Single-Step, One-Pot, DNA Amplification & Electrochemical Detection via Loop-Mediated Isothermal Amplification (LAMP)

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Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification technique that is a useful alternative to polymerase chain reaction (PCR) for low-cost or point-of-care diagnostics for infectious disease. The technique can be coupled with reverse transcription (RT-LAMP) for detection of RNA targets, e.g. RNA viruses [1,2]. LAMP (and RT-LAMP) is generally regarded as highly specific and highly sensitive, but a major challenge for LAMP in point-of-care applications is the detection of amplification without requiring cumbersome manipulations or elaborate instrumentation. Furthermore, the available detection mechanisms used in LAMP are not easily amenable to multiplexing to distinguish multiple targets in a single reaction, e.g. for syndromic panels or variant strains of pathogens.

To address these challenges, we recently developed a single-step, closed-tube, and multiplexable fluorescence-based detection method for LAMP and RT-LAMP, termed QUASR [3]. Here we report the extension of this technique for electrochemical detection as a non-optical alternative for RT-LAMP assays. This effort was carried out using MS2 phage as a model RNA viral target. The approach utilizes an electroactive molecular beacon bound to a gold electrode surface with the complement to one of the loop primers (LoopF) as the target.

Two approaches for surface immobilization were evaluated: alkane-thiol bond on gold, and a covalent approach utilizing maleimide-functionalized aryl diazonium chemistry. Between the two approaches, the covalent linkage was stable to the 63 °C used for the LAMP incubation, and can thus be used for a truly *in-situ* detection method. The alkane-thiol on gold linkage was not thermally stable, and thus the amplification must be performed in a separate chamber from the electrochemical detector, with a sample transfer step required. This adds complexity to the operation and adds risk of amplicon contamination. As shown in **Figure 1**, electrochemical detection of LAMP amplicon by differential pulse voltammetry yields a drop in current of approximately 25% (**Fig 1B**). Based on the calibration with a synthetic oligonucleotide target (**Fig1A**), this suggests a concentration of the probe complement of 0.1 µM. The detection limit for this system was 3 nM (5σ), with a normalized relative standard deviation of 8.4%, showing the potential of this system for low-cost or point-of-care diagnostics for infectious disease.

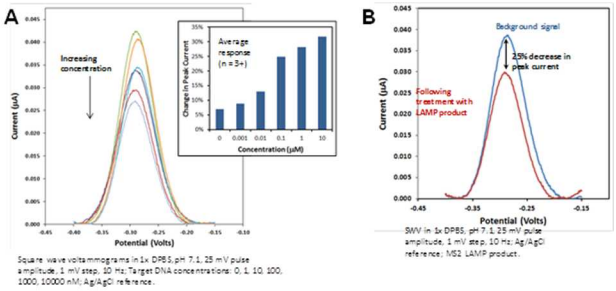

Figure 1. Electrochemical detection of (A) a synthetic oligonucleotide and (B) RT-LAMP amplicon with an electrode-bound stem-loop probe.

1. Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. *Nucl. Acids Res.* **2000**, *28* (12), e63–e63.
2. Parida, M.; Posadas, G.; Inoue, S.; Hasebe, F.; Morita, K. *J. Clinical Microbiol.* **2004**, *42* (1), 257–263.
3. Ball, C. S.; Light, Y. K.; Koh, C.-Y.; Wheeler, S. S.; Coffey, L. L.; Meagher, R. J. *Anal. Chem.* **2016**, *88* (7), 3562-3568.