# Development of a handheld multiplex point of care diagnostic for differentiation of Lassa fever, Dengue fever and Ebola Hemorrhagic Fever

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## ABSTRACT

**Background:** Lassa virus is a zoonotic virus causing severe disease and hemorrhagic fever (HF), infecting hundreds of thousands of people each year. Dengue virus is a pandemic mosquito born virus causing 50-100 million infections and several hundred thousand cases of HF each year. Ebola virus infection is rare but severe and a cause of HF with sporadic outbreaks in Central Africa. The symptoms and causes of HF can be difficult to distinguish but necessitate different treatment, isolation and epidemiological responses. There is a clear need for diagnosis of viral HF in endemic and austere environments, in military zones or biothreat scenarios. We have developed the Nanomix POC IVD Panel, a handheld electronic, carbon nanotube biosensor multiplex assay for the detection of Lassa, Dengue and Ebola virus hemorrhagic fevers.

**Methods:** The POC IVD Panel assay consists of a reader/processor and sealed, disposable assay cartridges containing the necessary biological and chemical reagents. Cartridges were prepared with reaction pads coated with capture antibodies specific for Lassa, Dengue and Ebola. Low volume samples were mixed with a reporter pellet containing lyophilized HRP-conjugated antibodies and injected into the cartridge. The reader/processor performed the assay and wash steps and reported nano-voltage results in ten minutes. Lassa positive samples were also assayed with the ReLASV™ Lassa antigen detection ELISA. Samples included non-infectious recombinant proteins (Lassa, Ebola), inactivated viral culture supernatants (Dengue) and infectious human samples collected at Kenema Government Hospital, Sierra Leone.

**Results:** Lassa, Dengue and Ebola antigens were successfully detected with the assay with no cross reactivity. The mean voltage of Dengue antigen positive samples was 1417 compared to mean voltages less than 50 for LASV negative and malaria positive samples, p<0.0001. The mean of Ebola antigen positive samples was 3208 compared to means below 100 for negative control, Lassa negative and Malaria positive samples, p<0.0001. Lassa fever patient serum and plasma samples show strong specific Lassa signals. The mean of Lassa positive clinical samples was 5267 as opposed to means of 163 and 58 for negative and malaria positive samples, p<0.0001. The optical density results from the ReLASV™ ELISA correlated well with voltage results on the POC assay. Multiple antigens can be detected in single spiked patient samples. When Lassa and Dengue antigens are co-detected, the mean voltages are 8838 and 1167 respectively. When Ebola and Lassa antigens are co-detected, the mean voltages are 3092 and 3350. No interference or cross reactivity was observed in patient samples positive for Malaria antigen, or Chickungunya and Dengue antibodies or patients treated with ribavirin.

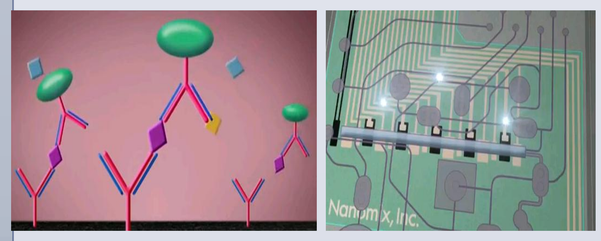
**Conclusion:** We successfully detected hemorrhagic fever viruses in a rapid, multiplexed point of care assay. Lassa, Dengue and Ebola antigens were specifically detected singly or mixed in a variety of human samples. Operation of the assay was not affected by antigen and antibodies specific for other infectious diseases or treatment with antivirals. Further development of the device will entail definition of normal and cut-off levels, optimization of antibody pairs and cartridge assembly, optimization of sensitivity and further testing on authentic infectious human samples.

## INTRODUCTION

- Three possible causes of Hemorrhagic Fever (HF) are infection with Ebola Virus (EBOV), Dengue Virus (DENV) or Lassa virus (LASV).
- The symptoms and causes of Hemorrhagic Fever can be difficult to distinguish but necessitate different treatment, isolation and epidemiological responses.
- There is a clear need for diagnosis of viral HF in endemic and austere environments, in military zones or biothreat scenarios.
- We have developed the Nanomix POC IVD Panel, a handheld electronic, carbon nanotube biosensor multiplex assay for the detection of Lassa, Dengue and Ebola virus hemorrhagic fevers.

## MATERIALS AND METHODS

- The Nanomix POC IVD Panel operates like a very sensitive antigen capture ELISA
- LASV, DENV or EBOV specific capture antibodies and positive and negative controls were coated on reaction pads
- Liquid sample mixed with multiplexed lyophilized HRP-conjugate reporter pellet
- Sample is injected-system takes over operation and pumps labeled sample onto reaction pads
- Wash steps performed in cartridge
- Optimized TMB is injected by system, reaction between captured HRP and TMB releases electrons.
- Resulting electrons are focused by carbon nanotubes onto the Biosensor and measured in nanocoulombs
- Results available within 10 minutes



- The ReLASV™ ELISA is an antigen capture ELISA in development for the detection of LASV NP protein in serum
- Chickungunya virus positive samples were assayed for virus specific IgM with commercially available kits
- Malaria positive samples were assayed for *Plasmodium falciparum* antigen with commercially available kits
- Research samples were obtained from consenting patients at Kenema Government Hospital, Lassa Fever Program
- Recombinant antigens for LASV NP and EBOV Zaire VP40 and culture supernatants from DENV culture were used to spike negative samples where described

## RESULTS

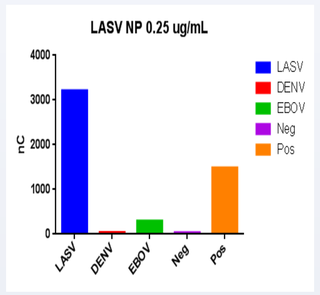



**Figure 1. Recombinant LASV antigen is detected by the reader**
Recombinant LASV Np antigen spiked into serum in specifically detected by the POC system.

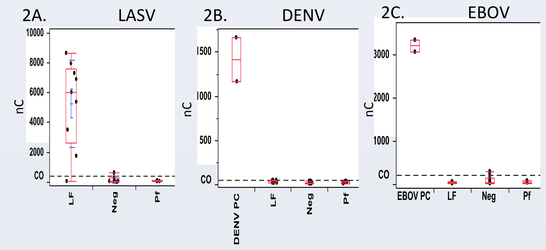



**Figure 2. LASV, DENV and EBOV do not cross react**
(2A) LASV antigen positive patient serum, (2B) DENV recombinant antigen spiked serum and (2C) EBOV antigen spiked serum are detected specifically with no cross reactivity., all p<0.0001

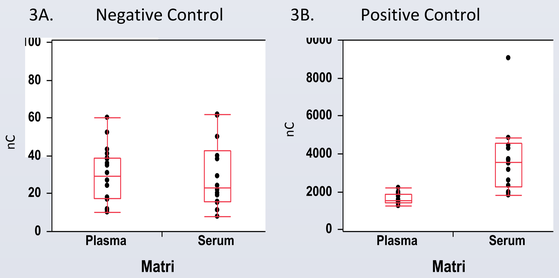



**Figure 3. Plasma or Serum can be used as samples**
Plasma or Serum yield similar results in both (3A) negative control and (3B) positive control pads

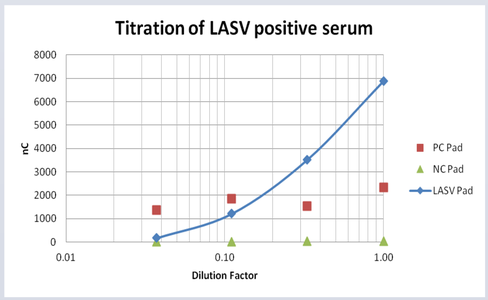



**Figure 4. Diluted serum maintains positive signal**
Serially diluted LASV antigen positive serum reacts in a dose dependent manner but does not alter negative or positive control values

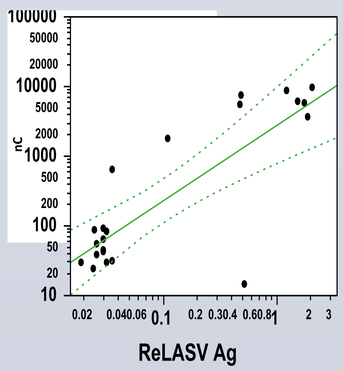



**Figure 5.POC system and ELISA values correlate**
Readings from the POC system correlate with O.D. values obtained from the ReLASV™ antigen detection ELISA

## RESULTS

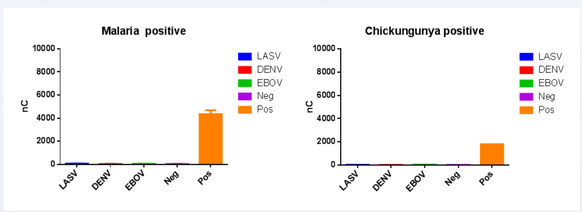



**Figure 6. Malaria and Chickungunya virus do not cross react**
(6A) Malaria antigen positive human serum and (6B) Chickungunya virus IgM positive human serum do not cross react with the LASV, DENV, or EBOV detectors

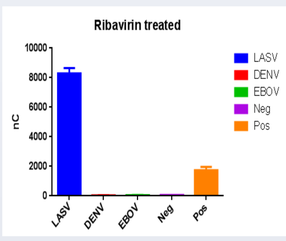



**Figure 7. LASV signal is maintained during drug treatment**
LASV signal in positive patient serum is not affected by treatment by the antiviral drug Ribavirin

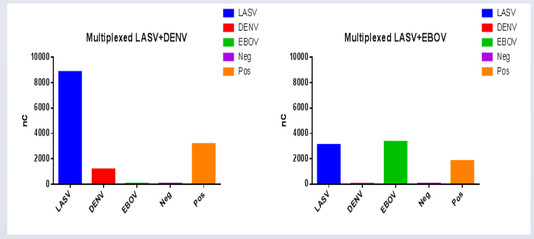



**Figure 8. Multiple viruses can be detected at once**
(8A) LASV and DENV and (8B) LASV and EBOV in serum can be co-detected in multiplexed assays on the POC reader

## CONCLUSIONS

- Hemorrhagic fever viruses were successfully detected in human samples in a rapid, multiplexed point of care assay.
- Lassa, Dengue and Ebola antigens were specifically detected singly or mixed in a variety of samples.
- Operation of the assay was not affected by antigen and antibodies specific for other infectious diseases or treatment with antiviral drugs.
- Further development of the device will entail:
  - -definition of normal and cut-off levels
  - -optimization of antibody pairs
  - -optimization cartridge assembly
  - -optimization of sensitivity
  - -further testing on authentic infectious human samples

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