

TOEHOLD SWITCH ENABLED VIRAL DETECTION VIA ROUTINE GLUCOSE MONITORING TECHNOLOGY

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ABSTRACT

Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) is a virus with 35% mortality rate, considered to be one of the most likely to cause major epidemics. Attempting to strengthen the response provisions in case of an outbreak, this project aims to develop an easy-to-use, reliable and safe, rapid test for MERS-CoV diagnosis. The proposed diagnostic kit, intended for use in point-of-care and emergency testing facilities, encloses all the necessary elements for sample processing, virus detection (biosensor) and signal generation. The engineered biosensor is based on the toehold switch mechanism. Toehold switches are mRNA molecules with riboswitch functionality. In this case, the designed switches regulate the expression of trehalase, an enzyme which hydrolyzes the disaccharide trehalose to glucose and acts as the reporter of the system. Trehalase expression is allowed only when the virus is present in the sample. Thus, overall, the viral load in the sample triggers glucose production, which is measured by a common commercial glucometer, signaling the diagnosis. An alternative reporter system, an engineered split trehalase, is also tested. This system lowers the structural complexity of the toehold switches, which is considered to accelerate the diagnosis. The two split fragments assemble to a functional enzyme through coiled-coil interactions. The proposed diagnostic workflow is easily customizable for the detection of other viruses threatening global health, aiming to contribute to the fields of travel medicine and diagnostics.

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