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

An Analysis Of Isothermal Amplification Chemistries For Use In Point-of-need Diagnostic Devices

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The health and wellbeing of warfighters and other military personnel is of foremost concern, whether stationed on base or in the field. When stationed in low resource areas, the use of current state of the art diagnostics are not practical; most infectious disease diagnostics rely on polymerase chain reaction (PCR), which is difficult to implement in the field due to its dependance on thermal cyclers and thus lack of portability, higher cost, longer time to result, and requirement for a power-source. Isothermal amplification is a viable alternative to conventional amplification chemistries such as PCR. Most isothermal amplification chemistries rely on enzymatic activity at a single temperature for amplification rather than temperature cycling, making them an attractive alternative for use in point-of-need diagnostics. Furthermore, many isothermal chemistries function at relatively low temperatures, making their use in the field even more feasible.

Here we present an analysis of isothermal amplification chemistries for use in point-of-need diagnostic devices, considering amplification time and temperature, cost, reaction kinetics, and ease of chemistry optimization. Eighteen isothermal chemistries were considered, and the four most promising chemistries were selected to be tested in-house. Of the in-house tested chemistries, Recombinase Polymerase Amplification (RPA) proved to be the most favorable for point-of-need diagnostics due to its characteristically high amplicon yield, low amplification temperature, and rapid amplification time.

We've demonstrated that RPA is a robust chemistry, with a limit of detection (LoD) similar to that of PCR. Product amplification using RPA occurs rapidly and works at a wide range of incubation temperatures, making it a more appealing chemistry than alternatives for field applications. Furthermore, RPA is compatible with a variety of reverse transcriptases, allowing both DNA and RNA to be used as reaction templates. With further chemistry optimization, we expect that RPA can be conducted near ambient temperature, indicating RPA may not require external heating or can utilize human body temperature as a heat source.

Effective point-of-need diagnostics provide warfighters with the tools necessary for mitigating the spread of disease to remain fit for duty. Isothermal amplification chemistries are cost effective and can be optimized for use in the field and other low-resource settings, making them promising for portable diagnostics and an auspicious alternative to conventional PCR.

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