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Improving Point-of-need Diagnostic Readouts Across Amplification Chemistries

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The COVID-19 pandemic has highlighted the gap in our capabilities to rapidly diagnose and address infectious disease transmission across the population. Sensitive point-of-need diagnostics are needed to ensure our preparedness to combat the rapid spread of communicable diseases among service members. We aim to improve overall test time and lower test costs in order to bring sensitive diagnostic capabilities to the point-of-need.

In this study, we evaluated two common isothermal nucleic acid amplification methods to identify mechanisms for improving reaction readout on a lateral flow assay (LFA). Because we can eliminate the need for expensive equipment to analyze test results by using an LFA, we focused our research on improving LFA readouts to enable more cost-effective diagnostics for the point-of-need market. Since 2020, there has been a dramatic increase in the use of isothermal amplification and LFA readouts for point-of-care diagnostics, resulting in cheaper tests that can be performed outside of central laboratories, thereby reducing the delay between sample collection and test result.

Standard recombinase polymerase amplification (RPA) and loop-mediated amplification (LAMP) reagents were purchased from Twist Dx and New England Biolabs, respectively. For both methods, the oligonucleotide primers were manufactured by IDT. For readout on an LFA, both primers in RPA and two of the six LAMP primers were modified on the 5’ end and purified by HPLC. Standard testing methods were employed to develop RT-RPA and RT-LAMP assays for compatibility with an off-the-shelf LFA. The standard method to evaluate amplification reactions on an LFA are to use 100% modified primers in the reaction. Here, we explored the impact of varying the ratio of unmodified to modified primers on reaction performance and LFA readout.

Adjusting the ratios of modified and unmodified primers in an amplification reaction improves reaction kinetics and therefore reaction sensitivity. An additional benefit of this approach is that it can reduce the cost of oligonucleotides per reaction. It also allows for a more sensitive LFA as there are fewer unincorporated modified primers to compete for binding sites on the LFA’s test lines.

Improving isothermal amplification reactions and LFA readouts by adjusting the ratios of modified and unmodified primers can be easily employed in other tests and can generate exciting results with point-of-need testing capabilities. Using the described method to enhance the sensitivity and lower the cost of relevant field tests could improve our ability to rapidly obtain medical answers and keep our service members healthy and responsive to the needs of their mission.

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