ReLASV® Pan-Lassa Antigen Rapid Test

(Lassa Virus Nucleoprotein)

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 nucleoprotein in whole blood, plasma or serum of suspected Lassa fever patients[3, 4]. The rapid test utilizes a mixture of LASV NP-specific antibodies raised against the three most prevalent lineages of LASV (lineage II and III in Nigeria, lineage IV in Sierra Leone, Guinea, Liberia, and Mali) to provide Pan-Lassa cross-reactivity to the ReLASV® Pan-Lassa Antigen Rapid Test.

The ReLASV® Pan-Lassa Antigen Rapid Test is performed as a dipstick immunoassay. Whole blood from finger stick or venous collection, plasma or serum samples is added to the Sample Pad. Inserting the dipstick into a test tube containing Sample Buffer initiates flow of sample through the reagent pads and across the nitrocellulose membrane. The LASV NP specific antibody is striped onto the nitrocellulose membrane in order to capture LASV NP antigen. The LASV NP specific antibody is also conjugated to gold nanoparticles which are deposited in one of the rapid test reagent pads. As the assay develops, the presence of LASV NP antigen in the sample forms immune-complexes with anti-LASV NP antibody–gold conjugate. As these complexes are captured by the anti-LASV NP antibody Test stripe, the deposition of the gold conjugate generates a pink to dark red signal which corresponds to the concentration of LASV NP antigen in the sample. Excess gold-conjugate is captured by the anti-rabbit IgG Control stripe, indicating a valid result. Visual interpretation is made between 15-25 minutes development time (refer to Visual Aid section). Permanent records of results by digital photography is recommended.

REAGENTS

Store at 2–8°C. Do Not Freeze.

Each ReLASV® Pan-Lassa Antigen Rapid Test contains the following reagents:

- 2 x 25 each Rapid Test Dipsticks (resealable foil pouch with desiccant)
- 2 x 7 mL Sample Buffer 1 (dropper bottles)
- 2 x 0.25 mL Negative Control (negative human plasma, lyophilized)
- 2 x 0.25 mL Positive Control (recombinant NP antigen spiked in negative human plasma, lyophilized)

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. ReLASV® Pan-Lassa Antigen Rapid Test controls are prepared with pooled negative human plasma. The Positive Control is negative human plasma to which recombinant LASV NP antigen has been added.
2. Human source material used to prepare the 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. All human blood derivatives, including patient samples, should be handled as potentially infectious material.
3. Do not pipette by mouth.
4. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
5. When testing in facilities with limited biocontainment equipment, wear disposable gloves while handling samples and kit reagents and wash hands thoroughly afterwards.
6. 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.
7. When testing in facilities with limited biocontainment equipment, wear rubber boots while handling samples and kit reagents and decontaminate with bleach solution after use.
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

Whole Blood from Finger Stick or collected in EDTA Vacutainers are the preferred sample matrixes for the ReLASV® Pan-Lassa Antigen Rapid Test dipsticks. Serum and plasma (EDTA) can also be tested with ReLASV® Pan-Lassa Antigen Rapid Test dipsticks.

INSTRUCTIONS FOR USE

MATERIALS PROVIDED:

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

MATERIALS REQUIRED BUT NOT PROVIDED:

- For fingerstick: Disposable lancets, cotton balls, alcohol wipes
- Precision pipettors capable of delivering between 10µL and 100µL, with appropriate tips
- Laboratory grade water
- In austere testing conditions: disposable gloves, gowning, safety googles, face shields, respiratory masks, and boots that can be decontaminated

PROCEDURAL NOTES

- 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.
- Visual interpretation of assay results must be conducted within 15-25 minutes signal development time.
- Incubation temperatures above or below ambient temperature (18-30°C) may contribute to inaccurate results.
- Do not use kit components beyond expiration date.
- Do not use kit components from different kit lot numbers.

ASSAY PROCEDURE – For Fingerstick Whole Blood (See Visual Instruction Aid on page 6)

1. Remove appropriate number of dipsticks for testing the required fingerstick whole blood samples.
2. Add 4 drops of Sample Buffer to test tubes provided with the kit.
3. Perform finger stick using disposable lancet (not included), allow large drop of blood to develop from the fingerstick.
4. Touch sample pad of the dipstick to the drop of blood to transfer sample
5. Insert the ReLASV® Pan-Lassa Antigen Rapid Test dipstick (arrows down) into tube containing sample buffer. Replace tube cap. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
6. Allow ReLASV® Pan-Lassa Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation. Refer to visual aid on page 5.
7. To guard against missing slow developing positives, all negative results should be confirmed at 25 minutes.
8. If test strip produces an invalid result, user should repeat test with a new test strip.

ASSAY PROCEDURE – For Venous Whole Blood, Plasma, or Serum. (See Visual Instruction Aid on page 5)

1. Remove appropriate number of dipsticks for testing the required venous whole blood, plasma, and serum samples.
2. Add 4 drops of Sample Buffer to test tubes provided with the kit.
3. Transfer 30µL of venous whole blood, plasma, or serum onto the center of the Sample Pad using a pipettor.
4. Insert the ReLASV® Pan-Lassa Antigen Rapid Test dipstick (arrows down) into tube containing sample buffer. Replace tube cap. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
5. Allow ReLASV® Pan-Lassa Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation. Refer to visual aid on page 5.
6. To guard against missing slow developing positives, all negative results should be confirmed at 25 minutes.
7. If test strip produces an invalid result, user should repeat test with a new test strip.

ASSAY PROCEDURE – For Negative or Positive Control. (See Visual Instruction Aid on page 6)

1. Remove appropriate number of dipsticks for testing the required one Negative and one Positive Control.
2. Reconstitute one lyophilized Negative Control and one Lyophilized Positive Control each with 0.250 mL of laboratory grade water for minimum of 5 minutes at ambient temperature. Shake or agitate vial to ensure complete reconstitution of controls. Rehydrated controls are stable for 30 days at 2-8ºC.
3. Add 4 drops of Sample Buffer to test tubes provided with the kit.
4. Transfer 30µL of Negative or Positive Control onto the center of the Sample Pad using a pipettor.

5. Insert the ReLASV® Pan-Lassa Antigen Rapid Test dipstick (arrows down) into tube containing sample buffer. Replace tube cap. **DO NOT** shake, agitate, or invert test tubes during rapid test signal development.
6. Allow ReLASV® Pan-Lassa Antigen Rapid Test to develop for 15-25 min before performing a visual interpretation. Refer to visual aid on page 5.
7. To guard against missing slow developing positives, all negative results should be confirmed at 25 minutes.
8. If test strip produces an invalid result, user should repeat test with a new test strip.

RESULTS INTERPRETATION – Refer to included visual aid on page 5.

1. The ReLASV® Pan-Lassa Antigen Rapid Test results should be compared to the Visual Aid included to assist with the interpretation of the results.
2. For a positive patient result on the ReLASV® Pan-Lassa Antigen Rapid Test, a pink to dark red line should form across the Test Line, and a pink to red line should form across the Control Line.
3. For a negative patient result, no line should be detected across the Test Line, and a pink to red line should form across the Control Line.
4. Absence of a pink to red line forming across the Control Line is considered as an “invalid” result which requires sample retesting.
5. If available a permanent record should be made by digital photography.

QUALITY CONTROL – Refer to included Visual Aid

1. The ReLASV® Pan-Lassa Antigen Rapid Test should form a pink to red line across the Control Line indicating the dipstick is performing properly.
2. Failure of the Control Line to develop constitutes an invalid result and requires retesting.
3. The appearance of pink to red background, streaks or spots in the Test or Control Line area may be due to improper flow of reagents and constitutes an invalid result and requires retesting.
4. The development of partial width or variable intensity Test Line does not constitute an invalid result but retesting may be considered.

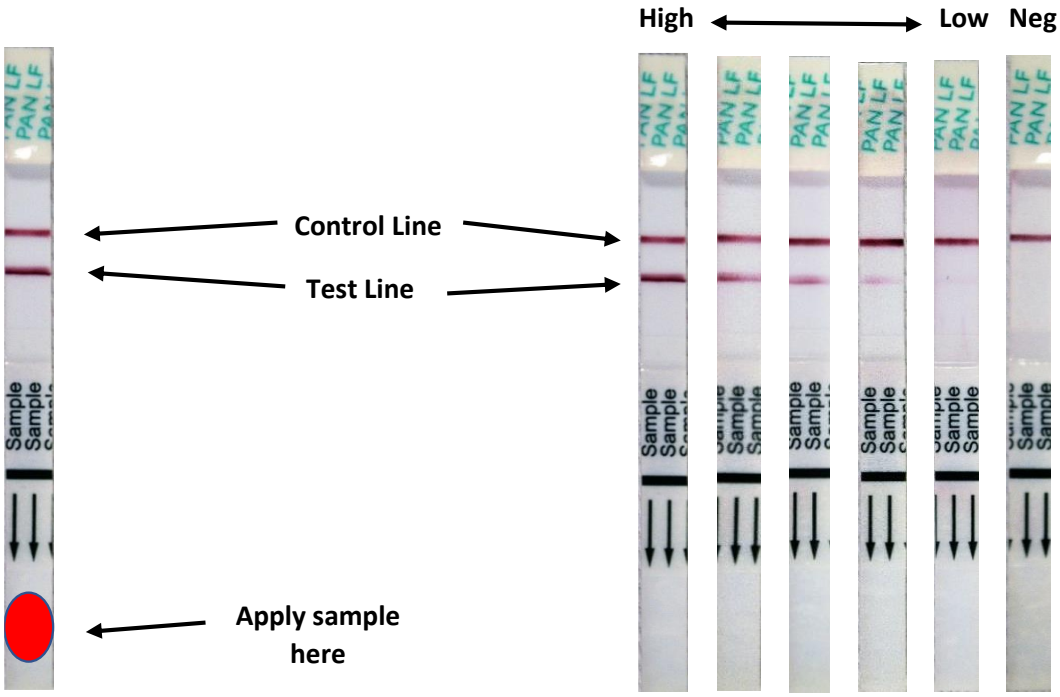
LIMITATIONS OF THE TEST – FOR RESEARCH USE ONLY – Not for use in Diagnostic Procedures.

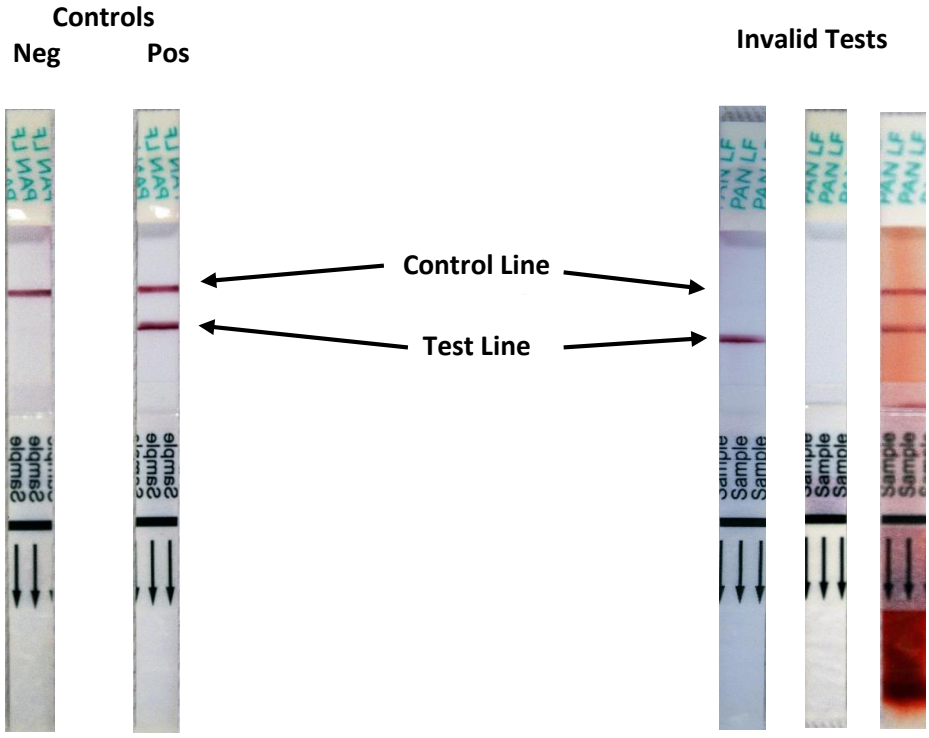
ReLASV® Pan-Lassa Antigen Rapid Test detects the presence of LASV NP in suspected LF patient whole blood, plasma, or serum. On-going studies indicate that circulating LASV NP may be absent or undetectable if the patient has progressed in their humoral immune response and anti-LASV NP antibody titers have developed.

A negative ReLASV® Pan-Lassa Antigen Rapid Test result does not eliminate the possibility that the patient has LASV. The patient may be antibody positive by ELISA or potentially positive by PCR. Patients suspected of Lassa fever should undergo additional testing as part of a thorough assessment of the patient’s symptoms.

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

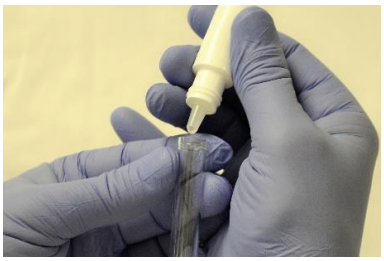
ReLASV® Pan-Lassa Antigen Rapid Test Visual Aid Chart





ReLASV® Pan-Lassa Antigen Rapid Test Instructions

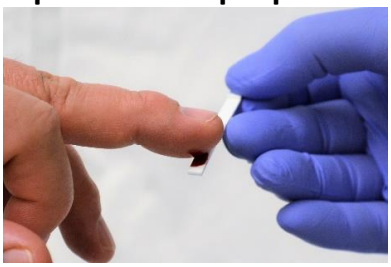
1. **Add 4 drops sample buffer to plastic tube**



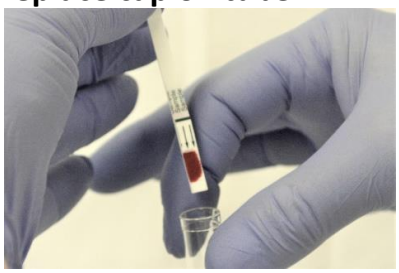
2. **Use safety lancet to perform finger stick**

3. **Allow large drop of blood to develop on finger**

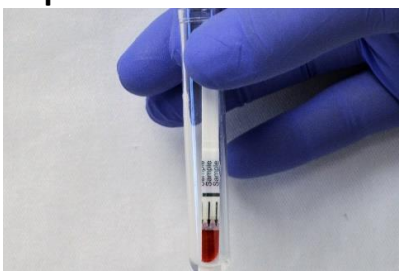
4. **Transfer drop of blood to ReLASV® Pan-Lassa Antigen Rapid Test sample pad**



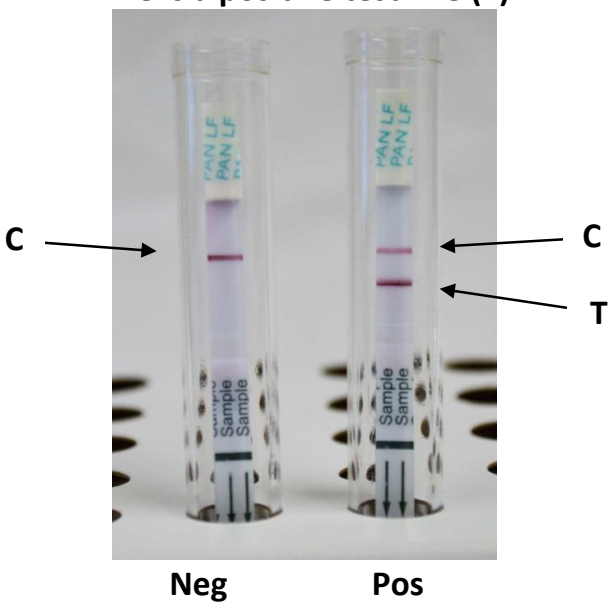
5. **Place ReLASV® Pan-Lassa Antigen Rapid Test into tube containing sample buffer and replace cap on tube**



6. **Allow ReLASV® Pan-Lassa Antigen Rapid Test to develop for 15-25 min before visual inspection**



7. **Visual interpretation: top line is control stripe (C); bottom line is a positive test line (T)**



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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

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

WARRANTY

This product is warranted to perform as described in this package insert. Zalgen Labs, LLC disclaims any implied warranty of merchantability or fitness for a particular use, and in no event shall Zalgen Labs, LLC be liable for consequential damage.

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Doc. No.: PI-01R 00
Effective: 2019-10-08