INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

CRISPR Cas13 For Sensitive And Specific Detection Of RNA Viruses

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Harnessing the exquisite specificity of Leptotrichia buccalis (Lbu) Cas13a guide RNA for its target RNA and its subsequently activated non-specific nuclease activity, we have developed an 11-plex lateral flow diagnostic device for respiratory viruses. The ten viruses detected include SARS-CoV-2, Influenza A (H1N1, H3N2, H5N1), Pan-Influenza B, RSV A, MERS, SARS, hPIV and hMPV as well as a human RNase P as a positive control. The device takes a nasal pharyngeal or other swab containing virus transport media, saliva, etc. as the sample input type. The swab is inserted into the device where it is mixed with a single-step extraction buffer. The extracted RNA is then split and delivered to the eleven separate reaction chambers containing free Cas13a and magnetic bead-bound hybrid DNA/RNA reporters. If the target RNA is present in the sample, Cas13a guide RNA specifically binds the target and Cas13a enzyme non-specifically cleaves the reporter away from the bead allowing it to flow downstream to a synthetic transcription/translation reaction chamber. This results in the translation of a unique dual-epitope peptide which then flows into the lateral flow strip, binds to the test line, and illuminates the line with colloidal gold for a visual positive detection signal. The device takes less than 45 minutes from sample to result and the current limit of detection with no pre-amplification is 5,000 copies of input target viral RNA. Optimizing the device and assay performance is ongoing.

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