

Electrochemical Redox Label Multiplexing on Single Carbon Electrodes for the Real-Time Detection of Bacterial Wound Infections

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Summary:

We introduce redox label multiplexing and demonstrate the first real-time electrochemical duplex LAMP assay [1] enabling simultaneous detection of two nucleic acid targets on a single carbon electrode. Distinct redox labels combined with universal surface probes enable multiplexed signal-on detection within one reaction volume. Preliminary validation with patient wound swab samples demonstrates robust and highly specific performance in complex biological matrices. The presented platform establishes a scalable route toward low-cost, highly multiplexed point-of-care molecular testing.

Keywords: electrochemical detection, LAMP, multiplex diagnostics, point-of-care, biosensors

Background, Motivation and Objective

Electrochemical detection of loop-mediated amplification (LAMP) reactions is a promising approach to make sensitive, miniaturized molecular diagnostics available at the point-of-care. Emerging probe-based LAMP methodologies have adopted universal designs that increase analytical specificity and significantly simplify the development process [2]. However, these approaches are limited to the detection of only one target per working electrode. Multiplexed detection at the point-of-care remains challenging when sample volumes are limited, and robust multiplexing methods within a single reaction cavity are lacking [1]. Here we introduce redox label multiplexing on a single electrode using generic surface probes to overcome this limitation, demonstrating duplex detection with methylene blue and anthraquinone labels and thereby expanding the electrochemical mediator displacement (eMD) LAMP toolbox.

Description of the New Method or System

We demonstrate redox label multiplexing with the first real-time electrochemical duplex LAMP assay using distinct redox-active molecules on a single carbon working electrode. The platform

approach builds on a generic surface functionalization strategy employing universal DNA capture probes, enabling modular assay design independent of target-specific surface modifications. Target-specific amplification is coupled to signal generation via labeled mediator strands displaced during the LAMP cascade. Each target is associated with a displaced mediator modified with a distinct redox label, producing separate and identifiable electrochemical signals at defined potentials when hybridized to the surface probes (see Fig. 1). This label-based multiplexing strategy allows simultaneous, real-time detection of multiple amplification reactions within a single chamber. The presented approach overcomes key limitations of existing electrochemical LAMP systems by combining (i) real-time signal-on detection, (ii) multiplex capability without geometric or electrode-based partitioning, and (iii) compatibility with simple and scalable PoC device architectures.

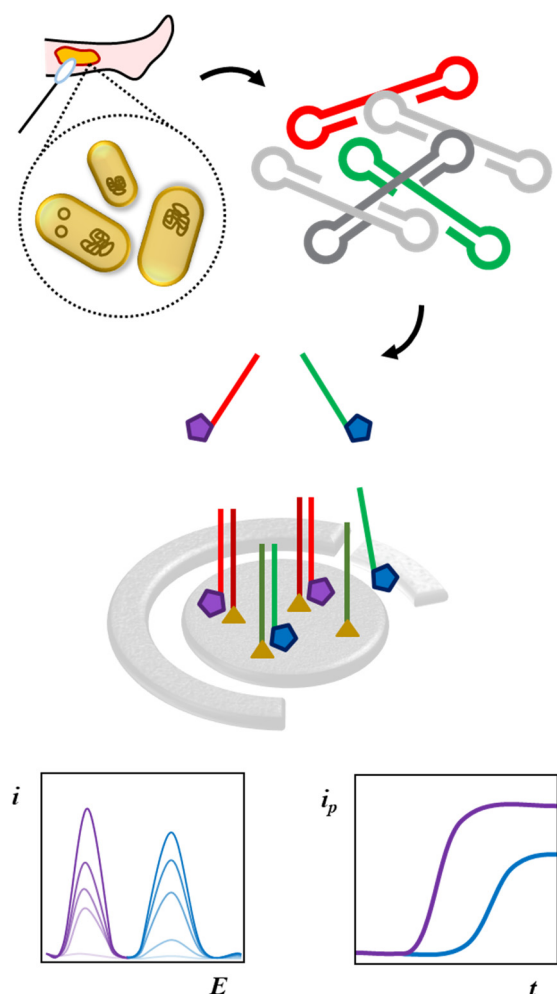


Fig. 1. Schematic visualization of the electrochemical duplex eMD LAMP realized on a single carbon electrode with redox label multiplexing. LAMP detection of lysed bacteria from patient wound swab samples (top) causes target-specific displacement of redox-labeled mediator sequences (red and green). Hybridization of displaced mediators to complementary surface probes allows for the co-detection of anthraquinone (purple) and methylene blue (blue) redox labels on a single electrode (middle). Both labels have discreet redox potentials, allowing for the simultaneous resolution in real-time (bottom left). Plotting the label-specific peak currents over time (bottom right) reveals the LAMP amplification plot, allowing for real-time electrochemical duplex LAMP detection. Figure adapted from [1].

Results

The *uidA* *E. coli* housekeeping gene and the CTX-M1 antibiotic resistance gene found in ESBL *E. coli* are both successfully detected in singleplex down to 10 CFUs per reaction and show semi-quantitative amplification signals. Our novel electrochemical real-time LAMP assays show equal sensitivity and similar time-to-positive as their optical counterparts while improving the signal-to-noise ratio for the used primer sets. The combined duplex LAMP allows for detection of both targets within 40 min, and its

clinical matrix compatibility was preliminarily validated using five clinical wound swab samples (see Fig. 2). The duplex LAMP specifically detects the *E. coli*-positive sample in presence of eleven other wound-associated bacterial species. Our label-based real-time multiplexing approach doubles the detection capabilities per working electrode and presents the first real-time probe-based electrochemical signal-on duplex LAMP detection, paving the way for miniaturized sample-to-answer point-of-care devices, with further redox labels available for an even higher degree of multiplexing. In combination with electrode-based multiplexing, highly multiplexed assay formats become possible that can far exceed what is currently possible in a single reaction volume.

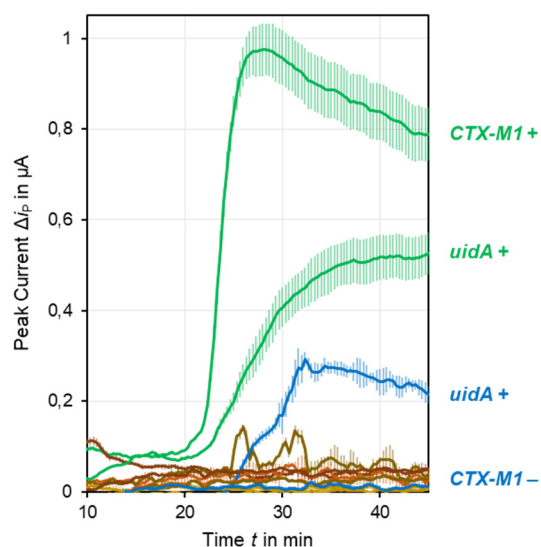


Fig. 2. Patient wound swab samples tested with eMD LAMP duplex assay. One *E. coli*-negative matrix sample with spiked-in CTX-M1 (10^2 CFUs/rx.) and *uidA* (10^4 CFUs/rx.) lysate (green) was used as the control reaction. In addition, one *E. coli*-positive wound swab sample (blue) and four *E. coli*-negative wound swab samples with various other wound colonizing bacterial strains (gold-brown hues) were tested. The eMD LAMP correctly identified the positive *E. coli* sample and did not yield false positive signals within 45 min, demonstrating diagnostic sensitivity and specificity in clinical samples. Figure adapted from [1].

References

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