can cause irritation to the eyes and skin. Absorption through the skin is possible. Use gloves when handling substrate and wash thoroughly after handling. Keep reagent away from ignition sources. Avoid contact with oxidizing agents.
8. Certain components are labeled with the following: Irritating to eyes (R 36). Avoid contact with skin and eyes (S 24/25). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S 26). If swallowed, seek medical advice immediately and show container or label (S 46). Warning  . Biological Risk  .

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma (EDTA) are the preferred sample matrices. Blood should be collected by venipuncture, allowed to clot, and the serum separated from the cells by centrifugation. If not tested immediately, specimens should be stored at 2–8°C. If specimens are to be stored for more than 1 week, freeze at -20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, icteric, or lipemic serum as these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

If plasma (EDTA) is to be used, blood should be collected by venipuncture and the plasma separated from the cells immediately by centrifugation following the blood tube manufacturers recommendations. The supernatant must be carefully removed after centrifugation to avoid contamination with platelets. Repeating the centrifugation and separation steps may be advisable in order to minimize platelet contamination. If not tested immediately, plasma samples should be stored as previously described for serum.

INSTRUCTIONS FOR USE

MATERIALS PROVIDED:

ReLASV® Pan-Lassa Antigen ELISA Kit; see “Reagents” for a complete listing.

MATERIALS REQUIRED BUT NOT PROVIDED:

- Laboratory grade water
- Graduated cylinders
- Precision pipettors capable of delivering between 10 µL and 1000 µL, with appropriate tips
- Miscellaneous glassware appropriate for small volume handling
- Flask or bottle, 1 liter
- Wash bottles for manual plate washing or an automated or semi-automated washing system
- Disposable gloves
- Plate reading spectrophotometer capable of reading absorbance at 450nm (with a 620nm reference if available)
- Multichannel pipettors capable of delivering to 8 or 12 wells simultaneously

PROCEDURAL NOTES

1. Bring samples and kit reagents to ambient temperature (18-30°C) and mix well before using; avoid foaming. Return all unused samples and reagents to refrigerated storage as soon as possible.
2. The plate reader should be programmed for blank subtraction.
3. Good washing technique is critical for optimal performance of the assay. An automated microtiter plate washing system should be used be used with bleach added to the waste reservoir.
4. IMPORTANT: Failure to adequately remove residual Wash Solution can cause inconsistent color development of the Substrate.
5. Use a multichannel pipettor capable of delivering to 8 or 12 wells simultaneously when possible. This speeds the process and provides more uniform incubation and reaction times for all wells.
6. Careful controlled timing of all steps is critical. All Reference, Controls, and samples must be added within a five-minute period. Batch size of samples should not be larger than the amount that can be added within this time period.
7. For all incubations, the start of the incubation period begins with the completion of reagent or sample addition.
8. Addition of all samples and reagents should be performed at the same rate and in the same sequence.
9. Incubation temperatures above or below ambient temperature (18-30°C) when required may contribute to inaccurate results.
10. Avoid contamination of reagents when opening.
11. Do not use kit components beyond expiration date.
12. Do not use kit components from different kit lot numbers.

REAGENT PREPARATION

1X ELISA Wash Solution: Measure 30 mL of Wash Concentrate (33X) and dilute to 1 liter with laboratory grade water. Store unused Wash Solution in the refrigerator at 2–8°C . Discard if the solution shows signs of microbial contamination.

Lyophilized Controls: Reconstitute Reference and Normal Control with 0.250mL laboratory grade water. Mix gently for several minutes until completely dissolved. Unused portion should be store at 2-8°C for up to 7 days or stored frozen (-20°C or less) for longer periods.

ASSAY PROCEDURE

1. Remove any microwell strips that will not be used from the frame. Store them with the desiccant pouch in the resealable bag provided.
2. Prepare a five-point NP Antigen Reference curve: Label five tubes for Reference 1 – 5.

   In tube #1, prepare a 1:10 dilution of Reference in Sample Diluent (yellow) by adding 100 µL reference to 900 µL Sample Diluent.

   Add 500 uL of Sample Diluent (yellow) to tubes # 2-5.

   Remove 250 uL from dilution tube #1, transfer to dilution tube #2 and mix well.

   Repeat this 3-fold serial dilution series through tube 5.

   The value of the NP Antigen Reference is indicated on the vial label. The value of Reference dilutions 2 – 5 are calculated by dividing Reference value by each Reference dilution factor (DF).

   Example:

Dilution #	DF	Volume to Add		Sample Diluent		Reference Value
1	-	100 uL Reference	+	900 uL	=	3.3 (example)
2	3	250 uL Dilution #1	+	500 uL	=	1.1
3	9	250 uL Dilution #2	+	500 uL	=	0.37
4	27	250 uL Dilution #3	+	500 uL	=	0.12
5	81	250 uL Dilution #4	+	500 uL	=	0.04

3. A reagent blank control should be run in duplicate on each plate. These wells will be treated the same as sample wells in subsequent assay steps.
4. Duplicate determinations are recommended. Prepare a 1:10 dilution of the controls and samples in Sample Diluent 1 (yellow), e.g., 50 µL sample added to 450 µL Sample Diluent equals a 1:10 sample dilution.
5. Mix thoroughly and add 100 µL of each sample (Reference dilutions, controls, samples, and reagent blank) to the appropriate microwells.
6. Incubate 60 minutes at ambient temperature (18-30°C).
7. After the incubation is complete, wash 4 times (300uL/well) with 1X ELISA Wash Solution. Blot on absorbent paper to remove residual wash fluid.
8. Add 100 µL of the Anti-NP HRP Conjugate Solution (orange) to each well.
9. Incubate for 30 minutes at ambient temperature (18-30°C).
10. Wash 4 times as in step 7.
11. Add 100 µL ELISA Substrate (TMB, H2O2) to each well and incubate for 10 minutes at ambient temperature (18-30°C). Blue color will develop in wells with positive samples.
12. Add 100 µL ELISA Stop Solution (2% methanesulfonic acid) to each well to stop the enzyme reaction. Blue substrate will turn yellow and colorless substrate will remain colorless. Air blank or zero the plate reader. Read the O.D. of each well at A450 nm (and A620 nm reference if available). The O.D. values should be measured within 5 minutes after the addition of Stop Solution.

RESULTS

1. Calculate the mean O.D. values for the duplicates of the Reference dilutions, Reagent Blank, Controls and samples. Subtract mean O.D. A620nm reference from mean O.D. A450nm.
2. Estimate the concentration of NP antigen by plotting the mean O.D. obtained for each NP Antigen Reference (y axis) against the corresponding NP Antigen Reference value (x axis) using curve fitting software. A 4-Parameter curve fit calculation is recommended.
3. Ensure that all quality control parameters have been met (see Quality Control) before reporting test results.
4. A new calibration curve should be prepared with every test run.

QUALITY CONTROL

1. The mean O.D. of the reagent blank (zero point) should be less than 0.150. Readings greater than 0.150 may indicate possible reagent contamination or inadequate plate washing.
2. O.D. values for the duplicates of the controls or patient samples should be within 25% CV of the mean O.D. value for samples with absorbance readings greater than 0.250.
3. Each laboratory should determine their own normal range for the appropriate population.

NORMAL CUT-OFF

To be determined experimentally by the end user within a study population. Cut-off range has not been established by manufacturer.

LIMITATIONS OF THE TEST – FOR RESEARCH USE ONLY – NOT FOR USE IN DIAGNOSTIC PROCEDURES

Estimated LASV NP antigen levels obtained with this assay are not for use in diagnostic procedures.

ReLASV® Pan-Lassa Antigen ELISA Kit is designed to detect circulating LASV NP titers during viremia stage of illness. LF patient samples that have progressed to a humoral immune response stage may not have detectable LASV NP antigen titers. These samples may still be positive by LASV PCR or by Lassa virus specific antibody detection assays such as ReLASV® Pan-Lassa NP IgG/IgM ELISA Kit.

Strain variability of Old World Arenaviruses or LCMV may affect performance of the assay.

Testing LF samples containing excess hemoglobin, lipids, and/or bilirubin is not recommended as these substances may interfere with the results of the assay.

The performance characteristics of this test have not been established.

REFERENCES

1. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, et al. Lassa Fever in Post-Conflict Sierra Leone. PLoS Negl Trop Dis. 2014;8(3):e2748. doi: 10.1371/journal.pntd.0002748.
2. Hartnett JN, Boisen ML, Oottamasathien D, Jones AB, Millett MM, Nelson DS, et al. Current and emerging strategies for the diagnosis, prevention, and treatment of Lassa fever. Future Virology. 2015;10(5):559-84.
3. Branco LM, Boisen ML, Andersen KG, Grove JN, Moses LM, Muncy IJ, et al. Lassa hemorrhagic fever in a late term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. Virol J. 2011;8:404. doi: 10.1186/1743-422X-8-404. PubMed PMID: 21843352; PubMed Central PMCID: PMC3177908.
4. Boisen ML, Hartnett JN, Shaffer JG, Goba A, Momoh M, Sandi JD, et al. Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever. Sci Rep. 2018;8(1):5939. doi: 10.1038/s41598-018-24246-w. PubMed PMID: 29651117; PubMed Central PMCID: PMCPMC5897328.

SYMBOL LEGEND

								
Manufacturer	Batch Code	Use by/ Expiry Date	Temperature Limitations	Warning	Caution	Biological Risk	Catalog Number	Consult Instructions for Use

WARRANTY

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Doc. No.: PI-04R 02
Effective: 2020-02-20