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Ultra-sensitive and high-throughput quantification of RNA virus using digital RT-PCR in dropbased microfluidics

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Epidemics of RNA virus diseases continue to pose a threat to public health despite the advances of medicine. This is mainly because RNA viruses are the most rapidly evolving organisms on earth, which enables them to escape immune systems, resist treatments and switch between hosts. At the time of evolution through mutations, each mutant virus contains unique information and may cause a new epidemic; therefore, these rare mutants must be individually detected and characterized. We present for the first time a digital one-step RT-PCR in pico-liter drop-based microfluidics to compartmentalize, amplify, count and isolate single target RNA viruses, at concentrations as low as one in a million, and acquire their genomic information one by one. This platform will not only facilitate experimental and clinical detection of RNA virus, but can be generalized to detect and sequence any type of rare RNA molecule and may be applied to other fields such as emerging drug resistance in viral diseases and the regulation of biological functions in rare cells, especially in cancer.

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