B112 Direct Amplification and Commonly Encountered Crime Scene Substrates

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After attending this presentation, attendees will better understand how to obtain DNA profiles by direct amplification of bloodstains on substrates commonly encountered at crime scenes. Attendees will also learn that it is possible to obtain complete DNA profiles from body fluids without extraction or pretreatment of the stains.

This presentation will impact the forensic science community by providing information concerning how to generate DNA profiles within a very short time and without labor-intensive and time-consuming steps.

Items of evidence retrieved from crime scenes often contain blood. In order to generate Short Tandem Repeat (STR) profiles from such evidence, extraction, purification, and quantification prior to amplification of DNA is necessary. In this research, autosomal STR profiles were generated via direct amplification of bloodstains deposited on simulated crime scene substrates.

Blood samples from deceased males were collected in sterile tubes containing Ethylenediaminetetraacetic Acid (EDTA) to prevent DNA degradation and were kept frozen until ready for analysis. There were four stages in this study. The blood samples from the deceased donors were first extracted using the BioRobot EZ1 workstation and EZ1 DNA investigator kit in order to generate reference profiles. In the second stage, a measured volume (0.1µL) of blood was deposited on each of the ten substrates and dried for 24 hours at room temperature. The objects included: cigarette butt, drinking straw, dry brown leaf, woodchip, leather, and five different types of fabrics often encountered as evidence from crime scenes. None of these objects contained any lysing agent. Several of these objects contained potential inhibitors. The deposited bloodstains were punched using a Harris 1.2mm micro-punch on nine of the listed substrates. The tenth substrate, the woodchip, was too difficult to punch. Therefore, a minute piece approximating the 1.2mm punch was shaved off after depositing the blood on the woodchip. Each of the stains was extracted using the forensicGEM™ tissue extraction protocol. The samples generated from the two extraction methods were quantified and amplified using the amplification kits used for direct amplification.

In the next step of the project, each of these punched substrates containing the bloodstains created from 0.1µL of blood was amplified directly after pretreatment with reagents and buffer. All the substrates containing the stains were treated with the Prep-n-Go™ buffer prior to amplification with the GlobalFiler® Express Amplification kit. SwabSolution™ and PunchSolution™ were used for treatment of the stains prior to amplification with the PowerPlex® Fusion and PowerPlex® 18D Systems.

In the fourth and final stage of the research, each stain was amplified directly without any pretreatment with the reagents and buffer mentioned earlier. During the third and the fourth stages of this research, the substrates remained in the amplification reagents during the thermal cycling steps. Each bloodstain was amplified with the three direct autosomal STR amplification kits: GlobalFiler® Express Amplification kit, PowerPlex® Fusion, and PowerPlex® 18D Systems. Capillary electrophoresis was performed on a 3130xl Genetic Analyzer and data were analyzed using **GeneMarker**® software version 2.7.1.

Reference profiles were obtained from blood extracted using the traditional (EZ1 extraction) method. These profiles were compared to the profiles generated from the stains deposited on the various substrates and extracted with the forensicGEM™ tissue extraction protocol. The profiles were found to be concordant and consistent between and within each substrate and each amplification kit. These profiles were then compared to the profiles generated from each of the stains amplified directly with and without pretreatment as described in the third and fourth stages of the research.

Complete and concordant autosomal STR profiles were successfully obtained from the bloodstains deposited on the ten challenging substrates when they were amplified directly using GlobalFiler® Express Amplification kit, the PowerPlex® Fusion, and the PowerPlex® 18D Systems. The research indicated that the pretreatment of the bloodstains with the reagents and buffer did not enhance the quality of the profiles. Complete and concordant profiles were obtained when the stains were not subjected to any pretreatment and while the substrates remained in the reagents during the amplification steps.

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At this time, the forensic community requires that forensic DNA analysts perform quantification prior to amplification. This study with direct amplification using simulated casework-type samples may be of limited value. Currently, with instruments such as the RapidHIT™ system from IntegenX® or the DNAScan Rapid DNA Analysis System from NetBio, analysts are already performing direct amplification of body fluids. It is expected that this procedure, which involves challenged substrates and direct amplification, would be acceptable in the near future.

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