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The Effects of Specific Latent Fingerprint and Questioned Document Examinations on the Amplification and Typing of the HLA DQ alpha Gene Region in Forensic Casework

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ABSTRACT: The apparent stability of DNA in forensic samples has permitted the successful application of several techniques such as polymerase chain reaction (PCR)-based and restriction fragment length polymorphisms (RFLP) analysis to forensic cases. PCR-based typing of the HLA-DQ alpha region in forensic casework has been shown to be a valid and reliable technique. This inherent stability of DNA in forensic evidence has led us to address the question of whether DNA could successfully withstand certain evidence processes such as latent fingerprint and electrostatic detection apparatus (ESDA) processing and still yield a sufficient quantity and quality of DNA for PCR HLA DQ alpha typing. Through testing done with biological material on simulated and casework envelope, stamp, and cigarette butt type evidence, it was determined that samples could be processed for specific latent fingerprint and ESDA examinations and still yield sufficient DNA for conclusive HLA DQ alpha typing results.

KEYWORDS: questioned documents, pathology and biology, polymerase chain reaction (PCR), HLA DQ alpha typing, electrostatic detection apparatus (ESDA)

Envelope and stamp evidence, which is commonly encountered in mail bomb, extortion, and kidnapping cases, could provide useful forensic information in certain criminal investigations. These types of evidence samples may require special handling due to the nature of the material and the potential forensic significance. Questioned document examinations, latent fingerprint examinations, and DNA analysis of epithelial cells in saliva are all potential approaches for the examination of these types of evidence. However, it is necessary to determine the order in which these examinations should be performed, as well as whether or not they can all be performed. The forensic application of polymerase chain reaction (PCR)-based typing of the HLA DQ alpha region of evidence samples has been shown to produce valid and reliable results [*1–7*]. The overall

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research objective of this study was to determine if HLA DQ alpha analysis by PCR could be successfully performed on DNA extracted from saliva epithelial cells on envelope flap, stamp, and cigarette butt samples after the samples had been subjected to specific questioned document and latent fingerprint examinations.

In criminal cases such as extortion, one of the initial examinations done by questioned document examiners is the use of the ESDA method to visualize indented writing on envelopes or other specific surfaces using electrostatic detection [*8–10*]. ESDA has also been used for visualizing faint footwear impressions and latent fingerprint impressions. The technique requires that the evidence be placed between a brass plate and a piece of polymer film. An electrostatic charge is placed on the "sandwich" and a fine spray of powder or pellets coated with toner are used to visualize impressions. The ESDA technique has been shown to have no subsequent effect on latent fingerprint examinations [*8*]. One of the goals of this research was to determine if ESDA processing had any effect on the HLA DQ alpha typing of DNA.

Typically, after ESDA processing, envelopes and related types of evidence are sent to latent fingerprint examiners for processing. Envelopes and stamps are routinely processed by the FBI Latent Fingerprint Section using three separate processes: (1) 1,8-Diazafluoren-9-one (DFO), (2) ninhydrin and (3) physical developer [*11–15*]. Another goal of this study was to determine if any or all of these processes and the chemicals used in these processes would affect DQ alpha typing results.

The processes cited above present a variety of factors, such as, heat, steam, electrostatic forces and chemicals which could effect the DNA in evidence samples. The determination of any effects of the specific processes outlined above will aid the forensic scientist in deciding if and when the HLA DQ alpha typing technique of DNA may be utilized.

Material and Methods

Three separate latent fingerprinting processes: (1) DFO, (2) ninhydrin and (3) physical developer were considered in this study. DFO is a fluorescent chemical process used to visualize latent fingerprints. The chemical solvents generally used in this process are acetic acid, ethyl acetate, methanol, 2-propanol, acetone, xylene and petroleum ether [*11–13*].

Ninhydrin processing, which is designed to react with amino acids and other compounds found in human finger secretions in latent fingerprints, uses a variety of chemical solvents including acetone, methanol, ethanol, ethyl ether, ethylene glycol, petroleum ether, naphtha, and Freon 113 [*16–20*]. In the FBI Laboratory Latent Fingerprint Section ninhydrin is used in combination with acetone or petroleum ether mixed with methanol and 2-propanol. These FBI Laboratory ninhydrin preparations were used to process the envelopes, stamps and cigarette butts in the present study. Heat and steam were also used to enhance the development of ninhydrin processing. This heat and steam (approximately 100°C) is generally applied to the surface treated with the ninhydrin using a household iron, although an oven set at 100°C could also be used. If a household iron is used, direct contact with the evidence sample is avoided. Samples processed by the DFO and ninhydrin methods both with and without applied heat and steam were analyzed in this study.

Physical developer is a chemical process used to develop latent fingerprints on porous surfaces such as paper. This process is generally performed after the DFO and ninhydrin processing and is done in lieu of the conventional silver nitrate method [*15*]. The chemical solutions used in the physical developer process include: (1) detergent solution consisting of N-dodecylamine acetate and Synperionic-N, (2) silver nitrate solution, (3) redox solution consisting of ferric nitrate, ferrous ammonium sulfate and citric acid and (4) maleic acid solution.

The simulated forensic samples were prepared by FBI Laboratory staff from six different individuals who had different known HLA DQ alpha genotypes. For the simulated evidence studies, a total of 24 envelopes, 18 stamps, and 6 cigarette butts were tested. For the envelopes, approximately one-half of each envelope flap was used for extraction. The envelopes were white standard sized mailing envelopes and, when necessary, steam was used to assist in removing an affixed stamp or unsealing the envelope flap. Three 50 μl aliquots of distilled water were pipetted up and down on the flap and the fluids were combined in a microcentrifuge tube. One to three tubes were collected for each flap sample. For the stamps tested, three 50 μl aliquots of distilled water were also used for pipetting up and down on the adhesive surface of the stamp and these fluids were combined in a microcentrifuge tube. For DNA extraction, an approximately 5% solution w/v in sterile water of Chelex 100™ resin (100 to 200 mesh, sodium form, BioRad 143-2832, Richmond, CA) was then added to the combined fluids from the envelopes and the stamps. The Chelex extracts were then processed using Centricon (Amicon, Danvers, Mass.) concentration [*21*]. For the cigarette butts (Marlboro light 100's-white filter), a 5-mm-wide ring of the paper at the filter end was removed, cut into several smaller pieces, and placed in a microcentrifuge tube. The samples were then extracted using Chelex [*21–23*] with slight modifications [*2*]. The quantity of DNA in the samples was approximated by using a slot blot quantification method [*24*]. For each set of extractions performed, one sample of all test reagents without the extracted DNA was processed as a reagent blank control. The extracted DNA and control samples (positive and negative amplification controls and reagent blank controls) were amplified and typed using the AmpliType® HLA DQ alpha Forensic DNA Amplification and Typing kit (Perkin-Elmer Corp., Norwalk, Conn.) according to the manufacturer's instructions. If sufficient DNA was extracted from a sample, duplicate sets of amplifications were performed. Each set consisted of a serial dilution of the DNA (2 ng, 1 ng, 500 pg, 250 pg, and 125 pg). Bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, MO.) [4 μl of a 4 mg/ml solution in an amplification mixture of approximately 105 to 144 microliters] was added to the amplification mixture of one set of samples in order to determine if BSA could enhance the typing results by possibly counteracting potential inhibition of the amplification process.

Forensic casework samples tested were handled in a manner similar to the simulated forensic samples.

Results

Questioned Document Examinations-ESDA Effects on DNA DQ alpha Analysis

Two envelopes, each licked and sealed by the same individual of known HLA DQ alpha type, were subjected to the ESDA processing and DNA was extracted from the envelopes. The DNA extracts from the two envelopes were combined since the saliva samples were known to be from the same individual and the total yield of DNA from each envelope was less than 2.0 ngs. The combined extract was then equally divided and tested. The addition of BSA to one set of amplified samples produced slightly greater amplification product as determined by the post-amplification test gel; however, the resulting DQ alpha typing strips exhibited dots of approximately equal intensities. The expected DQ alpha types were detected for all the extracted DNA analyzed (Fig. 1).

Latent Fingerprint Examination-Latent Fingerprint Processing Effects

One of the determinations to be made was whether or not the latent fingerprint processes effected the extraction of sufficient quantities of DNA for typing from simulated

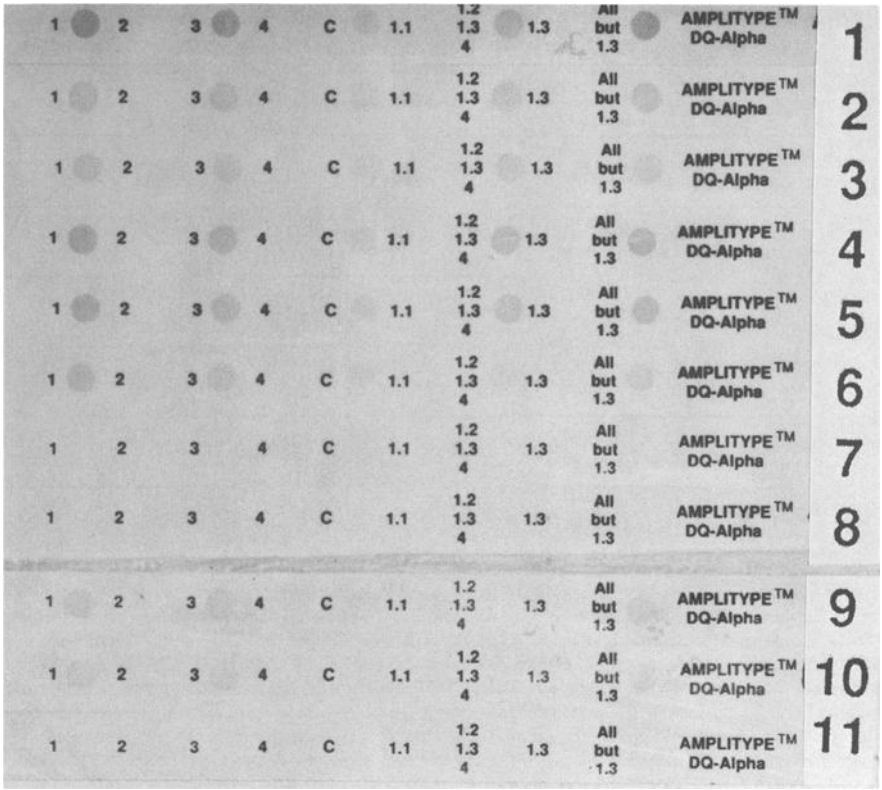

FIG. 1—*Rows 1 through 11 (top to bottom), Rows 1,2,3-Envelopes ESDA processed, approx. 500, 250, 125 pgs extracted DNA, respectively, without BSA; Rows 4,5,6-Envelopes ESDA processed, approx. 500, 250, 125 pgs extracted DNA, respectively, with BSA; Rows 7,8-Reagent blanks, without and with BSA respectively; Rows 9,10-positive genomic DNA control with BSA added, approx. 500 and 250 pgs DNA respectively; Row 11-negative amplification control.*

forensic samples subjected to latent fingerprint processing. Twelve envelopes, eighteen stamps and six cigarette butts from three different individuals who had different known HLA DQ alpha genotypes were subjected to three latent fingerprint processes as performed by the Latent Fingerprint Section of the FBI Laboratory: (1) DFO, (2) ninhydrin with acetone and ninhydrin with petroleum ether and (3) physical developer. Based on the slot blot results (Fig. 2), processing with ninhydrin with acetone or petroleum ether and DFO did not effect the yields of DNA which were consistent with samples not subjected to the latent fingerprint processes, thus indicating that latent fingerprint processing using these methods did not significantly decrease the amount of recoverable human DNA. No detectable DNA was recovered from specimens processed by the physical developer. This physical developer processing consists of numerous chemicals (see introduction) and is routinely done in a tray about an inch deep in liquid solution. Either the types of chemicals used have a degrading effect on the DNA or the DNA is diluted by the processing to the point that no DNA is recovered. Based on these studies, the physical developer processing was determined to have a disadvantageous effect on DNA extraction and consequently was not tested any further in this study.

The same series of extractions were performed on a second set of samples (6 envelopes, 3 stamps and 3 cigarette butts from three different individuals of known HLA DQ alpha

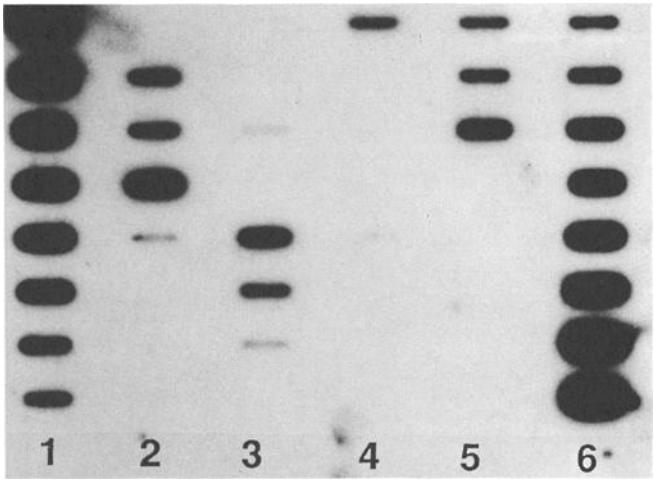

FIG. 2—*Columns 1 through 6 (left to right), Column 1-top to bottom = standards of genomic DNA top to bottom 40, 20, 10, 4, 2, 1, .4 and .2 ngs DNA; Column 2-top to bottom = no sample, unprocessed envelope, unprocessed envelope, unprocessed cigarette butt, unprocessed stamp, no sample, envelope processed by physical development, cigarette butt processed by physical development; Column 3-top to bottom = stamp processed by physical development, no sample, envelope processed by DFO, no sample, envelope processed by ninhydrin with petroleum ether, cigarette butt processed by ninhydrin with petroleum ether, stamp processed by ninhydrin with petroleum ether, no sample; Column 4-top to bottom = envelope processed by ninhydrin with acetone, no sample, reagent blank (no DNA), reagent blank (no DNA), reagent blank (no DNA), no sample, no sample, no sample, Column 5-top to bottom = unprocessed envelope, unprocessed envelope, unprocessed envelope; no sample, no sample, no sample, no sample, no sample, Column 6-top to bottom = standards of genomic DNA top to bottom .2, .4, 1, 2, 4, 10, 20 and 40 ngs DNA.*

type) which had been subjected to the same three latent fingerprint processes with similar results being obtained.

The amount of DNA extracted from the envelopes and stamps ranged from 1 to 4 nanograms. The results for the latent fingerprint processing for ninhydrin (with and without heat) and DFO and ninhydrin (with and without heat) treated envelopes showed the expected DQ alpha types consistent with the known control types of the three different individual donors (Fig. 3). Random samples of the reagents used by the Latent Fingerprint Section for DFO processing, ninhydrin processing with acetone, and ninhydrin processing with petroleum ether were collected and amplified (40 μl) and typed using the AmpliType® HLA DQ alpha kit. The results were negative, that is, the chemicals used for the latent fingerprint processing did not demonstrate the presence of any detectable DNA HLA DQ alpha type or typable contaminant.

For some of the DNA extracted from the envelopes and stamps, the addition of BSA to the amplification mixture enhanced dot blot detection (Fig. 4). Based in part on these results, BSA is routinely used by the FBI Laboratory in envelope, stamp and cigarette butt PCR HLA DQ alpha amplifications. BSA may potentially enhance a weak result, and there have been no apparent deleterious or artifactual effects attributable to BSA.

Casework Applications

As of November 1992, 28 envelopes, 6 stamps and 40 cigarette butts had been processed for HLA DQ alpha types by the FBI Laboratory. For the envelopes, 15 of 28 (54%) had

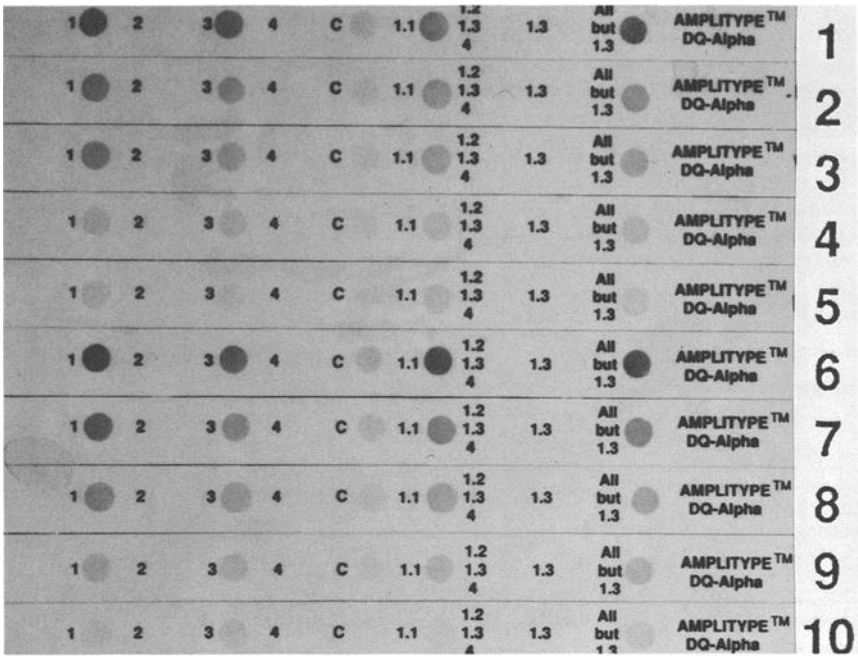

FIG. 3—*From top to bottom rows 1 through 10, Rows 1 through 5-Typing results from DNA extracted from DFO and ninhydrin without heat processed envelope using approximately 2 ngs, 1 ng, 500, 250 and 125 pgs respectively for amplification; Rows 6 through 10-Typing results from DNA extracted from the same DFO and ninhydrin without heat processed envelope using approximately 2 ngs, 1 ngs, 500, 250 and 125 pgs respectively for amplification with the addition of BSA. Reagent blank, positive and negative controls functioned correctly and were identical to the results as depicted in Figure 5, rows 7, 8 and 9, reagent blank, positive genomic and negative amplification controls respectively.*

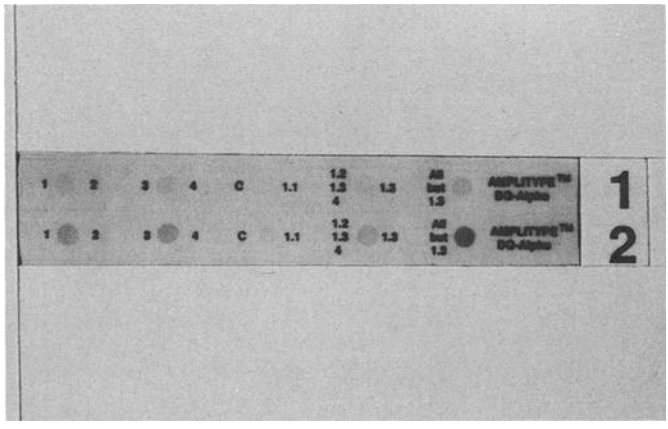

FIG. 4—*Row 1-HLA DQ alpha results from amplification without BSA of approximately 500 pgs. of DNA extracted from ninhydrin with heat processed envelope flap; Row 2-From a duplicate sample, HLA DQ alpha results from amplification with BSA. Reagent blank, positive and negative controls functioned correctly and were identical to the results as depicted in Figure 5, rows 7, 8 and 9, reagent blank, positive genomic and negative amplification controls respectively.*

sufficient DNA for HLA DQ alpha typing and the remaining 13 had no detectable DNA. Sufficient DNA quantities for HLA DQ alpha typing were generally found to be approximately 250 picograms and greater. The quantity of DNA generally extracted from the envelope flaps was in the range of approximately 1 to 2 nanograms. For the stamps, 1 of 6 (16%) had sufficient DNA for HLA DQ alpha typing with the remaining 5 having no detectable DNA. The quantity of DNA generally extracted from stamps in casework has been from 250 picograms to approximately 1 nanogram. It is noted that some envelope flaps and stamps encountered in casework may not have been licked and consequently there would be no nucleated cells with DNA present. For the cigarette butts, 20 of 40 (50%) had sufficient DNA for HLA DQ alpha typing. The remaining 20 cigarette butts had no detectable DNA or less than 250 picograms total of detectable DNA.

In routine casework, envelope, stamp, and cigarette butt evidence are generally processed first for latent fingerprints using DFO and ninhydrin. Envelopes in some cases will be processed by ESDA first and then processed for latent fingerprints. After ESDA, DFO and ninhydrin processing, DNA is extracted from the evidence and this DNA is subjected to HLA DQ alpha analysis. The actual evidence can then be resubmitted to the latent fingerprint examiner for physical developer processing, and thereby, the evidence can routinely be processed for latent fingerprints using all three separate fingerprint development processes: DFO, ninhydrin, and physical developer.

For example, in a death investigation case, an envelope and adhering stamp were processed for latent fingerprints using DFO and ninhydrin. They were then processed for DNA and typed using the AmpliType® HLA DQ alpha kit with the addition of BSA

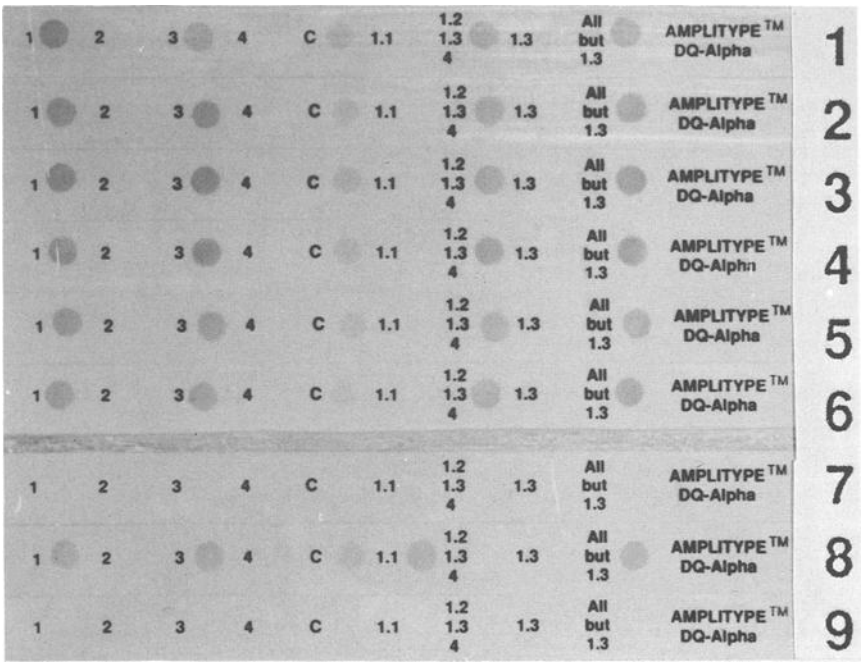

FIG. 5—*From top to bottom rows 1 through 9; Rows 1, 2-HLA DQ alpha results from DNA extracted from DFO and ninhydrin processed stamp, and duplicate; Rows 3, 4-HLA DQ alpha results from DNA extracted from DFO and ninhydrin processed envelope, and duplicate; Rows 5, 6-HLA DQ alpha results from second DNA extraction from DFO and ninhydrin processed envelope, and duplicate; Row 7-Reagent blank control sample; Row 8-positive genomic DNA control; Row 9-negative amplification control.*

to the amplification mixture. The envelope and stamp revealed HLA DQ alpha type 1.2, 3 (Fig. 5). The evidence could then be returned to latent fingerprints for physical developer processing. Using the detected DQ alpha types for comparison with the known samples could exclude or include potential suspects as the individual(s) who licked the envelope and stamp evidence. It can also be determined if the DNA and fingerprint comparisons were consistent with each other.

Discussion

Conclusive HLA DQ alpha results (that is, dots equal to or greater than the C dot and consistent with the known types) were obtained on both simulated and actual casework samples with DNA quantities ranging from approximately 250 picograms to 2 nanograms as approximated by slot blot quantitations. No simulated evidence samples tested were subjected to both ESDA and latent fingerprint processing; however, two envelopes from two different actual cases have been subjected to both ESDA and latent fingerprint processing and both produced conclusive HLA DQ alpha typing results.

Conclusion

The results of the study presented here demonstrate that DNA can successfully withstand certain evidence processing such as DFO and ninhydrin processing or questioned document ESDA processing and still yield a sufficient quality and quantity of DNA for HLA DQ alpha typing. The physical developer latent fingerprint processing may dilute and/or degrade DNA in a sample so there is not sufficient quality and/or quantity for HLA DQ alpha typing. No false inclusion or exclusion results were detected, regardless of any extraneous dots present. Extraneous dots, i.e. dots other than the expected from the known control sample, were detected in two simulated specimens tested, but these dots did not affect the ability to detect the expected and correct DNA DQ alpha type.

It was also determined that for some of the DNA extracted from the envelopes and stamps, the addition of BSA to the amplification mixture enhanced dot blot detection. It appears that BSA may potentially enhance a weak result with no apparent deleterious or artifactual effects to the DQ alpha typing results.

Testing performed with simulated and actual casework envelope, stamp and cigarette butt evidence demonstrated that recovered DNA could be successfully typed by PCR HLA DQ alpha after specific latent fingerprint and questioned document examinations have been performed.

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