Rapid Detection of Viral Hemorrhagic Fever (VHF) Agent RNA at Point of Need by Isothermal Amplification

1Chad Mire, 1Thomas W. Geisbert, 1Krystle G. Agans, 2Yogesh Chander, and 2Michael J. Moser
1Galveston National Laboratories, Univ. of Texas Medical Branch, Galveston, TX 77550 USA, 2Lucigen Corporation, Middleton, WI 53562 USA

Abstract
Emerging RNA viruses are infectious agents that pose an ongoing and significant threat to human health and well-being. Nucleic acid tests (NATs) to target the RNA component of the virus are now the diagnostic gold standard to detect these important pathogens. NATs allow higher specificity and sensitivity compared to alternatives based on antibodies, and as new infectious agents emerge, NATs are fast and easily rapidly developed. Treatment and management of diseases caused by pathogens like influenza, the common cold, Dengue Fever, and HIV could be significantly improved by rapid detection and typing of the viruses that cause them at point of care (POC). Unfortunately, expensive equipment, highly-trained personnel, and the need for a clinical laboratory setting preclude routine nucleic acid based testing (NAT) for infectious disease in most of the developing world and even in resource-limited parts of the United States, leading to wide disparities in health care worldwide. A fast, sensitive, low cost but facile NAT method for robust detection of infectious agents at point of care (POC) would help bring the power of molecular diagnostics to anyone, anywhere.

We have demonstrated feasibility of a fast-appropriate NAT diagnostic system by developing a system for rapid detection of viral hemorrhagic fever (VHF) RNA directly within the ESI-L laboratory at Galveston National Laboratories. The technology that is the basis of this platform is a thermostable polymerase with innate transcriptase (RT) activity that can also perform an isothermal NAT alternative to PCR called loop mediated isothermal amplification (LAMP). Since LAMP is isothermal, it does not require thermocycler instrumentation plus it is much faster than PCR. A novel polymerase has been developed at Lucigen that combines thermostable strand displacement activity for LAMP-based amplification and RT activity to amplify directly from RNA.

LAMP assay systems designed to detect three well-known VHF agents were tested: Ebola Virus, Marburg Virus, and Lassa Fever Virus. The performance characteristics of these three test systems with both purified viral RNA and from clinical specimens obtained from animal subjects from VHF vaccine trials ongoing at GNL will be presented.

We have made significant progress developing rapid tests for VHF pathogens that do not require any instrumentation. Our current assays are based on single enzyme, isothermal RNA amplification using a novel RT-LAMP polymerase and detection by lateral flow. We have developed a test methodology that detects Ebola, Marburg, and Lassa Fever RNA after 5-20 minutes with results read out on a closed system lateral flow test cassette. These VHF tests have been verified externally using nucleic acids extracted from cultured virus and from residual sample obtained from experimentally infected animal subjects at GNL. Our goal is a fully integrated form of these tests that will be highly reliable and as simple to operate and interpret as a home pregnancy test.

Abstract

10 to 20 Minute Isothermal Amplification of VHF RNA OmniAmp™ DNA Polymerase

VHF Background
Lassa Fever Virus (LaV)
Lassa fever is caused by an arenavirus endemic in parts of West Africa. The reservoir of LaV is a rodent known as the multi-mammate rat of the genus Mastomys. LaV infections are estimated at 100,000 to 300,000 annually causing about 5,000 deaths. Estimates are crude, because surveillance for cases of the disease is not uniform. In some areas of Sierra Leone and Liberia, it is known that 10%-16% of people admitted to hospitals have Lassa fever, showing the enormous impact of the disease on health in this region. Accurate diagnosis of LaV infection remains difficult but is important since ribavirin therapy within the first week has been found to effective against Lassa fever. Methods include ELISA, virus isolation and RT-PCR, Immunohistoschemistry is used to confirm cause of death post mortem.

Marburg Virus (MaV)
Marburg hemorrhagic fever (MVF) is a rare, severe type of HF which affects both humans and non-human primates. Caused by a unique zoonotic virus of the Filovirus family, its recognition in 1967 led to the creation of this virus family. Other well known members of the Filovirus family are the five subtypes of Ebola virus. Some studies implicate the African fruit bat Rousettus aegyptiacus as the reservoir host of the MVa. Cases of MVa HF have been reported from Uganda, Zimbabwe, Congo, Kenya, and Brazil. Marburg HF has occurred outside Africa, primarily among laboratory workers and hospital staff. MaV HF has been diagnosed by immunohistochromy, virus isolation, or PCR of blood or tissue specimen from deceased patients.

Ebola Virus (EV)
There are five subtypes of Ebola virus, a Filovirus. Four have caused disease in humans: Ebola-Zaire, Ebola-Sudan, Ebola-Ivory Coast and Ebola-Bundibugyo. The fifth, Ebola-Reston, has only caused disease in nonhuman primates. Confirmed cases of EV HF have been reported in the Congo, Gabon, Sudan, Ivory Coast, and Uganda. Infections with EV are acute and risk from exposure to body fluids from infected individuals is severe. Nitric oxide transmission occurs frequently during EV HF outbreaks. ELISA, IgM ELISA, RT-PCR, and virus isolation can be used to diagnose a case of EV HF within a few days of onset. Late in the course of the disease or after recovery testing for IgM and IgG antibodies is used; the disease can also be diagnosed in deceased patients by using immunohistochemistry, virus isolation, or RT-PCR.

Conclusions
- Detection of VHF in 20 minutes at point of need by RT-LAMP and NALF
- OmniAmp for fast, sensitive, specific RT-LAMP
- OmniAmp is suitable for detection of RNA viruses

About Lucigen
At Lucigen, we deliver advanced molecular biology products and services to life scientists by inventing solutions to the most difficult problems in Next Gen Sequencing, DNA cloning, amplification, and protein expression.

Acknowledgements
This work was supported by NSF and NIH grants