B67 The Development of New Enzymes for 20-Minute Rapid Direct Amplification of Crime Scene Samples and Its Application in Presumptive DNA Screening

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After attending this presentation, attendees will understand how new inhibitor-resistant, rapid polymerases may be used to type blood, saliva, and semen.

This presentation will impact the forensic science community by showing how ultimately the development of direct, rapid Polymerase Chain Reaction (PCR) -based systems will greatly expand the capabilities of police, custom agents, and first responders to process crime scene samples and mass disasters, providing information on a timely basis that otherwise might take hours to obtain. Because these procedures involve existing commercial systems, issues such as injection, loading, and reliability have already been worked out through extensive marketplace testing. The analytical systems have very small footprints and the chips and PCR tubes are disposable and easy to transport. These systems can be implemented immediately and incorporated into current forensic labs with only the addition of a fast thermal cycler and no complex training or engineering design. The overall result will be a massive improvement in the current processing of submitted DNA samples and in the capability to perform remote testing and screening.

Current DNA typing methods provide the best biometric information yielding potential identity, kinship, and geographical origin, but they are not sufficiently fast to permit rapid identity of a suspect. There are situations in which it is very important to rapidly screen crime scene samples and unknown individuals who may have been involved in a crime. Examples include seized evidence potentially linked to a suspect, commingled bone samples buried in a mass grave, or the determination of which bloodstains present at a crime scene may be probative. In these situations, many samples may need to be run in order to create probable cause for detaining a suspect. The goal of this project is to develop a rapid and direct method for determining DNA from a wide variety of crime scene samples. Rape kit screening will also be possible.

In the first phase of this study, a rapid thermal cycling procedure combined with a direct amplification was tested and optimized on control DNA standards, K562, 9948, and 9947a cell lines, and paper saliva punch samples from 20 individuals. Optimization included testing different polymerases, buffer compositions, salt concentration, pH, and varying concentrations of magnesium and dNTPs, as well as thermal cycling parameters. It was demonstrated that the rapid direct PCR using rapid DNA polymerases (Z-Taq™ Polymerase) and direct PCR buffer (AnyDirect™ F buffer with 30 cycles at 98°C for five seconds, 60°C for ten seconds, and 72°C for 15 seconds) permitted a high-speed amplification of a 7-locus multiplex that required no extraction step. A 1.2mm punch of samples from FTA® paper was directly added to the rapid direct master mix and amplification performed in 15 minutes and 54 seconds on rapid thermal cyclers. This was then coupled with a short 1.8cm microfluidic electrophoresis system and it was shown that the entire procedure from paper punch to genotype can be completed in under 25 minutes.

In the second phase of this study, mutant taq polymerases that are inhibitor resistant were tested on replicate samples spiked with hematin or phenol chloroform. Different volumes of the OmniTaq™ polymerases were tested on control DNA with varying amounts of inhibitors. The new enzymes were capable of partially overcoming the inhibition induced by hematin (15µM) and phenol chloroform (0.4µl).

In the third phase of this project, the proven high-speed thermal cyclers and rapid enzymes capable of performing 28 cycles of amplification in 14 minutes or less, in combination with the new inhibitor resistant enzymes on different biological fluids, will be tested. This procedure will be coupled with high-speed genotyping using microfluidic chips or standard capillary-based systems modified for faster run times.

Rapid direct PCR procedures can greatly speed the processing time but they primarily work with saliva.[1,2] The goal is to perform high-speed DNA detection for a wider variety of sample types including blood, bone, and sexual assault kits. Specially engineered enzymes will be used, high-speed thermal cyclers, and chip-based electrophoresis, as well as inhibitor-resistant DNA polymerases to achieve sample genotypes in less than 20 minutes for a wide variety of sample types.[3-5] The goal will be to provide methods for any crime laboratory to obtain genotypes in less than 40 minutes using off-the-shelf, high-speed thermal cyclers and novel polymerases and in less than 20 minutes using a commercial microchip prototype.[1]

This project is interdisciplinary involving the efforts of instrument manufacturers, biochemistry research, and law enforcement.

References:

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Rapid, Direct PCR, Inhibition, DNA Polymerase