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Clustered Regularly Interspaced Short Palindromic Repeat/cas9-Based Lateral Flow and Fluorescence Diagnostics for Rapid Pathogen Detection

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Abstract : Clustered, regularly interspaced short palindromic repeat (CRISPR/Cas) proteins can be designed to bind specified DNA and RNA sequences and hold great promise for the accurate detection of nucleic acids for diagnostics. Commercially available reagents were integrated into a CRISPR/Cas9-based lateral flow assay that can detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) sequences with single-base specificity. This approach requires minimal equipment and represents a simplified platform for field-based deployment. A rapid, multiplex fluorescence CRISPR/Cas9 nuclease cleavage assay capable of detecting and differentiating SARS-CoV-2, influenza A and B, and respiratory syncytial virus in a single reaction was also developed. These findings provide proof of principle for CRISPR/Cas9 point-of-care diagnosis that can detect specific SARS-CoV-2 strain(s). Further, Cas9 cleavage allows for a scalable fluorescent platform for identifying respiratory viral pathogens with overlapping symptomology. Collectively, this approach is a facile platform for diagnostics with broad application to user-defined sequence interrogation and detection.

Keywords : CRISPR/Cas9, lateral flow assay, SARS-Co-V2, single-nucleotide resolution
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