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Space-coded microchip for multiplexed respiratory virus detection via CRISPR-Cas12a and RPA

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ABSTRACT

Multiple infections of respiratory viruses are common in patients with clinical respiratory diseases, but current detection methods still have problems such as complex equipment and long detection time. Rapid, low-cost, and on-site detection of human respiratory viruses is crucial for both clinical diagnosis and population screening. In this research, we created a space-coded microfluidic chip (SC-Chip) for the recognition of nine respiratory viruses: influenza A virus, influenza B virus, severe acute respiratory syndrome coronavirus 2, human coronavirus OC43, human coronavirus NL63, human coronavirus HKU1, human respiratory syncytial virus, human parainfluenza virus, and human metapneumovirus. For the first time, a comprehensive sequence comparison among these viruses was performed to design the recombinase polymerase amplification (RPA) primers and Cas12a-crRNAs. The SC-Chip partitions samples amplified by RPA into spatially coded wells preloaded with CRISPR-Cas12a detection reagents, enabling the identification of all nine viral targets in a single test using a single fluorescence probe. The chip-based assay displays 9 respiratory viruses in less than 40 min with a minimum detection limit at a concentration of 10^{-18} M (~1 copy/reaction). Additionally, the efficacy of the method was assessed through its application to 35 clinical patient samples identified as being at risk for respiratory virus infection, yielding a sensitivity of 90 % and a specificity of 100 %. In summary, this space-coded microfluidic CRISPR system offers several advantages, including ease of operation, cost-effectiveness, and rapid data acquisition, thereby holding great potential for multiplexed detection of nucleic acid targets in a clinical setting.

1. Introduction

The susceptibility of certain populations, such as children, the elderly, and immunocompromised individuals, to severe respiratory infections induced by the human respiratory syncytial virus is well-documented in the literature [1,2]. Respiratory viruses can cause bronchiolitis and pneumonia, leading to serious incidence rate and even death [3,4]. The recent coronavirus disease 2019 (COVID-19) pandemic has further exacerbated the risk of co-infections with other respiratory pathogens, underscoring an increased public health concern regarding dual viral infections, where respiratory viruses and other viruses may simultaneously cause respiratory tract infections, complicating diagnosis and treatment [5]. Rapid and multiplexed detection of the nine common respiratory viruses - including influenza A (FLUAV), influenza B (FLUBV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), human respiratory syncytial virus (HRSV), human coronaviruses OC43 (HCoV-OC43), NL63 (HCoV-NL63), human parainfluenza virus (HPIV-3), HKU1 (HCoV-HKU1), and human metapneumovirus (HMPV)–is crucial for several reasons [6,7]. Firstly, these viruses often present with similar clinical symptoms, making it challenging to

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