TECHNICAL NOTE

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An Assessment of DNA Contamination Risks in New York City Medical Examiner Facilities

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ABSTRACT: DNA evidence holds an important position in criminal investigations and proceedings. The polymerase chain reaction (PCR) is often utilized to amplify polymorphic regions of DNA which are subsequently typed to produce distinct genotypes. The sensitivity of PCR-based techniques provides a major advantage over other DNA or conventional serological typing systems. Samples containing quantities of DNA in the picogram range are often typed. However, the unprecedented sensitivity of PCR is often cited as a criticism. One concern is that the interpretation of PCR typing can be affected by DNA contaminants from foreign sources. In this report, the level of DNA contamination in New York City Medical Examiner facilities and its potential affects on HLA-DQA1 typing were assessed. Two related studies conducted over a five week period measured and typed HLA-DQA1 from accumulated DNA on autopsy room and Forensic DNA Laboratory structures. The potential for DNA contamination from airborne sources was also evaluated in the autopsy suites. This study demonstrated the presence of small amounts of DNA on structural surfaces, but little evidence of airborne DNA contamination.

KEYWORDS: forensic science, DNA typing, genetic markers, polymerase chain reaction, medical examiner facility

Due to the sensitivity of PCR-based methods, it is extremely important that cross-contamination of samples from crime scene to laboratory is minimized to ensure protection against misinterpretations and potential legal challenges against the use of this technology.

The HLA-DQA1 system has been used as a model for studying the effects of autopsy room and laboratory contamination on PCR amplifiable systems. The HLA-DQA1 test involves PCR amplification of the polymorphic HLA-DQA1 locus followed by genotyping of samples using sequence-specific oligonucleotide probes in a reverse dot blot format. The HLA-DQA1 reverse dot blot test has been validated as a reliable method for typing DNA samples in forensic casework (1–5).

In this report, we assess the degree of contamination present in New York City Medical Examiner facilities and determine the extent to which it can potentially influence HLA-DQA1 typing. Two parallel studies were designed to assess contamination, both from accumulated DNA on surfaces and from airborne sources (presumably from aerosols generated during autopsies) in New York City Medical Examiner facilities. Each study was conducted over the course of five weeks. We present data collected from the New York City Medical Examiner offices in Manhattan (including the Forensic DNA Laboratory), Queens, Brooklyn, and the Bronx.

Materials and Methods

Sampling

Each of the four borough medical examiner's offices (Manhattan, Queens, Brooklyn, and the Bronx) share a basic arrangement of structures and facilities in their autopsy rooms. The autopsy tables occupy the center of the room, with one scale suspended over each table. Sinks, cutting drains, X-ray viewers, and chalkboards are located at the head of the tables. The perimeter of the room is lined by shelves, cabinets, hoods, refrigerators, and freezers. Windows and ledges are in the back of the room. In addition to the stationary structures, mobile scales, carts, and air vents are dispersed throughout the rooms as necessary. Overhead structures include shelves, cabinets, lights, and air vents.

For the cumulative DNA contamination study, 18–20 structural units in each autopsy room were chosen to be swabbed. In order to isolate areas which presented the greatest potential for DNA contamination, structures closest to the autopsy tables were selected. These structures included scales, sinks, cutting drains, and autopsy table surfaces. In addition, certain sites which were not easily accessible to pathology staff were selected as control sites. Such structures included overhead lights and sink eaves, corner cabinets, and ceiling overhangs.

Cumulative DNA present on autopsy room (Manhattan, Queens, Brooklyn, and the Bronx) and Manhattan Forensic DNA Laboratory structures was collected by the use of a sterile cotton-tipped swab moistened with deionized water. In the autopsy rooms, each site was swabbed once at the start of the study, and once again five weeks later. In order to ensure that the exact site was swabbed five weeks later, outlines were drawn on surfaces after the initial

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swabbing. Each swab site was approximately 4 by 4 cm. The sites in the Forensic DNA Laboratory were swabbed only once, at the start of the study. None of the areas sampled either in the autopsy rooms or the DNA laboratory were specifically cleaned before initial sampling. After each surface was swabbed, the cotton-tipped swabs were thoroughly air-dried before being packaged. In the interval between collection and analysis, the specimens were stored in individual plastic bags at −20°C in the Forensic DNA Laboratory.

In the Forensic DNA Laboratory, 55 sites were swabbed; of these, 45 were located on laboratory work areas and desk spaces. Areas that typically contain amplified DNA such as the PCR hybridization rooms were tested. The remaining ten sites were distributed among light boxes and stationary cameras in the laboratory darkroom, where staff members photograph electrophoresis gels and DNA hybridization strips. All laboratory areas are cleaned with bleach and ethanol after use. Swabbings were taken at approximately 2 h after cleaning. Any DNA found represents accumulation during routine use covering a period of approximately one year (the age of the laboratory).

For the airborne contamination study, six sampling sites were chosen in each autopsy room. When selecting sites for the airborne contamination study, priority was given to structures which presented the potential for airborne contamination, including overhead lights and cabinets, scales, and ceiling areas. At each site, several cotton-tipped swabs were hung. All swabs were suspended in air with the wooden handle attached to site surfaces. Every 2–3 days for a period of five weeks, one swab was removed from each site and stored at −20°C. Airborne DNA contamination was not measured in the Forensic DNA Laboratory.

Analysis of Swabs

DNA was extracted from the swabs using a chelex-based method (6). Unused control swabs taken from boxes of swabs used during the swabbing phases of the cumulative study and the setting up portion of the air-borne study were also extracted. After extraction, 20 μL of each chelex-extracted sample was submitted for quantiblot analysis (7). Once quantified, the volume of sample containing 2 ng of DNA was determined and amplified without BSA.

Using a Perkin-Elmer Cetus model 480 thermal cycler, PCR amplification of the HLA-DQA1 locus was performed (8). Genotyping was accomplished by reverse dot blot hybridization (8). All swabs collected were analyzed.

Results

Cumulative DNA Study

The QuantiBlot procedure quantifies DNA in increments of 0.00, 1.5, 3.1, 6.2, 12.5, 25.0, and 100.0 (ng) per total Chelex extract (200 μL). Only those swabs containing quantities of DNA at least 1.5 ng/200 μL are considered significant for these studies, because laboratory standard operating procedure at the New York City Medical Examiner Office requires this minimum value for amplification of the extract. The study demonstrates no significant difference between boroughs. Figures 1A and 1B represent the number of swabs found to contain each of the above listed quantities of DNA, at initial and 5-week collections, respectively.

From the samples collected initially, 52 out of 77 (67.5%) autopsy room swabs contained significant amounts of DNA. Of the samples collected five weeks later, 31 of the 77 (38.3%) autopsy

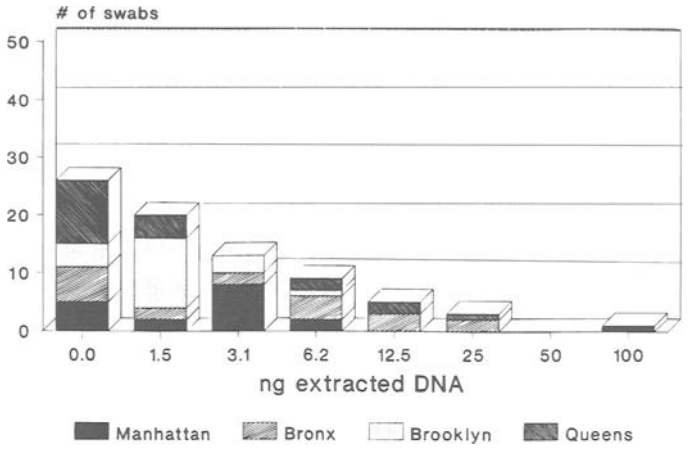

FIG. 1A—*Number of swabs from initial collection (cumulative study) yielding respective quantities of extracted DNA.*

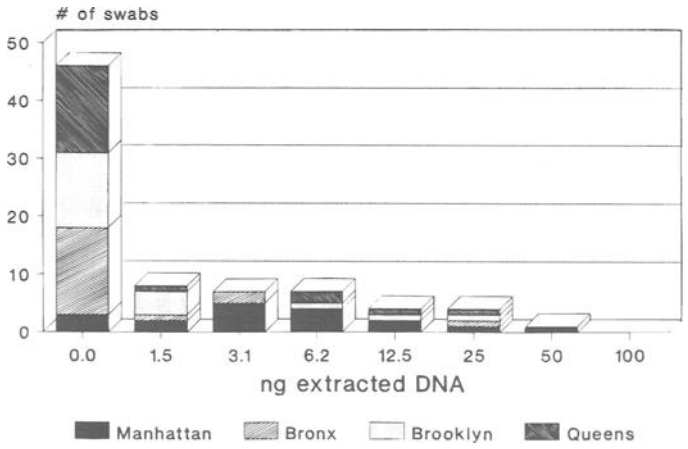

FIG. 1B—*Number of swabs from 5 week collection (cumulative study) yielding respective quantities of extracted DNA.*

room swabs contained significant quantities of DNA. This reduction indicates that DNA did not accumulate during the five weeks between collections to the level present in the initial swabbing. In the Forensic DNA Laboratory, only 2 out of 55 (3.64%) swabs contained significant amounts of DNA, both having 1.5 ng. Control swabs did not contain DNA.

Of the 52 swabs collected initially from the autopsy rooms which contained significant quantities of DNA, only 17 produced typeable alleles. Only 4 of the 17, however, yielded results that could be construed as a single genotype (containing 1 or 2 alleles), whereas the other 13 yielded more than 2 alleles (indicating a mixture of DNA). Of the 35 swabs which did not produce typeable alleles, only 5 yielded alleles below the control threshold although the other 30 produced completely blank strips.

Of the 31 swabs collected 5 weeks later from autopsy rooms which contained significant quantities of DNA, only 11 produced typeable alleles. Only 1 of the 11, however, yielded what appeared to be a single genotype, whereas the other 10 showed a mixture of DNA. Of the 20 which did not produce typeable alleles, 19 yielded completely blank strips.

The two swabs from the DNA Laboratory which contained measurable quantities of DNA did not produce typeable alleles. Both swabs, however, produced alleles below the control threshold. Three swabs taken from the PCR hybridization room which did

not show any measurable DNA were also amplified. Subsequent hybridization produced no HLA-DQA1 alleles.

Table 1 lists the number of autopsy room swabs with respective amounts of DNA and the number of each group with and without typeable alleles. Sites where detectable, quantities of DNA were found are also given. Those structures which consistently accumulated DNA include counter tops, autopsy tables, cutting drains, sinks, and scales. These structures are central to each autopsy procedure, and are natural sites for the accumulation of genetic material. Structures surrounding these, such as hoses over autopsy tables, sink eaves, and cabinets over counter tops demonstrated sporadic contamination.

The majority of swabs which contained measurable amounts of DNA but did not produce HLA-DQA1 alleles contained DNA in the 1.5–6.2 ng range However, four swabs containing a large accumulation of DNA, three with 12.5 ng and one with 50 ng, also did not yield HLA-DQA1 alleles. To test the possibility that a PCR inhibitor exists in these samples, aliquots of the respective chelex extracts were diluted 10:1 and retested. In all four samples, no HLA-DQA1 alleles were detected. The lack of results may be due to degradation of the DNA. Potential causes of degradation include close proximity to overhead UV light sources or exposure to residual bleach, which is used daily to clean all surfaces. In the case of the 50 ng sample, which was taken from the surface of a hot air vent, repeated exposure to heat may have caused substantial DNA degradation leading to untypeable results. In all instances in which HLA-DQA1 alleles were not detected, the age of the DNA present was not known. Swab extracts yielding DNA in quantities below 12.5 ng and which did not produce HLA-DQA1 alleles were not retested.

Airborne DNA Contamination Study

During the five-week study, there were 275 autopsies performed in Manhattan, 224 in Queens, 315 in Brooklyn, and 187 in the Bronx for a total of 1001 autopsies.

A total of 348 swabs were collected throughout the four autopsy rooms over the five week airborne study. Only 4 of the 348 swabs (1.1%) yielded quantifiable DNA. All 4 contained 1.5 ng of DNA and were collected from an unused sink (1 swab), the eaves of a cutting drain (2 swabs), and a light over an autopsy table (1 swab). No HLA-DQA1 alleles were detected after amplification and hybridization. The concentration of DNA did not accumulate over time in any of these areas. Control swabs were negative for DNA.

Discussion

The results of this study demonstrate that the facilities at the New York City Medical Examiner's Office (Manhattan, Queens, Brooklyn, and the Bronx) contain moderate quantities of DNA typeable contamination. Cumulative typeable contaminating DNA was found on 17.7% of the swabs examined. Though DNA was shown to accumulate on structural units in the autopsy rooms, most of the swabs with the greatest amounts of DNA are located in areas peripheral to the center of autopsy room activities (e.g., hoods, air vents). These structures are generally exposed to less contact by medical and technical staff in the autopsy rooms, and may not be cleaned as frequently or meticulously as more central structures.

Those swabs yielding only 2 typeable alleles or in the case of an apparent homozygote, 1 allele, have a greater potential for false inclusions or exclusions than do those swabs presenting a mixture of types. Definitive matches cannot be easily ascertained if more than 2 alleles are present. Of the 17 swabs collected initially in the cumulative study which produced typeable HLA-DQA1 alleles, only 4 yielded 1 or 2 alleles, whereas the other 13 (76.5%) yielded three or more alleles. Of the 11 swabs collected 5 weeks later which yielded typeable HLA-DQA1 alleles, all but one presented a mixture of alleles. The mixture of alleles is an indication of contamination building up over time.

Analysis of swabs collected during the airborne study indicate that DNA contamination from airborne sources is not evident. None of the 4 swabs from the airborne study, which had detectable levels of DNA, yielded typeable HLA-DQA1 alleles.

TABLE 1—*Number of autopsy room swabs found to contain quantifiable DNA at initial and final collections in the cumulative study. Quantiy of DNA was determined by QuantiBlot analysis, in the increments of 1.5, 3.1, 6.2, 12.5, 25, 50, and 100 ng. The number of swabs yielding typeable alleles combines those yielding two (apparent single source) and three or more alleles (mixture of DNA). Structural units represent autopsy room sites where swabs containing DNA were collected. Twenty-five swabs collected initially and 46 swabs from the final collection did not show any detectable DNA.*

Amount DNA Isolated (ng)	Collection	Number of Samples: Without Typeable Alleles	Number of Samples: With Typeable Alleles	Structural Units
1.5	Initial	20	0	Ledge, file cabinet, shelf, moveable air vent, sink, hood, X-ray viewer, autopsy table, scale
	5-Week	7	1	Counter top, cabinet over counter top, window ledge, wall, cutting drain, X-ray viewer
3.1	Initial	9	4	Vent, scale, sink, cabinet, autopsy table, ledge, refrigerator, X-ray viewer, air vent
	5-Week	6	1	Autopsy table, scale, hood, shelf, sink, moveable air vent
6.2	Initial	5	5	Scale, counter top, cabinet over counter top, overhead sink eaves, autopsy table, window ledge, wall, light, shelf, moveable air vent
	5-Week	4	3	Autopsy tables, vent, shelf, light, ledge, moveable air vent
12.5	Initial	1	4	Scale, autopsy table, cabinet, cutting drain, freezer door
	5-Week	2	2	Hose over autopsy table, shelf, scale, ledge
25	Initial	0	3	Autopsy table, hose over cutting drain
	5-Week	0	4	Cart, hood, moveable scale, air vent
50	Initial	0	0	
	5-Week	1	0	Hot air vent
100	Initial	0	1	Shelf
	5-Week	0	0	

Results from swabs taken in the Forensic DNA Laboratory show no demonstrable evidence that the Forensic Biology laboratory is contaminated with extraneous DNA. No typeable HLA-DQA1 alleles were detected in the 2 swabs which had significant quantities of DNA. Both swabs were collected from areas of the laboratory which are not used for evidence or specimen processing and handling.

Autopsy rooms represent locations in which specimens containing genetic sources are processed. Naturally, these are areas where a large quantity of DNA might accumulate on structural units because of the large number of autopsies performed (1001 in 5 weeks). DNA does accumulate slowly on peripheral structural units by contact with contaminated surfaces despite daily cleaning with bleach. However, the DNA is not airborne and should not cross-contaminate samples as long as samples are collected into clean containers and the samples are not placed directly in contact with contaminated surfaces.

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