Title:

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

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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.