TECHNICAL NOTE

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Threat Mail and Forensic Science: DNA Profiling from Items of Evidence After Treatment with DFO

REFERENCE: Zamir A, Oz C, Geller B. Threat mail and forensic sciences: DNA profiling from items of evidence after treatment with DFO. J Forensic Sci 2000;45(2):445–446.

ABSTRACT: Two cases of threatening letters with their accompanying envelopes were received to the Division of Forensic Identification unit of the Israel Police. The envelopes, including the stamps, and the letters were initially examined for latent fingerprints by the DFO reagent, known to cause degradation of DNA. Although no latent fingerprints could be visualized on any of the items, the biology laboratory using organic DNA extraction, was successful in defining genetic profiles from all the items employing six STR loci, even after treatment with DFO.

In a controlled experiment, a known donor attached a stamp, by licking, to an envelope. This item was treated with DFO and then profiled using STR loci. The results showed that previous DFO treatment on the control stamp before DNA analysis had no negative effects on obtaining the DNA profile of the known donor using STR loci.

KEYWORDS: forensic science, threat mail, latent fingerprints, DFO, DNA profiling, polymerase chain reaction, STR

Of all categories of physical evidence discovered from crime scenes, fingerprints and biological material providing DNA profiles are the most incriminating. Modern methods of fingerprint detection and comparison, as well as the advances in DNA-profiling techniques allow for a relatively fast and reliable identification of the suspect. Problems can arise when the same item of evidence must be examined by both forensic laboratories employing different methods. When biological material is removed for DNA testing, latent fingerprints can be damaged. Also, some chemical reagents used in latent fingerprint detection (personal communication, Andrson J) can interfere with further DNA profiling procedures (1). One such reagent is DFO (1,8-diaza-9-fluorenone), known for its sensitivity to amino acids and used widely in fluorogenic detection of latent fingerprints from dry porous surfaces (2,3).

This report presents two cases where successful DNA results for six STR loci, after phenol/chloroform extraction were achieved from threat mail items after treatment with DFO for latent fingerprint detection.

Case Histories

Two cases of threatening letters were received by Israeli VIPs and subsequently turned over to the Division of Identification and Forensic Science of the Israel Police for forensic analysis. The letters and their accompanying envelopes were initially examined for latent fingerprints. Before completing their examination, portions of the envelope flap and the area containing the stamps were transferred to the biology laboratory. Biological material found on these items could possibly provide a genetic profile of the sender.

Following is a description of the work done by the two forensic laboratories and their results. The forensic biology laboratory was able to define biological characteristics from all the items. Neither the envelopes nor the letters provided any fingerprints by the DFO method. There are still no suspects in these cases.

The Latent Fingerprints Laboratory

Preliminary non-destructive tests of observation with white and green light, using PolilightTM, showed no observable fingerprints from any of the items. These items were then immersed for 2 min in a DFO solution (0.025% DFO in ethanol containing 2% acetic acid) and then heated at 80°C in a chamber for 30 min. No finger-prints were developed by this process.

Before continuing with ninhydrin, a portion of one of the envelope flaps and other portions of the envelopes with the stamps still attached were removed and transferred to the biology laboratory. The remainder of the envelopes and the letters were then treated by ninhydrin. This treatment also failed to provide latent fingerprints from any of the items.

The Forensic Biology Laboratory

In order to determine that previous treatment with DFO does not interfere with DNA profiling, and/or alter a DNA profile of a known donor using STR's, a controlled experiment was carried out. A stamp was licked once and attached to an envelope by a known donor. After a period of seven days the envelope was treated by DFO in the latent fingerprints laboratory by the procedure reported earlier. DNA profiling was carried out using the following procedure. DNA was extracted from the envelope portion containing the stamp using the phenol-chloroform extraction method (4). The extracted DNA was amplified using the PCR method for the following short tandem repeat (STR) markers: CSF1PO, TPOX, THO1, F13A, FESFPS, VWA, using the CTT and FFV kits from Promega (5). The products of these amplifica-

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Received March 1999; and in revised form 1 June 1999; accepted 2 June 1999.

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TABLE 1—Geneti	c profile obtained	from items o	f evidence after	treatment with DFO.

	CSF	TPOX	THO1	F13A	FESFPS	VWA
Control envelope with stamp	10,12	8,8	9,9	3.2,5	10,11	17,18
Known control donor	10,12	8,8	9,9	3.2,5	10,11	17,18
Envelope with stamp— Case 1	10,10	8,11	6,9	7,7	10,12	14,17
Envelope with stamp— Case 2	11,12,13	8,9,11	6,7,9.3	5,6,7	_	16,17,18,21
Envelope flap Case 2	9,11,13	8,11	6,7,9.3	5,6	10,11,12	16,21

tions were run on polyacrylamide gels and visualized using silver staining (5). The profile achieved was compared to the known profile of the donor and observed to be identical. Once it had been established from the control experiment that previous DFO treatment does not affect DNA profiling using STR loci, the same procedure was employed on the items of evidence from the two cases of threat mail.

It was thought that perhaps the senders of the letters had sealed the envelopes and attached the stamps by licking them and thereby left behind their biological identification in the form of DNA. Because it was considered unimportant whether the source of any biological material found on the items originated from saliva or epithelial cells, no preliminary testing was undertaken to determine the presence of saliva in order not to use up small amounts of DNA that might have been deposited on these items.

Table 1 shows the results of the DNA products amplified by PCR for six STR loci, after phenol-chloroform extraction from the control experiment and the two actual cases.

Discussion

The control experiment demonstrated that previous DFO treatment of items of evidence had no negative or altering effects on subsequent DNA profiling using STR loci after phenol-chloroform extraction.

Table 1 represents our results from the control experiment, in addition to the STR results from two actual cases of threat mail. As can be seen from Table 1, the item from Case 1 provided a DNA profile for all six STR loci. Regarding the items from Case 2, a DNA profile was obtained from five of the six loci examined regarding the portion of the envelope containing the stamp, and all six loci in relation to the envelope flap. Most of the markers provided a multiple profile, indicating a mixture of more than one person's genetic material.

This mixture could possibly be explained by the involvement of more than one person or reuse of the envelope. A possible profile could arise from the licking of the stamp and the envelope flap, and/or from epithelial cells left behind from general handling of the items. Two quantities of DNA from all items were amplified and typed and the same profile was noted for both quantities. This was done in an effort to avoid allele drop out. We also cannot disregard the possibility that a partial profile may occur as a result of DNA degradation. In any case, the allele VWA 21 is exceptionally rare in the Israeli population (0.00%), and accordingly regarding the American population (6). The definition of VWA 21 on the two items will greatly assist in linking at least one future suspect to these items. It must also be stated that the multiple profile defined is not a result of contamination by either of the forensic examiners of these items. This is based on a comparison of the known profiles of both examiners.

It is known that PCR DNA typing of both stamps and envelopes is possible, (6,7) and that DFO can be a destructive reagent in DNA profiling (1). We were unable to locate any references regarding STR analysis after DFO treatment in threat mail. In conclusion, we present these cases to illustrate that items of evidence which have previously been treated for latent fingerprints by DFO, can still be successfully typed by conventional DNA methods to obtain a DNA profile by STR analysis.

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