REVOLUTIONARY DIAGNOSTICS – NONTRADITIONAL APPROACHES FOR DEVELOPING BREAKTHROUGH CAPABILITIES AGAINST EMERGING THREATS

Overcoming Challenges Of Using Saliva As A Biospecimen In Rapid Diagnostic Tests

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The COVID-19 pandemic highlighted the need for rapid and frequent screening of infection. This concept applies to many infectious diseases that are common among military populations and can be imperative for preserving the health of a unit. Diagnostic tools that use non-invasive and accessible biospecimens, such as saliva, enable routine screening and can provide rapid, actionable results to infected individuals to limit the spread of infectious disease.

Saliva is a non-invasive, easily obtained biospecimen which has been shown to contain pathogen genomes and host RNA biomarkers of infection. Here we've explored the feasibility of using saliva as a biospecimen for infectious disease diagnostics and have identified multiple challenges with using crude saliva extracts as a template in enzyme-based amplification reactions. Challenges arise from the highly variable nature of saliva; large ranges in pH can interfere with downstream assays, and variability in high-density mucin concentration and viscosity can reduce robustness of complex microfluidic devices. Additionally, the abundance and activity of RNases and the presence of contaminating bacterial nucleic acids can negatively impact nucleic acid amplification-based tests.

Using a combination of chemical, enzymatic and mechanical treatments, methods to overcome these challenges were assessed. Various materials for the collection and filtration of saliva were evaluated for ease of collection, sample retainment, and reduction of viscosity. Methods for rapid sample lysis and preservation of nucleic acids were established using either a short heat treatment or exposure to an optimized lysis buffer combined with filters to clear cellular debris. The efficiency of sample collection, lysis, and nucleic acid preservation was directly quantified using a TapeStation RNA assay and RT-qPCR. Based on our evaluations, we developed a non-powered, rapid saliva processing technique compatible with isothermal nucleic acid amplification, applicable for use in the field. By identifying challenges and viable solutions to using saliva as a biospecimen, we have enabled the use of saliva in point-of-need and lab developed tests for the frequent monitoring of health status and rapid detection of infectious disease.

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