

Fast, Accurate, and High-Sensitivity Virus Detection: Zika and Ebola Assays for Droplet Digital PCR Systems

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Droplet Digital PCR

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Abstract

Virus quantification and detection are critical for effectively identifying, monitoring, and mitigating viruses. Viral infections can ebb and flow seasonally in the population or spread rapidly in a seemingly sudden and intense outbreak, such as the recent outbreaks of Zika and Ebola viruses. During such outbreaks, testing becomes important for understanding and managing the spread and treatment of that virus. Sensitivity and specificity are important for responding effectively. However, RNA viruses, such as Ebola and Zika, have high variability and need to be detected in high backgrounds of DNA/RNA. Droplet Digital PCR (ddPCR) is a useful tool for viral detection, offering several key advantages over qPCR alone; for example, ddPCR enables accurate quantification of standards for qPCR. Additionally, ddPCR can verify the accuracy of a qPCR assay for cases where the virus contains sequence variants, which is common in RNA viruses. Ultimately, for low-level quantification, digital PCR provides easy, accurate results with high confidence. Droplet Digital PCR results with the new Zika and Ebola ddPCR assays are shown and discussed.

Introduction

When a viral outbreak occurs it is important to be able to rapidly detect and monitor the virus accurately. Droplet Digital PCR (ddPCR) technology is ideal for detecting and quantifying viruses. Relatively high nucleotide variant levels within these RNA viruses can be problematic for accurate quantification when using older PCR methods. RNA viral detection with digital end-point detection allows for direct counting of virus without a standard curve and can reliably measure low numbers of virus within a sample (Figure 1). Lab-to-lab and day-to-day reproducibility with ddPCR means you can be confident in your results (Figure 2). Low PCR efficiencies can occur when nucleotides vary from outbreak to outbreak (Strain et al. 2013). However, digital PCR counts positives as positives, whether they are low signal or high signal (Figure 3). Consequently, digital PCR lets you count more of the virus that's in your sample.

Two new ddPCR assays for detecting and quantifying the RNA viruses Zika and Ebola are discussed. Example data are shown for results using RNA or cDNA with our Expert Design Zika and Ebola Assays (Zika:dEXD90907415 and Ebola:dEXD65177188).

Quantifying and Detecting Zika and Ebola with ddPCR

In 2007 the first recorded outbreak of Zika occurred. In 2015 large outbreaks occurred in Brazil. Zika virus is spread mostly by mosquitos (*Aedes aegypti* and *Aedes albopictus*). When passed from a pregnant woman to her fetus it can cause serious birth defects. There is no vaccine or medicine for Zika, and symptoms can be mild or nonexistent. This means that accurate tests for Zika infection are important for identifying where outbreaks are occurring and whether someone has been infected. Additionally, since Zika can persist in the body for long periods and can be sexually transmitted, it is vital to have a sensitive and accurate assay to test for the virus.

Dangerous Outbreaks: Ebola

In 2014 the largest Ebola outbreak on record occurred. Due to the size of that outbreak Ebola has a larger reservoir than ever, meaning more frequent future outbreaks are predicted. As is common with RNA viruses, genetic variation will occur within and between outbreak strains (Gire et al. 2014). Results from a temperature gradient of the Ebola VP24 gene assay demonstrate that the assay is robust across a wide range of conditions. The clear distinction between positive and negative droplets is all that is needed for accurate counting of virus.

Materials and Methods

Synthetic RNA (Integrated DNA Technologies) was added to either the One-Step RT-ddPCR Advanced Kit Supermix for Probes (Bio-Rad catalog #1863021) or the iScript Select cDNA Synthesis Kit (Bio-Rad catalog #1708897) followed by ddPCR Supermix for Probes (No dUTP) (Bio-Rad catalog #1863024) following the manufacturer’s instructions. Gene-specific cDNA synthesis was done using the reverse primer

and the gene-specific protocol for the iScript Select cDNA Synthesis Kit. RNA or cDNA was added to the reaction and 20 µl of the solution used to create droplets. The droplets were run through a thermal cycler and read using the QX200 Droplet Digital PCR System (Bio-Rad catalog #1864001) according to the manufacturer’s instructions.

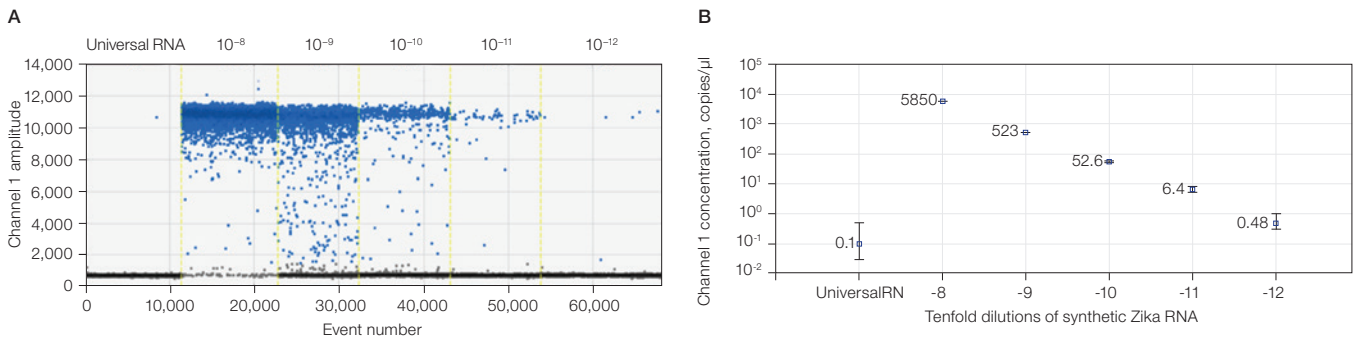


Fig. 1. Zika assay targeting NS5 gene titration series shows linearity across a concentration gradient. Titration of synthetic Zika RNA ultramer (Integrated DNA Technologies) added directly to the One-Step RT-ddPCR Advanced Kit for Probes (Bio-Rad catalog #1863022). Each titration of RNA was spiked into Universal Human Reference RNA (UniversalRN) (Agilent). **A**, 1-D droplet plot of assay Zika NS5 titration series. **B**, concentration plot shows linearity of the assay when serial dilutions were tested across five tenfold dilutions.

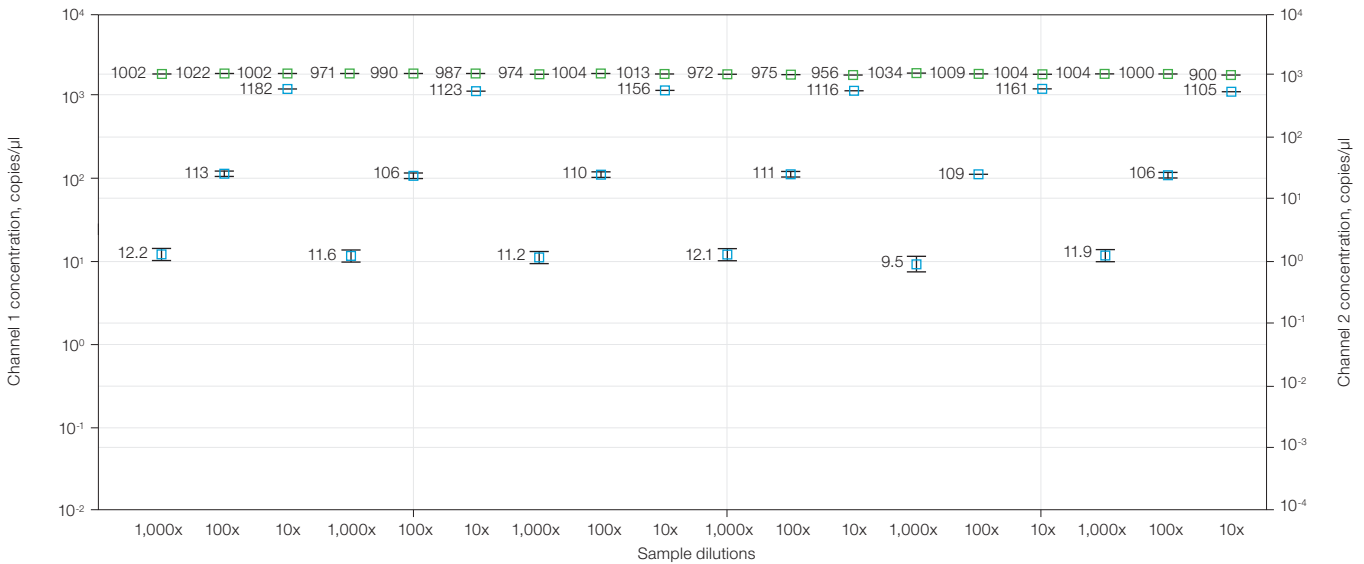


Fig. 2. Reproducibility of replicate wells demonstrates robust assay results. Replicate wells of a titration of cDNA made from synthetic RNA ultramer in a background cDNA made from Universal Human RNA. 2-D plot of the ddPCR Zika Assay (□) duplexed with the GUSB PrimePCR ddPCR Probes Assay (dHsaCPE5050189) (◻) using iScript Select cDNA Synthesis Kit (Bio-Rad catalog #1725037) shows reproducibility.

Conclusions

Droplet Digital PCR is a powerful method for accurately quantifying nucleic acids and detecting low levels of nucleic acids like viruses in a large background of other DNA or RNA (Figure 1). This method of measuring DNA and RNA has unparalleled accuracy and reproducibility of quantification (Deprez et al. 2016, Bhat and Emslie 2016). The robust and repeatable results of direct quantification can easily transfer to any lab (Figure 2). The QX200 System has options for measuring RNA viruses in a two-step (ddPCR Supermix for Probes (No dUTP), Bio-Rad catalog #1863024) or a one-step (One-Step RT-ddPCR Advanced Kit for Probes, Bio-Rad catalog #1863022) ddPCR reaction. Robust results can be achieved even if PCR efficiency is decreased. Decreased efficiency can result from samples containing inhibitors (Dingle et al. 2013), from nucleotide variants common to RNA viruses (Strain et al. 2013), or from thermal cycling temperature (Figure 3). The QX200 System and chemistry are compatible with standard reverse transcription kits, as is shown here using Bio-Rad Laboratories' iScript Select cDNA Synthesis Kit.

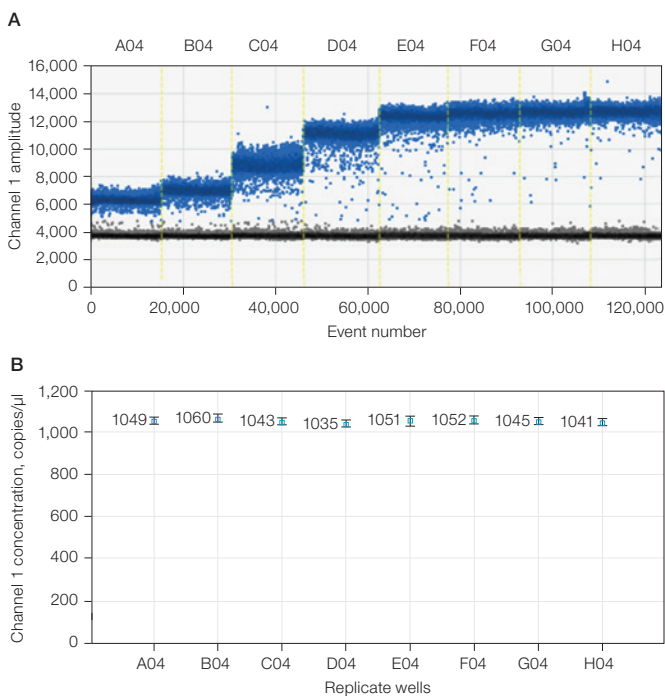


Fig. 3. A temperature gradient of the ddPCR Ebola Virus Assay demonstrates robust quantification with low and high PCR efficiency. **A**, a 1-D droplet plot of an annealing/extension temperature gradient 65–55°C corresponding to wells A04–H04 was generated to demonstrate the robust quantification and assay performance of Droplet Digital PCR. **B**, direct counting of molecules allows the concentration to be counted accurately even when amplification efficiency is low.

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