Rapid point of use OmniAmp® Ebola assay using the Douglas Scientific® AmpliFire®

ABSTRACT

The AmpliFire by Douglas Scientific using OmniAmp isothermal amplification chemistry from Lucigen® provides a simple and portable tool to perform genetic analysis at the point of use. This paper describes a proof-of-concept experiment demonstrating the ability of the AmpliFire to detect various concentrations of the Ebola virus (EBOV) RNA using the OmniAmp Ebola assay.

INTRODUCTION

Douglas Scientific has developed a portable, point of use testing solution for rapid genetic analysis using OmniAmp isothermal nucleic acid amplification chemistry in combination with the AmpliFire instrument.

- **AmpliFire Point of Use Instrument** (Figure 1)
  The AmpliFire point of use instrument supports genetic analysis of up to eight samples simultaneously. Samples are incubated at a constant temperature using an internal heat block and fluorescence is read in real time by an integrated multichannel fluorescence detection system. Data then can be displayed and analyzed on the touch screen interface as the reaction progresses, or exported for further analysis.

Figure 1. AmpliFire Point of Use Instrument

- **OmniAmp Isothermal Chemistry**
  The OmniAmp Ebola assay is based on loop-mediated isothermal amplification (LAMP) and utilizes patented OmniAmp DNA polymerase. OmniAmp is a thermostable viral enzyme that enables point of use viability by overcoming important barriers to isothermal amplification. It is tolerant of inhibitors, which enables the use of crude samples. OmniAmp exhibits high thermostability for efficient amplification of highly structured genome regions and it rapidly amplifies either DNA or RNA targets with a typical reaction time less than 30 minutes.

MATERIALS AND METHODS

The reagents (primers, enzyme, and intercalating dye) were added to 0.2 mL PCR tubes at a volume of 20 µL per tube. RNA from the Guinea (2014) EBOV strain was serially diluted to create a series of six dilutions ranging from 56,000 copies per microliter to 350 copies per microliter. Five microliters of each dilution was added to separate reagent tubes for a total reaction volume of 25 µL per tube. These dilutions and two NTCs were run in the AmpliFire for 40 minutes at 72 °C. The amplification curves were monitored in real time for all of the samples. Data for the reactions were exported and analyzed.

RESULTS

Figure 2 shows the data from the OmniAmp Ebola assay run in the AmpliFire. The presence of EBOV RNA was successfully detected at concentrations as low as 350 copies per microliter. All reactions containing EBOV RNA showed strong positive signals easily differentiated from the NTC reactions in 30 minutes or less.

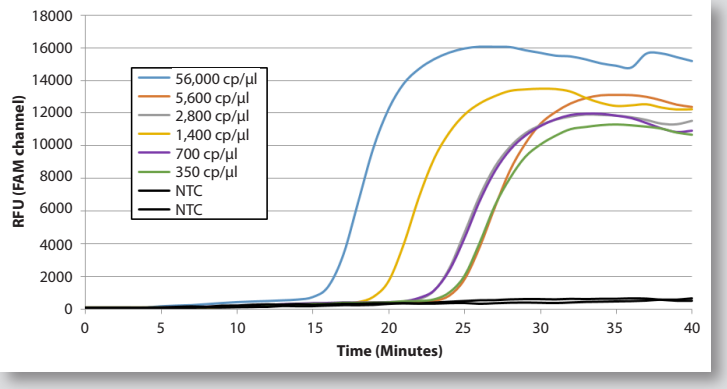

Figure 2. Exported results from the AmpliFire (Time vs. RFU) for various concentrations of EBOV RNA.

CONCLUSION

The AmpliFire successfully detected EBOV down to 350 copies per microliter. This experiment demonstrates the ability of OmniAmp Ebola assay to amplify a specific RNA target without interference from inhibitors or compromising sensitivity. With greater portability than real-time PCR instrumentation, the AmpliFire has the potential to become a very powerful tool for point of use applications in the continued fight against infectious disease. The AmpliFire produces rapid and accurate results in the field or in the lab without cumbersome equipment or reagents.

AMPAPP-6-2