IDENTIFICATION OF EBOLA VIRUS ON FIREFLY DX

The West African Ebola virus epidemic (2013–2016) was the most widespread outbreak of Ebola virus disease (EVD) in history, causing major loss of life and socioeconomic disruption in the region and there have been more cases this year (2017). Ebola has caused significant mortality during the epidemic, with the case fatality rate reported at slightly above 70%, while the rate among hospitalized patients was 57–59%. In addition, imported cases led to secondary infection of medical workers in the United States and Spain.

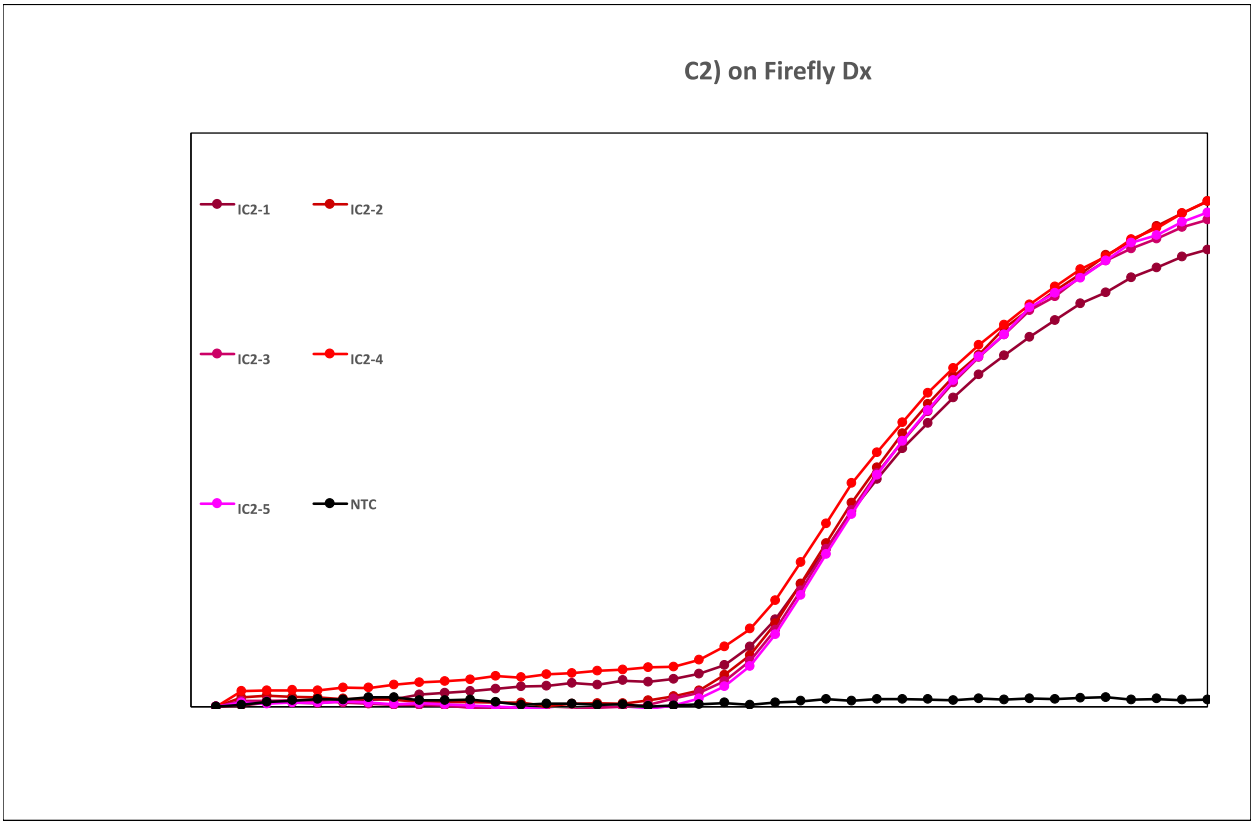
The outbreak left about 17,000 survivors of the disease, many of whom report post-recovery symptoms termed post-Ebola syndrome, often severe enough to require medical care for months or even years. An additional cause for concern is the apparent ability of the virus to "hide" in a recovered survivor's body for an extended period of time and then become active months or years later, either in the same individual or in a sexual partner. Rapid detection and diagnosis of Ebola virus infection along with vaccination is imperative to quickly containing an epidemic.

The recent outbreak of Ebola virus disease (EVD) in West Africa has highlighted both the importance of rapid and accurate diagnosis of this disease and the challenges around diagnostic testing. Throughout the 2014-2015 outbreak, diagnosis relied primarily on testing of venipuncture blood samples from symptomatic individuals in a biocontainment laboratory facility, leading to challenges with specimen collection and data management and often a prolonged turnaround time to final results. Consequently, the need for rapid and, particularly, for point-of-care diagnostics generated an unprecedented surge in development of new diagnostic methods for EVD[1].

Ebola is a RNA virus and require a reverse transcription (RT) step prior to PCR. Despite its potential diagnostic advantages, RT-PCR methodology (both conventional and real-time approaches) requires significant laboratory infrastructure, electrical power, multiple temperature-sensitive reagents, the operation and maintenance of specialized equipment, and technical expertise in molecular biology, potentially complicating deployment in resource-limited settings.

PositiveID/ExcitePCR is developing Firefly Dx, a handheld device offering rapid sample-to-result detection in less than 30 minutes using real-time polymerase chain reaction (PCR) chemistry. Firefly Dx is capable of multiplex assays and utilizes lyophilized reagents on a single-use, disposable cartridge for lab-quality results at the point of need (PON). The Firefly Dx system combines sample lysis, purification, real-time PCR analysis, and reporting of results. The system will process a variety of sample types, including whole blood, buccal and nasopharyngeal swabs, urine, and environmental field samples. Additionally, the use of lyophilized reagents eliminates the need for refrigeration of reagents needed in traditional laboratory-based protocols.

Recently in a collaborated effort with GenArraytion Inc., PositiveID successfully detected Ebola virus on the Firefly Dx prototype system. Ebola virus was tested at low number of copies via PCR and the growth curves showed a Ct of 21. The automated runs successfully synthesized cDNA using a proprietary reverse transcriptase (RT) step and then completed a 40-cycle PCR to produce the detected target results depicted in figure 1. Even in an early stage of development, the Firefly Dx system has consistently shown its effectiveness and repeatability through a wide range of organisms.



[1] Broadhurst MJ, Brooks TJG, Pollock NR. 2016. Diagnosis of Ebola virus disease: past, present, and future. Clin Microbiol Rev 29:773–793. doi:10.1128/CMR.00003-16.