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TECHNICAL NOTE

CRIMINALISTICS

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The Use of Hemastix® Severely Reduces DNA Recovery Using the BioRobot® EZ1

ABSTRACT: The choice of reagents for presumptive tests for blood, and subsequent extraction methodologies, can significantly affect both the quantity and quality of purified DNA. Blood samples directly tested with Hemastix® yielded <1% of the DNA recovered from untested samples when purified using the Qiagen BioRobot® EZ1 and EZ1® DNA Investigator kit. Full short tandem repeat profiles were obtained from both tested and untested samples, suggesting that the Hemastix® reagent(s) affect DNA binding, rather than produce DNA damage. The Hemastix® inhibition of DNA yield could be overcome by the addition of MTL buffer to the sample prior to extraction. Laboratories may wish to modify current procedures for extracting blood samples, utilize other extraction/purification methodologies, or inform their submitting agencies to avoid direct exposure of questioned bloodstains to Hemastix® reagents.

KEYWORDS: forensic science, blood test, DNA typing, DNA extraction, Hemastix®, phenolphthalein, polymerase chain reaction, short tandem repeats, MTL, D3S1358, D5S818, vWA, D13S317, FGA, D7S820, Amelogenin, D16S539, D8S1179, TH01, D21S11, TPOX, D18S51, CSF1PO, D2S1338, D19S433, capillary electrophoresis

One of the key tasks for a forensic DNA analyst is to extract DNA from biological stains for subsequent short tandem repeat (STR) analysis. Suspected bloodstains are routinely screened for the presence of blood using a catalytic test such as phenolphthalein (1) or other reagents, which do not affect the quantity of extractable, high molecular weight DNA (2–4). Fingerprint enhancement chemicals and techniques have also been evaluated with regard to minimizing effects on downstream STR analysis (5). Increasingly, premanufactured one-step tests such as the Hemastix®-testing strips (Siemens Healthcare Diagnostics, Inc., Washington, DC) are being utilized (4), as they afford increased sensitivity with minimal sample consumption.

Optimizing the sample preparation method is critical to successful STR analysis. Because biological stains are often of limited size and quantity, an analyst is frequently tasked with attaining the maximum DNA yield in a single extraction. However, the use of Hemastix®-testing strips has recently been shown to decrease DNA recovery when using DNA IQ™ magnetic bead technology (6). In contrast, no deleterious DNA testing effect was observed when using Hemastix® in conjunction with Chelex® method of DNA purification (4) or phenol–chloroform extractions (6). We report here the examination of Hemastix® effects upon DNA yield using the Qiagen EZ1® DNA Investigator kit (Qiagen, Venlo, Netherlands), an affinity-based DNA extraction system.

Materials and Methods

Samples

Whole blood was diluted in series using sterile deionized water. Diluted samples (15 μL) were spotted in duplicate onto the tips of sterile cotton swabs to create paired samples with eight dilutions ranging from 1:2 to 1:4374. Samples were dried prior to further testing.

Hemastix® Test

Hemastix®-testing strips were used as a screening test for blood. The reagent pads of the Hemastix® strips were lightly moistened with deionized water. One of each swab dilution pair was pressed directly onto the Hemastix®. Contact was maintained for 60 sec per manufacturer's directions. The other paired swab was left untested.

DNA Extraction

Swab heads were removed and incubated for 30 min at 56°C in 500 μL of lysis buffer (0.01 M Tris, pH 8, 0.01 M EDTA, 0.1 M NaCl, and 2% SDS) with 0.75 mg of Proteinase K. Cotton material was removed from the lysate using a spin basket (5 min/13 K) prior to purification using the Qiagen Biorobot® EZ1 and EZ1® DNA Investigator kit. MTL buffer was supplied by Qiagen (p/n 1020430). Final DNA elution volume was selected as 50 μL TE (10 mM Tris, pH 8, 0.1 mM EDTA, and 0.04% NaN_3).

DNA Quantification

DNA quantification was performed using the Quantifiler® Duo DNA quantitation assay (Applied Biosystems, Foster City, CA) on an ABI Prism® 7500 Sequence Detection System (Applied Biosystems). A 1 μL aliquot of extracted DNA or reference standard DNA was added to 11 μL of reaction mix (containing AmpliTaq® Gold polymerase [Applied Biosystems] and primer/probe solution). Amplification was carried out for 40 cycles per manufacturer's direction. Reference standards included the range of 50 to 0.0232 ng/μL.

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DNA Concentration

After quantification, DNA samples estimated to be <0.15 ng/μL were concentrated to approximately 10 μL using Ultracel YM-100 microconcentrators (Millipore, Billerica, MA).

PCR Amplification

DNA amplification was carried out using 1.5 ng of template DNA (where possible) and the AmpFlSTR® Identifiler® PCR Amplification kit (Applied Biosystems) in a 9700 thermocycler. Samples were amplified in a final volume of ~25 μL (10 μL reaction mix, 5 μL Primer set, 0.5 μL AmpliTaq® Gold DNA polymerase, and 10 μL of template DNA) following a hot start at 95°C/11 min, 28 cycles of denaturation at 94°C/60 sec, annealing at 59°C/90 sec, and extension at 72°C/90 sec, with a final nontemplate extension step at 60°C/45 min.

Capillary Electrophoresis

Electrophoresis of the amplified DNA fragments was performed using an ABI Prism® 3130xL Genetic Analyzer using the ABI Prism® 3130 Data Collection Software (version 3.0). Amplicons (1.5 μL) were denatured in 25 μL of Hi-Di Formamide and 0.25 μL of Genescan™ 500 (LIZ®; Applied Biosystems). Electrokinetic injection was performed at 5 kv for 20 sec using POP-4™ (Applied Biosystems) as the polymer. Sample file information generated by the 3130xL Genetic Analyzer was analyzed using GeneMapper® ID Software (version 3.2.1; Applied Biosystems).

Results and Discussion

All Hemastix®-tested swabs produced a positive blood test result as evidenced by a blue-green color produced on both the Hemastix® strips and on the tested swabs. This transferred material appears to contain a component that inhibits DNA extraction, as the yield of DNA from the Hemastix®-tested bloodstained swabs was severely reduced compared to the yield of DNA from the untested bloodstained swabs (Table 1). Even the most concentrated blood sample (whole blood) was affected by the Hemastix® test, yielding <1% of the DNA recovered from an untested swab. The Hemastix® reagent(s) completely impeded DNA recovery at a 1:18 dilution of whole blood, whereas untested swabs yielded measureable DNA up to a 1:486 dilution.

TABLE 1—*Quantitation results of Hemastix®-tested and untested blood samples.*

Blood dilution	Hemastix® Exposure	ng DNA*	% Yield
Whole	–	286.5	0.3
	+	0.75	
1:2	–	81	0.7
	+	0.55	
1:6	–	20	5.4
	+	1.1	
1:18	–	13.4	0
	+	0	
1:54	–	19.6	0
	+	0	
1:162	–	3.5	0
	+	0	
1:486	–	2.05	0
	+	0	
1:1458	–	0	–
	+	0	
1:4374	–	0	–
	+	0	

*Values reflect Quant Duo Human valuation. Zero values were ''undetermined'' value from Quant Duo.

– Denotes untested blood sample.

+ Denotes Hemastix®-tested blood sample.

TABLE 2—*STR profiling results.*

	STR Profile	
Blood Dilution	Untested	Hemastix® Tested
Whole	Full	Full
1:2	Full	Full
1:6	Full	Full
1:18	Full	Full
1:54	Full	Partial (21/25)
1:162	Full	Partial (4/25)
1:486	Full	Partial (5/25)
1:1458	Full	None
1:4374	Partial (8/25)	None

Despite the lower DNA yields, full STR profiles (25 alleles detected) were obtained for the Hemastix®-tested swabs, yet only at slight dilutions when compared to the full STR profiles obtained for the untested swabs (Table 2).

The successful quantification of Hemastix®-tested DNA samples, combined with the successful amplification of the samples, supports an earlier report (6) indicating that Hemastix® affects DNA binding to the paramagnetic beads. We found that adding MTL buffer (Qiagen) to the sample prior to robotic extraction could completely restore the DNA yields, and that full STR profiles could be obtained (data not shown). The composition of the MTL buffer is proprietary; however, the ability of the MTL buffer to reverse the inhibitory effect upon DNA yield further suggests that the Hemastix® reagents do not degrade the DNA, but rather affect DNA binding to the paramagnetic beads.

An indirect Hemastix®-testing approach is therefore recommended for laboratories employing the Qiagen BioRobot® EZ1 and EZ1® DNA Investigator kit. The use of an intermediate swab or filter paper to test possible blood stains will prevent the blood stain from being exposed to the inhibitory Hemastix® reactant(s). Alternatively, such laboratories might use an alternative extraction method or utilize the MTL buffer for Hemastix®-tested bloodstains. In addition to laboratory procedural changes, BioRobot® users may wish to caution their external evidence submitting agencies to avoid direct contact of the Hemastix® to the sample in order to prevent deleterious loss of otherwise recoverable DNA material.

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References

1. Cox M. A study of the sensitivity and specificity of four presumptive tests for blood. J Forensic Sci 1991;36(5):1503–11.
2. Hochmeister MN, Budowle B, Baechtel FS. Effects of presumptive test reagents on the ability to obtain restriction fragment length polymorphism (RFLP) patterns from blood and semen stains. J Forensic Sci 1991;36(3):656–61.
3. Gross AM, Harris KA, Kaldun GL. The effect of luminol on presumptive tests and DNA analysis using the polymerase chain reaction. J Forensic Sci 1999;44(4):837–40.

4. Tobe SS, Watson N, Daeid NN. Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular—weight DNA. J Forensic Sci 2007;52(1):102–9.
5. Fregeau CJ, Germain O, Fourney RM. Fingerprint enhancement revisited and the effects of blood enhancement chemicals on subsequent Profiler Plus fluorescent short tandem repeat DNA analysis of fresh and aged bloody fingerprints. J Forensic Sci 2000;45(2):354–80.
6. Poon H, Elliott J, Modler J, Fregeau C. The use of Hemastix® and the subsequent lack of DNA recovery using the Promega DNA IQ system. J Forensic Sci 2009;54(6):1278–86.

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