Loop-Mediated Isothermal Amplification Assay with Amplicon-Specific Visual Readout by Using Oligo-Gold Nanoparticles

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Background

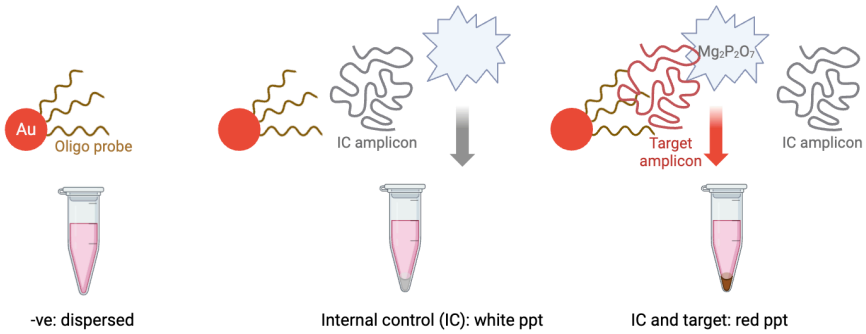
Nucleic acid testing (NAT) is a powerful method for a wide spectrum of applications such as medical diagnostics and food safety monitoring. Real-time polymerase chain reaction (PCR for deoxyribonucleic acid (DNA); reverse transcription-PCR (RT-PCR) for ribonucleic acid (RNA)), the current gold standard, offers high sensitivity and specificity, but requires bulky and expensive instruments in centralized laboratories. Decentralized testing therefore calls for low-cost and compact alternatives. Loop-mediated isothermal amplification (LAMP and RT-LAMP) with visual readout is a promising candidate, offering performance comparable to PCR but with faster turnaround and substantially simpler instrumentation requirements. However, existing LAMP visual assays use universal reporters, which means they cannot differentiate more than one target sequence in a single reaction (multiplex) or include an internal control (IC) to avoid false negatives. To address this, we developed a LAMP assay with amplicon-specific visual readout using gold nanoparticles functionalized with oligonucleotide probe (oligo-AuNPs). The dispersion/precipitation behavior indicates the absence/presence of a specific amplicon.

Methods

AuNPs were synthesized and functionalized with thiol-spacer-oligo. One primer of the target sequence served as the oligo probe AuNPs. Two functionalization methods (salt-ageing and freezing-driven adsorption) were compared for oligo loading and LAMP compatibility. Oligo-AuNPs were characterized, and their dispersion/precipitation behavior was evaluated in multiplex LAMP containing primers of both target and IC: (1) negative; (2) IC only; (3) IC and target.

Results and Discussion

The assay was amplicon-specific: oligo-AuNPs remained dispersed in negative and IC reactions whereas precipitated with magnesium pyrophosphate ($Mg_2P_2O_7$) crystals only in the presence of the target sequence. Figure below shows the readouts: (1) no precipitation in negative; (2) white precipitation in IC; and (3) red precipitation in IC and target. Freezing-driven adsorption yielded higher oligo loading and better NP stability in LAMP. Further sensitivity and specificity evaluation as well as mechanism study are needed.



Conclusion and Impact

This amplicon-specific visual reporter (oligo-AuNPs) combined with the intrinsic white $Mg_2P_2O_7$ precipitate as a positive IC enable simple multiplex LAMP readouts. It offers a fast, simple, on-site, and accurate alternative for detecting bacterial and viral targets, with potential impact in public health surveillance, environmental monitoring, and food safety testing. In addition, the platform could be further expanded for higher-order multiplexing by incorporating NPs of another color.

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