A19 Evaluation of Lyophilized Reagents for STR Analysis

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The goal of this presentation is to demonstrate a proof of concept experiment in which PCR reagents were prepared in and subjected to non-ideal temperature conditions.

This presentation will impact the forensic science community by providing a springboard for further exploration of lyophilized reagents designed for use in non-ideal forensic environments.

Recent literature indicates growing interest in the use of DNA as a biometric tool for applications in both the lab and the field. A factor which must be considered for field applications is the ability to maintain reagents in non-ideal conditions. Commonly used typing kits recommend storage of primers and reaction mix at 2 to 8°C and polymerase at -15 to -25°C. This would be problematic, for example, in a situation where a battlefield lab is deployed in a location such as Afghanistan. Suppliers may have difficulty reaching such locations and reagents would be exposed to a non-ideal, high temperature environment during shipping and at the field laboratory. An understanding of how PCR reagents will be affected by their environment is therefore necessary.

The purpose of this study was to evaluate the use of dried primers and stored at various non-ideal temperatures, along with commercially available lyophilized polymerases for use in STR analysis. Five temperatures were chosen for evaluation: room temperature, 4°C, 37°C, 50°C, 65°C, and 80°C. Several commercially available lyophilized polymerases already containing reaction mix were evaluated as well. Primers were dried down utilizing a speed vac and stored at their respective temperature for one week along with the lyophilized polymerase/reaction mix. A PCR amplification protocol was employed followed by capillary electrophoresis analysis. In addition, the effect of the high temperature environment on the dried primers was tested by using polymerase and reaction mix which had not been exposed to the non-ideal conditions. The latter reagents were stored according to manufacturer recommendations until amplification.

Full or partial profiles were obtained for temperatures closest to recommended storage conditions and some amplification was observed for several loci at temperatures up to 65°C. Artifacts such as incomplete adenylation and peak imbalance were observed and suggest that protocol improvements are necessary. Full profiles could be obtained with primers stored at temperatures up to 80°C when polymerase and reaction mix which had not been exposed to the non-ideal, high temperature environment were used for the PCR amplification. This suggests that the reagents which were most affected by the higher temperatures were the polymerase and reaction mix (in particular dNTPs). It is important to note that the polymerases which were chosen, although lyophilized, were not specifically designed for STR analysis in mind. Some reagents were tailored towards real time PCR applications, which likely contain non-ideal amounts of components such as $MgCl_2$ for end point PCR applications. This is suggested in these experiments by the presence of artifacts such as incomplete adenylation and non-specific PCR products even when reagents were stored in the conditions which were recommended by the manufacturer.

Results observed at higher temperatures believed to be incompatible with PCR reagents indicate that with optimization, there is potential for future development of lyophilized reagents for use in PCR field kits. If a product could be designed and tailored to STR analysis and the forensic practitioner's objectives, it is likely that the results obtained here could be improved in terms of profile quality.

Battlefield Forensics, STR Analysis, PCR

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