TECHNICAL NOTE

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A Novel Approach to Obtaining Reliable PCR Results from Luminol Treated Bloodstains

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ABSTRACT: In recent years the forensic scientist has been afforded great advances in technology both in the detection of latent bloodstains and in acquiring reliable DNA typing results from very small pieces of physical evidence. Scientists are now able to detect minute quantities of latent bloodstains by utilizing the luminol reagent, oftentimes indicating that an attempt has been made to conceal any evidence of bloodshed. With the introduction of PCR based technology to the forensic arena, scientists are now routinely able to obtain DNA typing results from previously insufficient amounts of biological material, items as small as a single hair, saliva on a cigarette butt, or a bloodstain the size of a pin head. We present here a merging of these two advances coupled with a new collection medium for post luminol treated latent bloodstains. The forensic scientist is now able to routinely isolate and recover an adequate amount of DNA suitable for PCR typing at all of the Promega GenePrint® PowerPlex™ 1.1 loci. In this study, several dilutions of latent bloodstains were prepared in an effort to simulate transferred bloodstains that are routinely encountered in a crime scene setting. The latent bloodstains were treated with luminol and subsequently collected using conventional cotton tipped swabs as well as a Puritan™ sponge tipped swab. PCR typing at the Promega GenePrint® PowerPlex™ 1.1 loci was then attempted upon all dilutions of the latent bloodstains for both collection mediums. The results clearly indicate that it is now routinely possible to recover adequate amounts of DNA suitable for PCR typing upon post luminol treated bloodstains.

KEYWORDS: forensic science, luminol, DNA typing, polymerase chain reaction, short tandem repeat, bloodstains, D16S539, D7S820, D13S317, D5S818, CFS1PO, TPOX, TH01, vWA

In all criminal investigations, the detection, collection and subsequent analysis of physical evidence recovered from the crime scene is critical in the identification of the responsible perpetrator. A complete and thorough analysis of the evidence may support or refute witness statements while aiding in the reconstruction of the events surrounding the crime. The limiting factor for biological analyses in the past has been the quantity and quality of the latent bloodstain.

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The ability of the forensic scientist to detect latent bloodstains has increased significantly with the introduction of the luminol (3-aminophthalhydrazide) reagent (1–4). Luminol, a presumptive test for the presence of blood, reacts with the heme component of blood producing free nitrogen and energy in the form of light through oxidation (5–9). Luminol has been found to react optimally with dried blood compared to liquid blood. This is due to an artifact of the drying process where the red blood cell breaks open exposing the heme group of the hemoglobin molecule, resulting in a more oxidized bloodstain (5–7,9,10). The dried blood consistently produces a more intense chemiluminescence lasting for a longer period of time, and as a result, is perfectly suited for crime scene applications.

The effect that luminol has on classic serological markers has already been established, with conventional testing of luminol treated bloodstains proving insufficient due to the adverse effect of the luminol reagent (6–12). Can the luminol reagent adversely interfere with the amount of DNA required in newer more sensitive techniques? The effect of luminol and other presumptive tests for blood on RFLP analysis was investigated with results indicating that the luminol reagent had no effect on the extraction of high molecular weight DNA from evidentiary samples (13). Again the limiting factor at a crime scene is the quantity of the latent bloodstain. However, if blood is not visible to the naked eye yet is evident with luminol analysis, the quantity of blood most likely is insufficient for RFLP based analyses.

With the advances in recent years of PCR based technology centered on the detection of short tandem repeat (STR) regions within the DNA molecule, the possibility of obtaining DNA typing results from post luminol treated bloodstains has increased. Additionally, advances in collection mediums for high molecular weight DNA have increased substantially over the past several years. The Alabama Department of Forensic Sciences has had great success implementing the Puritan™ sponge tip swab (Hardwood Products, Guilford, ME) for the collection of buccal samples for inclusion within the statewide DNA Databank (14). The success of the Puritan™ swab in the DNA Databank led us to inquire if this medium is better suited than the conventional cotton tip swab for casework applications such as the collection of post luminol treated bloodstains.

Materials and Methods

Sample Source and Preparation

The blood samples utilized were obtained from laboratory personnel. Dilutions of liquid blood were prepared in water ranging from 80 to 5% blood content to accurately represent latent blood-

stains that may be encountered in a crime scene setting. The dilutions were then placed upon a clean substrate of 2 × 2 ft vinyl flooring through a transfer stain mechanism, and allowed to air dry. The resulting dried bloodstains accurately represent what may be encountered in the field, with several of the dilutions producing dried stains not readily visible to the naked eye.

The latent bloodstains were allowed to dry for a minimum time period of 24 h and were subsequently treated with the luminol reagent [0.1g luminol, 0.7 g sodium perborate, and 5.0 g sodium carbonate per 100 mL]. Areas exhibiting greatest luminescence were swabbed and allowed to air dry. The conventional cotton tipped swab as well as the Puritan™ sponge tipped swab were evaluated in an attempt to determine if any advantage is gained in the recovery of typable DNA by employing one medium over the other.

DNA Extraction and Quantitation

DNA extraction was performed utilizing the organic extraction method in accordance with Alabama Department of Forensic Sciences' standard operating procedures. For every dilution of latent bloodstain studied, the entire tip of two cotton swabs was removed from the wooden applicators and placed within the extraction tube. For samples recovered with the Puritan™ sponge tipped swab, half of the sponge material was cut away from its plastic applicator and used in the extraction process. Due to the discrepancy in surface area between the sponge tipped swab and cotton tipped applicator, only one half of the sponge tipped swab was used in the extraction process to accurately compare these two mediums. All DNA samples were subsequently quantified using the QuantiBlot Human DNA Quantitation Kit with Chemiluminescent detection (Perkin Elmer Applied BioSystems, Foster City, CA).

DNA Amplification and Development

PCR amplification of template DNA was performed using the Perkin Elmer GeneAmp 9600 system (Perkin Elmer, Foster City, CA). Approximately 1 ng of template DNA was amplified for all samples, with all amplification reactions carried out in accordance with manufacturers guidelines and specifications.

Evaluation of amplified DNA was performed using 4% agarose horizontal gel electrophoresis run at 120 V for 45 min with the post-amplified DNA visualized with ethidium bromide staining and a UV light box.

Samples were denatured at 95°C for at least 2 min prior to loading upon the analytical gel. Analytical gel electrophoresis of each DNA sample was performed using 6% PAGE upon 32 cm plates, run at 40 W for 1.5 to 2 h. All samples and allelic ladders were run simultaneously with CXR internal lane standard. The visualization of alleles for the PowerPlex™ STR loci was accomplished by scanning the analytical gel upon the Hitachi FMBIO II (Hitachi, San Francisco, CA), which employs two color fluorescent detection. Allelic determinations were made both visually by two independent forensic scientists and by employing the Hitachi STaRCall software in accordance with departmental standard operating procedures.

Results and Discussion

The focus of this study was to determine if reliable DNA profiles could be obtained from post luminol treated bloodstains. Two recovery mediums were evaluated for a variety of latent bloodstains ranging in blood content from 5–80%. This range of dilutions was chosen to reflect possible dilutions that are encountered in crime scene settings. Both the conventional cotton tipped swabs and the Puritan™ sponge tipped swab demonstrated an ability to adequately recover a sufficient amount of DNA for PCR typing at the PowerPlex™ 1.1 STR loci.

Recovery and Quantitation

The amount of DNA recovered from the latent bloodstains was directly related to the dilution of the bloodstain and collection medium employed. The QuantiBlot film illustrating the amount of DNA recovered for each dilution and collection medium is given in Fig. 1. The conventional cotton tipped swab recovered DNA rang-

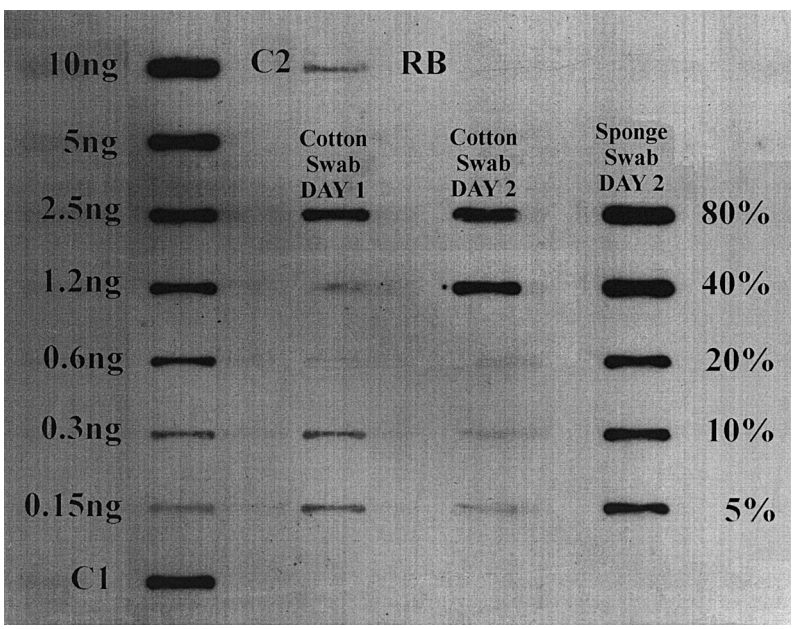

FIG. 1—*QuantiBlot film illustrating DNA recovery from the different collection mediums tested. Quantification standards ranging from 0.15–10 ng are shown in the first column followed by two calibrators (C1 and C2). Results for each collection medium at all of the dilutions of latent bloodstains tested (80–5% blood content) are illustrated in the final 3 columns.*

ing from 0–0.50 ng/μL for 10 total samples within two trials (2 samples/dilution) with an average yield of 0.16 ng/μL. The Puritan™ sponge tipped swab yielded between 0.25–2.00 ng/μL of DNA for the 5 dilution samples tested, with an average yield of 1.3 ng/μL. The Puritan™ sponge tipped swab proved to be a more consistent collection medium with DNA being successfully recovered for all blood dilutions (5 to 80%). Conversely, the cotton tipped swabs yielded inconsistent DNA quantities from the varied blood dilutions.

In terms of the amount of DNA isolated, the Puritan™ sponge again proved to be a more reliable collection medium. The sponge tipped swab consistently yielded more DNA than the cotton tipped swabs for all dilutions tested. This is most likely due to the larger surface area of the sponge and its increased absorbency over the conventional cotton swabs. Additionally, the possibility of DNA eluting off of the sponge material more efficiently than the cotton swab may also contribute to the discrepancy in the amount of DNA recovered. The consistency of the sponge tipped swab in recovering DNA for all dilutions is definitely a major asset, whereas the cotton tipped swab proved inconsistent, recovering little or no DNA.

Based upon the quantitation results, it is estimated that PowerPlex™ 1.1 typing results may potentially be obtained from dilutions containing as little as 1% blood content using the Puritan™ sponge tipped swab. The only minor drawback to employing the

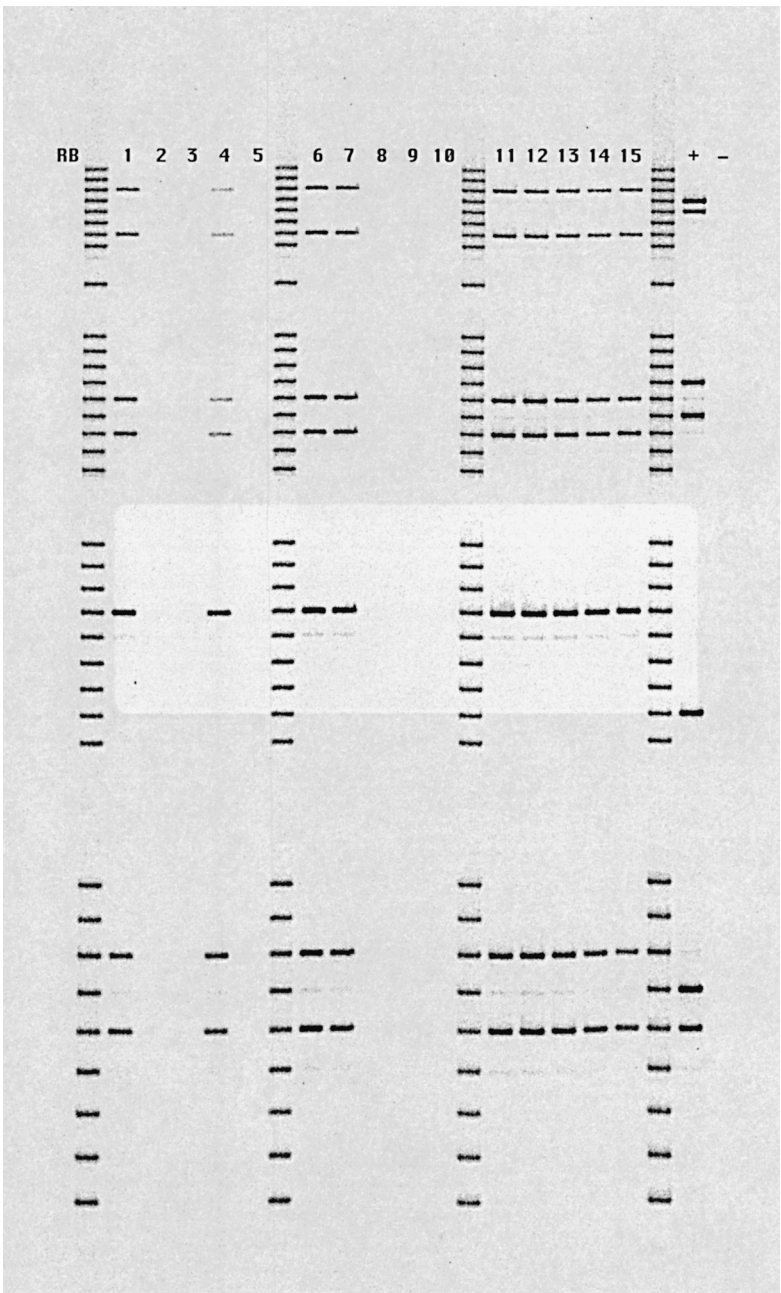

FIG. 2—*Vertical polyacrylamide gel displaying 505 nm scan for the following loci: D16S539, D7S820, D13S317, and D5S818. Lanes 1–5 and 6–10 represent DNA typing results obtained from the cotton swab collection medium for all dilutions tested. (Lanes 1 and 6 = 80%, Lanes 2 and 7 = 40%, Lanes 3 and 8 = 20%, Lanes 4 and 9 = 10%, and Lanes 5 and 10 = 5%). Lanes 11–15 represent DNA typing results obtained from the Puritan™ sponge collection medium. (Lane 11 = 80%, Lane 12 = 40%, Lane 13 = 20%, Lane 14 = 10%, and Lane 15 = 5%).*

sponge tipped swabs as the collection medium is the amount of time required for the sponge to dry. It is important for the sample to be dry prior to packaging because the presence of moisture can lead to DNA degradation through hydrolysis or bacterial processes. While the cotton swabs tend to dry fairly quickly, the sponge tipped swabs do require a substantially longer drying time, as long as a few hours.

PowerPlex™ 1.1 Results

Amplification of the PowerPlex™ 1.1 System was attempted employing 1 ng of template DNA. The maximum amount of sample (19.6 μL) was used in the amplification reaction by eliminating nuclease free water from the reaction mix in cases where 1 ng of template DNA could not be obtained. The volume of sample placed upon the analytical gel ranged from 0.60 to 1.50 μL, as determined after post-amp evaluation. If no amplification was detected on the post-amp gel, a sample volume of 1.5 μL was placed on the analytical gel to confirm the absence of amplified DNA. The DNA typing results for all dilutions of post luminol treated bloodstains are presented in Figs. 2 and 3. The genotype results were consistent for both collection mediums across all dilutions. Furthermore, the genotypes were consistent with those obtained from a blood standard without luminol treatment (data not shown).

It is interesting to note that the apparent cutoff for obtaining PowerPlex™ results is around 0.06 ng/μL of isolated DNA. No

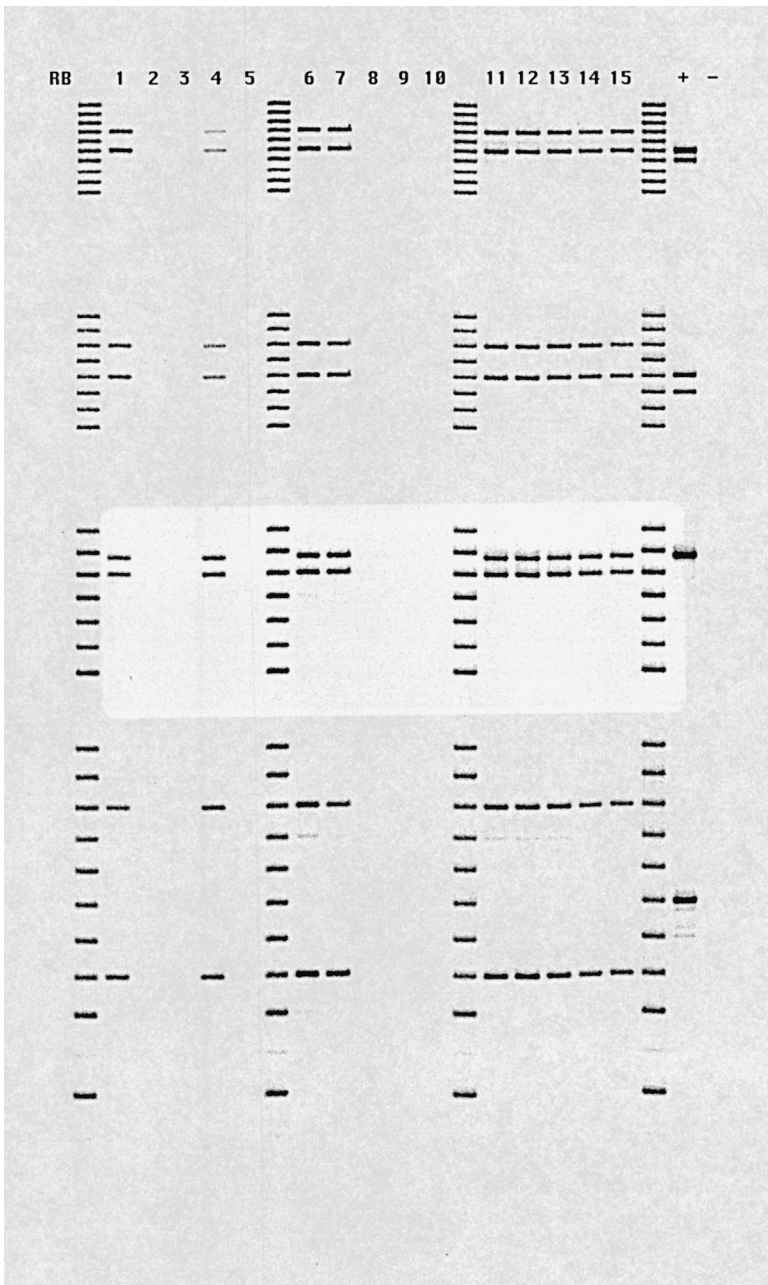

FIG. 3—*Vertical polyacrylamide gel displaying 585 nm scan for the following loci: CSF1PO, TPOX, THO1, vWA. Lanes 1–5 and 6–10 represent DNA typing results obtained from the cotton swab collection medium for all dilutions tested. (Lanes 1 and 6 = 80%, Lanes 2 and 7 = 40%, Lanes 3 and 8 = 20%, Lanes 4 and 9 = 10%, and Lanes 5 and 10 = 5%). Lanes 11–15 represent DNA typing results obtained from the Puritan™ sponge collection medium. (Lane 11 = 80%, Lane 12 = 40%, Lane 13 = 20%, Lane 14 = 10%, and Lane 15 = 5%).*

PowerPlex™ results were obtained when the amount of isolated DNA from the cotton swab was less than 0.06 ng/μL. The Puritan™ sponge tip swab consistently produced DNA results for all dilutions tested due to the greater amount of DNA that is recovered when employing this collection medium.

Conclusion

This project has clearly demonstrated that consistent DNA typing results can be obtained from post luminol treated latent bloodstains using the Promega GenePrint® PowerPlex™ 1.1 System. Employing the conventional cotton tip swab as the collection medium produced inconsistent results for the dilutions tested, with no DNA typing results obtained when the quantity of extracted DNA was less than 0.06 ng/μL. In contrast, the Puritan™ sponge always yielded a sufficient amount of DNA for amplification in all dilutions tested. DNA typing results were successfully obtained at all of the PowerPlex™ 1.1 loci for all dilutions when employing the Puritan™ sponge tip swab.

In conclusion, the luminol reaction continues to be a valuable presumptive test for the presence of latent bloodstains. Concerns regarding obtaining complete genetic profiles from post luminol treated bloodstains are not warranted with PCR based STR techniques. Consistent DNA typing results were obtained for all dilutions of post luminol treated bloodstains when the Puritan™ sponge tipped swab was used as the collection medium. Clearly, it is now possible to obtain consistent and reliable DNA typing results from post luminol treated bloodstains.

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