A78 Integrated Direct Amplification and Hybridization-Induced Aggregation for End-Point Detection of the Human TPOX Locus From Whole Blood

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After attending this presentation, attendees will gain an understanding of the development of single chamber, direct amplification of whole blood, followed by label-free visual detection of the product through hybridization-induced aggregation (HIA).

This presentation will impact the forensic science community by introducing a microfluidic method that presents the possibility for the direct amplification of the TPOX locus for the rapid verification of the presence of human blood. This work demonstrates the use of a micro-total analysis system for forensic onsite analysis.

Microfluidic devices offer numerous advantages to current forensic analyses, including low sample consumption and a reduction in analysis time, combined with the ability to deliver a closed "sample in-answer out" multi-process system within a single device, preventing sample loss and contamination.[1] Microfluidics presents an eventual economical option, with the ability to rapidly fabricate plastic microdevices using CO_2 laser ablation. In addition, using infrared-mediated heating and fan-assisted cooling, PCR is completed in half the conventional time, with instrumentation costing a fraction of that for a block thermocycler.[2] Progress toward a fully-integrated device has become simpler with the recent development of direct blood amplification, enabling the removal of the DNA extraction domain within the microdevice.

Here, a master mix capable of this chemistry, combing a commercially-available neutral proteinase and *Pwo* DNA polymerase, enabling customization of the components is presented. Initially, this was demonstrated in a conventional 25µL tube PCR reaction, amplifying the TPOX allele using 1µL of whole blood as the template. The PCR product was separated on a microchip electrophoresis platform, demonstrating successful amplification of the alleles within the TPOX locus. Since *Pwo* is a high-fidelity polymerase, no non-specific amplification was observed, which was seen as problematic with an alternative direct blood PCR kit. In addition, the successful amplification of the TPOX locus was achieved from 1µL blood stains on filter paper, cotton, cotton-polyester, and demin substrates. To confirm this amplification was human-specific, blood samples from a rhesus monkey, a rabbit, a mouse, and a dog were processed in the same manner, resulting in no peaks, confirming the specificity of the assay for human samples.

Translating this direct tube-based PCR method to a microdevice has the potential to significantly decrease the time required for the reaction, and if this could be linked to a label-free read-out, integration would allow for immediate results without further sample handling. Recently, a visual, label-free end-point detection that utilizes hybridization of biotinylated oligonucleotide probes bound to 1µm streptavidin-coated paramagnetic particles to the complementary (target) sequence was reported.[3] A rotating magnetic field agitates the particles, exposing the bead-bound oligonucleotide for hybridization to the target sequence to form an aggregate; visual detection of the aggregates confirms the presence of the target DNA. This is visually distinguishable from sample without target DNA where the particles remain dispersed. Cellular debris associated with the enzyme-based DNA liberation step did not result in any false aggregation, confirming that the particles only aggregate when adequate target copies are available as a result of successful amplification. This circumvents the requirement for fluorescent tags, an amplicon separation step, and skilled labor, while providing a highly sensitive, accurate method for specific DNA sequence detection in less than two minutes.

In summary, the rapid detection of a fragment of the TPOX gene directly from whole blood on a single device is facilitated by a microfluidic network that links the PCR and an aggregation domain, and requires no external apparatus to control fluid flow. This work presents a total micro analysis system, capable of identifying the presence of the TPOX allele from human blood using a plastic microdevice, integrating single chamber, infrared-mediated, direct PCR and visualized aggregation, in 60 minutes.

References:

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3. Leslie, D.C., et al., New Detection Modality for Label-Free Quantification of DNA in Biological Samples via Superparamagnetic Bead Aggregation. Journal of the American Chemical Society, 2012. 134(12): p. 5689-5696.

Direct PCR, Microdevice, Human Identification

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