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Implementation of Forensic DNA Analysis on Casework Evidence at the Palm Beach County Sheriff's Office Crime Laboratory: Historical Perspective

Cecelia A. Crouse

Palm Beach County Sheriff's Office Crime Laboratory, West Palm Beach, Flo, USA

Palm Beach County is the largest of the 64 counties in the state of Florida, USA, with most of the area uninhabited and the population concentrated near the coastal region. The Serology/DNA Section of the Palm Beach County Sheriff's Office (PBSO) Crime Laboratory serves a community of approximately one million residents, and an additional million tourists visit Palm Beach County every year. In addition to the unincorporated county regions, there are thirty-four city police agencies, the Florida State Highway Patrol, several university security agencies, the local Federal Bureau of Investigation, and the county Medical Examiners Office that all use the PBSO Serology/DNA Laboratory for the analysis of casework evidence. The purpose of this manuscript is to provide laboratories that are in the process of initiating DNA analysis on casework with practical information regarding the decision-making processes that occurred during the development of the DNA testing program at PBSO. Many of the concerns addressed in the early 1990's are still a guide to the development of a quality forensic DNA analysis program in the year 2001. Issues, such as personnel, laboratory space, internal standard operating procedures, implementation of DNA analysis on casework evidence, and building a relationship with law enforcement personnel are discussed.

Key words: *DNA; DNA fingerprinting; equipment and supplies; forensic medicine; laboratories; polymerase chain reaction; software; United States*

The decision to implement a forensic DNA analysis program in a crime laboratory must take into account several important factors, such as the type of DNA technology to be employed, the location and the layout of the facilities, the number of personnel, training protocols, and annual budgetary needs. In 1992, Palm Beach County Sheriff's Office (PBSO) elected not to implement the technology of restriction fragment length polymorphism (RFLP) but to develop a polymerase chain reaction-based (PCR) analysis for casework evidence. RFLP was the DNA technology of choice in 1992 and finding information regarding construction of a forensic PCR DNA program was challenging. At this time, the only forensic PCR-based DNA typing genetic marker that had been validated and commercially available in a kit format was the HLA-DQA1 locus (previously DQalpha) provided by Roche Molecular Systems (1). Results of an HLA-DQA1 analysis are presented as "blue dots" on a nylon membrane. This first PCR-based test allowed the forensic community to become acquainted with the nuances of the PCR process and the import of training DNA analysts in all aspects of DNA typing, including preparation of DNA extracts, conducting PCR in designated areas of the laboratory, DNA profile interpretation issues, reporting DNA profile frequencies, and reporting findings through court testimony. The HLA-DQA1 test was an important contribution to the forensic community. Even though the eventual implementation of a second "blue dot" forensic DNA typing kit called "PM/DQA1", which tests for six genetic markers, was initiated in many laboratories, the genetic markers were of low polymorphism and DNA mixture interpretation was challenging (2). By 1990, the international community was evaluating genetic markers within the human genome that demonstrated high polymorphism, were amenable to PCR analysis, and could easily be multiplexed in a single reaction, thereby conserving biological stain materials (3,4). These genetic markers, known as microsatellite or "short tandem repeat" (STR) DNA sequences, have become the standard in forensic DNA typing. STRs are genetic regions containing repetitive sequences, usually between 3-5 base pairs in length, thereby allowing for separation of the PCR STR fragment lengths. As a result, interpretation of DNA mixtures is not as difficult as the "blue dot" typing method.

The purpose of this manuscript is to provide the forensic community with issues encountered by the PBSO DNA laboratory while initiating a PCR-based forensic DNA program. In addition, it is hoped that the resources presented will act to guide laboratories through the challenges of meeting the needs of the citizens being served, as well as maintaining a high quality DNA program capable of withstanding courtroom scrutiny.

Forensic DNA Quality Assurance and Quality Control Literature

Regardless of the type and scale of the forensic DNA program to be implemented, the first assessment that must be considered is how to initiate and maintain a quality DNA testing program. This must be a documented Quality Assurance/Quality Control (QA/QC) program. The recognition for the need for DNA typing standards within the forensic community in the United States resulted in the formation of a national group of forensic scientists in the late 1980's called the Technical Working Group on DNA Analysis Methods or TWGDAM. These scientists eventually published a series of DNA forensic typing guidelines, including *Guidelines for a Quality Assurance Program for DNA Analysis 1991,* and *1995* (5,6).

PBSO used the TWGDAM guidelines from 1991 as a guide to initiating a DNA typing program and later implemented the updated 1995 TWGDAM Guidelines that contained additional information on preparing a validated PCR forensic DNA program.

Using the TWGDAM Guidelines as a template, the national DNA Advisory Board was commissioned in the mid-1990's to recommend universal DNA standards to be implemented in forensic laboratories in order to gain certification/accreditation. The effective date for implementing these National Forensic DNA Testing Standards (7), also known as the Federal Bureau of Investigation (FBI) Standards or National Standards, in publicly funded forensic DNA laboratories was October 1, 1998. These standards include an outline listing the essentials of internal DNA testing programs addressing quality assurance, organization and management, personnel, facilities, evidence control, validation of laboratory methods, analytical procedures, equipment calibration and maintenance, reports, review of data, proficiency testing, corrective action, audits, and safety and subcontractor standards. A copy of these standards may be found at *www.for.swg-swg.org*.

The Scientific Working Group on DNA Analysis Methods (SWGDAM), previously TWGDAM, has developed a laboratory DNA audit document, which is currently being used to conduct internal and external audits of forensic DNA laboratories using the National Standards recommendations. This audit document not only lists the National Forensic DNA Testing Standards, but also provides a guide to the interpretation of each standard. The purpose is to be sure there is uniformity in the understanding of the intent of each of the standards. This document is highly recommended for laboratories either initiating a DNA program or for those laboratories that may need a template to evaluate an existing forensic DNA typing laboratory.

The SWGDAM has also generated subcommittees to provide publications outlining in greater detail interpretation of the National Standards relating to DNA laboratory training programs, DNA profile interpretation guidelines, and genetic marker validation procedures. These very useful documents will be published in the near future on the FBI Forensic Communications website.

In 1992, the National Research Council (NRC) issued *DNA Technology in Forensic Science* (8) that provided valuable information on the state of forensic DNA analysis in the community. A second publication released in 1996 by the second NRC commission, *The Evaluation of Forensic DNA Evidence* (9), summarized the issues associated with forensic DNA typing both in the laboratory and in the courts. It offers recommendations for how evidence is handled, statistical evaluation of DNA profile frequencies, and many other aspects of DNA profiling.

When constructing a laboratory, whether a new facility or through renovation of an older facility, it is highly suggested that the laboratory obtain the publications listed above and request a visit to several established accredited forensic DNA laboratories for advice on how to implement a quality DNA testing program. It has been PBSO's experience that laboratories routinely share DNA protocol manuals, quality control documentation programs, and many other materials that aid in the development of a forensic DNA program.

Decisions on Forensic Typing Methods

The forensic community currently has extensive experience in using two well-established general molecular biology techniques for conducting DNA typing on casework evidence:

1) RFLP, initiated on casework evidence in 1985 (10); and 2) PCR-based analysis, with (a) HLA-DQA1 locus analysis initiated on forensic casework in 1986, (b) PM/DQA1 genetic marker analysis of six loci initiated on casework in 1993, and (c) minisatellite variant repeat loci analysis initiated on casework in the late 1980's (e.g., D1S80).

Mitochondrial DNA (mtDNA) typing ,sometimes referred to as "tiny DNA fingerprint typing", was used for the identification of missing persons in the late 1980's. This technique is conducted in a few selected laboratories throughout the world and is a powerful tool in the identification of individuals in addition to nuclear DNA testing or when nuclear DNA testing is not useful.

STR analysis was investigated predominantly in the European and Canadian forensic community in the late 1980's and is currently the international forensic PCR nuclear DNA typing standard.

PBSO was dedicated to PCR-based forensic DNA typing from the beginning of the development of the laboratory in 1992. At that time, PCR technology was the most innovative and widely used technique in the general scientific community. The advantage of being able to obtain a DNA profile from biological material from minute stains and relatively degraded samples in just a few days is instrumental in helping law enforcement obtain investigative leads.

After a year of development, on May 1, 1993, the HLA-DQA1 "blue dot" PCR-based kit was used on the first PBSO evidentiary stains. Evaluation of STR genetic loci for the use on forensic casework evidence began at PBSO in 1993, the same year when the European DNA Program (EDNAP) conducted an interna-

tional STR evaluation program. PBSO participated in this testing program, which consisted of obtaining PCR reagents and extracted DNA samples, conducting amplification, silver staining of amplified products, and reporting the resultant STR profiles. Shortly thereafter, internal validation of STR systems, provided by Promega Corporation, was initiated at PBSO on ten STR loci, with most of the STRs multiplexed as three or four STR systems. The first STR system validated at PBSO for the use on casework evidence was the CTT triplex, which included the genetic markers CSF1PO, TPOX, and THO1. After the genetic marker for sex type, called "Amelogenin", was included in the STR triplex, DNA samples were amplified with the CTAT reagents and the STR alleles were detected by the use of silver staining protocols optimized in the PBSO laboratory. Silver staining of amplified STRs is still widely used in laboratories throughout the world, as it is a robust and inexpensive methodology.

In 1995, after two and one half years of validating the manual silver staining technique at PBSO for the detection of STR markers, PM/HLADQA1 and a triplex of STR's markers were routinely used on forensic evidence. The STR markers offer genetic profiles from loci with reasonable or high polymorphism. The fact that STR markers are composed of sequence length differences makes the interpretation of STR DNA profiles from mixtures more straightforward than the "blue dot" DNA profiles.

An important aspect of PBSO's evaluation of the STR systems in the beginning was the unique scientific collaborations that were formed. Scientists from the FBI, paternity laboratories, state laboratories, and local laboratories worked together to provide tomes of data to substantiate the reliability of STR PCR DNA typing. A comprehensive list of STR literature references can be found at the website of the US National Institute of Standards and Technology (*http://www.cstl.nist.gov/biotech/strba se/*). There is a tremendous amount of experience in the forensic community from which laboratories, when considering STR technology, can elicit advice, provide collaborations, provide a visiting scientist exchange, and procure help on technical and administrative issues.

PBSO found collaborations especially informative when population studies were conducted. Guidelines for the initiation of DNA protocols for forensic human identification include the validation of each polymorphic locus for relevant regional populations. Once validation protocols have been completed, DNA profiling may be conducted on convicted offender databases and casework evidence. Laboratories need to have access to population STR loci allele frequency data. There is an enormous amount of STR allele frequency data available from many different populations from all over the globe. For example, in 1996, the FBI sponsored an 18-month STR study which involved over 20 laboratories using different fluorescent instrumentation for STR allele detection. One of the results from these studies was the generation of population data from many regions of the world. The PBSO found involvement in this 18-month study critical to understanding how STR analysis was to be conducted in other laboratories, the import of interpretation guidelines, and how statistics would be reported.

Setting up a Fluorescent STR-based Forensic DNA Program

Fluorescently-tagged STR PCR allele fragments, which are post-separation detected by lasers and DNA profiles are computer-generated, represented a significant advancement for forensic DNA programs. In early 1998, after two years of validating fluorescently-tagged STR PCR fragments, the PBSO DNA laboratory replaced the PM/HLADQA1 and CTAT kits with the Promega fluorescent PowerPlex1.1 GenePrint STR kit (11). The Hitachi FMBIO flatbed detection platform (Hitachi Software Engineering Company, Ltd., San Francisco, CA, USA) was purchased at PBSO to detect the fluorescent amplified STR alleles (12). Fluorescently tagged STR primers are multiplexed in commercially available megaplex kits with all of the necessary components for the PCR. Questions regarding which instrumentation to purchase in the mid 1990's depended on the source of the STR kits. For example, fluorescent amplified STR alleles generated from Perkin Elmer's Profiler, ProfilerPlus, and Cofiler fluorescently-tagged STR kits are predominantly used on Perkin Elmer ABI instruments and STR alleles generated from Promega Powerplex kits on the Hitachi FMBIO. Currently, Promega PowerPlex 1.1, Powerplex 2.1, and now PowerPlex16 STR kits are used on flatbed and capillary allele detection platforms.

The decision regarding which fluorescent instrumentation to purchase for a forensic laboratory is a personal one. The cost of the reagents and instruments usually plays important role. However, for some laboratories, data throughput is a critical consideration. In that case, multichannel capillary instrumentation, such as the PE ABI 3100 or 3700 instruments (Perkin Elmer), is available. There are other fluorescent instruments in the scientific community, which may also be used for forensic casework. The computerized read-out data format for the Hitachi FMBIO and the ABI 377 are separated STR allele fragments that are visualized in the form of bands and/or peaks. The ABI capillary format visualizes allele data as peaks (electropherograms).

The Hitachi FMBIO instrument was purchased by PBSO in 1996 because of the potential for high daily throughput of samples for casework evidence, approximately 10 gels/day with about 25 samples/gel or 250 STR profiles/day. An additional feature of the Hitachi FMBIO was the ease of use and customers' continuous high satisfaction with company support. The issue of pouring polyacrylamide gels has been alleviated in the PBSO DNA laboratory by the purchase of R3 disposable gels through Hitachi Software (12). Although expensive, the time saved more than compensates for the cost. Other laboratories have purchased pre-cut disposable glass, pour the gels, then discard after use.

It is highly recommended to visit laboratories using the various platforms before investing in a fluorescent STR program.

Physical Laboratory Facilities

Space truly is the last great frontier, especially for forensic science laboratories. The most important consideration when designing a PCR-based forensic DNA testing program, is workflow procedures. This includes but is not limited to where evidence is examined for biological stains, the location where DNA extraction, quantification and PCR preparation protocols will be conducted, a separate area/time for the amplification process, considerations for the handling of biohazardous and chemical materials, storage of submitted evidence, evidence stain cuttings, DNA extracts, and PCR products. When space is limited, it is especially critical that analysts be trained to observe laboratory standard operating procedures to maintain an organized DNA program. Each analyst should have personally assigned areas and reagents for certain aspects of the DNA procedures. Considering laboratory work area, report writing, and even areas for eating should average out to approximately 1,000 square feet per analyst.

Validation of STR Systems for Casework Evidence

Approximately two and a half years after starting the validation of the manual STR methodology at the PBSO laboratory, STR analysis was conducted on casework evidence. Likewise, there was nearly two years of validation completed before the fluorescent STR system PowerPlex 1.1 was used for casework. A 2001 survey by the STR Megaplex Advanced Research and Training group (SMART) reported that the average amount of time it now takes for a laboratory to completely validate a fluorescent STR system is one year. The most logical reason as to why the development of a forensic DNA program is much shorter now than in the past is due to the fact that laboratories are able to obtain valuable information from the more experienced laboratories.

As mentioned previously, many laboratories will provide validation study results and in addition, there are many validation studies for fluorescent STR studies that have been published in the literature.

Validation of forensic DNA programs involve many issues including designating the areas of the laboratory where each aspect of the analysis is conducted, purchasing the necessary reagents, materials and instruments, then conducting studies to confirm the validity of the process. There are three types of validation studies: developmental, imternal and corcondant validation.

Developmental Validation

The companies providing STR fluorescent kits will conduct these studies which include the design of the primers, amplification reaction conditions, and verification of the locus specificity. For laboratories synthesizing their own STR primers and providing all of their own reagents, developmental validation would be conducted internally.

Internal Validation

Internal validation is typically conducted at the forensic DNA laboratory implementing the methodology. Typical internal validation studies include but are not limited to:

1. Population studies using approximately 100 individuals from a designated racial/ethnic group.

2. Mixed specimen studies to include limited environmental studies to determine the effects of a variety of environmental conditions on the DNA and resultant effect on DNA typing.

3. Precision studies are imperative to understand the ability of the instrument's detection system to generate reliable results consistenly. These studies will also provide information regarding the accuracy of the instrument relation to the instrument's precision.

4. Non-probative evidence studies aid interpretation of DNA profiles from actual casework evidence.

5. Non human studies determine the species specificity of the DNA typing system.

6. Minimum sample studies help understand the sensitivity of the STR fluorescent allele detection system. Not all capillary instruments or flat bed scanners have the same sensitivity. This must be determined empirically. In addition, sensitivity studies help in the understanding of stochastic effects that may occur when amplifying low DNA template concentrations.

It is highly recommended that laboratories obtain SWGDAM validation guidelines and exchange validation information with other laboratories.

Concordant Validation

PBSO engages in a third type of validation, known as concordant validation, which is the validation of a DNA typing system by submitting DNA samples to other forensic laboratories for the purpose of comparing typing results from laboratory to laboratory. PBSO has participated in many concordant studies with a variety of laboratories when validating STR systems. Usually 15-25 bloodstains are prepared and samples are shipped to participating laboratories. PBSO finds it important to conduct concordant validation studies with laboratories using different instrumentation in order to verify STR profile results are the same regardless of the platform used for allele detection. Preparations of "challenging" samples, such those with known microvariants, partial null alleles, or three-banded allele patterns, help substantiate the robustness of the system. Concordant studies are usually requested formally through a documented letter to the participating laboratories. The laboratories conducting the DNA typing submit final profile results to the laboratory that prepared the samples. A final letter confirming that all allele calls are correct is then sent out to each of the laboratories.

Training Programs for DNA Analysts

The amount of time necessary to appropriately train individuals in the laboratory to aid or conduct forensic DNA analysis on casework depends on several factors:

1. The type of position available: clerical, technical aid, screening evidence, serology only, serology/DNA analyst, DNA analyst only, technical leader, supervisor, information systems analyst.

2. Analyst academic background: level of academic achievement (high school diploma, or associates, bachelors, masters, or doctorate degree).

3. Academic curriculum: biology, chemistry, or population genetics, etc. (the dates when the courses were taken may be important).

4. Technical experience: laboratory experience includes the types of techniques employed and job responsibilities.

Taking the above into consideration allows the laboratory to have a flexible training program. PBSO currently has many training modules depending on the position. Training programs include evidence handling, safety and biohazard measures, QA/QC protocols, serological analysis of casework evidence, DNA analysis, forensic statistics, report writing, and legal aspects of forensic science, which require hands-on analysis, reading from the literature, and/or final exams. The PBSO has separated the DNA written examinations into three major areas: molecular biology, forensic DNA analysis, and statistics. An example of the PBSO maintaining a flexible DNA training program is a case-in-point where an individual had a masters degree in which a comprehensive thesis project was defended. This individual was exempt from the written molecular biology exam. Flexibility in a training program is critical as long as any deviations from the training manual have been appropriately documented.

The SWGDAM Training Guidelines subcommittee has recently released a draft of training guidelines in order to improve the quality of forensic laboratories. The training program is divided into three modules, which include goals, tasks, reading assignments, and assessment. Topics include evidence handling, foundational scientific knowledge, applied scientific knowledge, laboratory analysis, report writing, legal issues, and final evaluation. The final evaluation is the successful completion of a qualifying test representing a mock case and preparing the case file according to laboratory policy.

All analysts need not repeat validation studies previously conducted for the initiation of DNA analysis on casework. During training at the PBSO, every analyst will generate data for minimum DNA template sensitivity, DNA template ratio mixture studies, a variety of forensic-like samples, precision, and non-probative casework evidentiary samples. In addition, exams are given where appropriate. A final in-house proficiency includes analysis of 25 samples in which the correct profile for all STR loci must be submitted for each sample. The final aspect of PBSO training involves the analyst conducting DNA analysis on a mock case, which has been constructed in the laboratory. The mock case usually consists of liquid blood from a "victim" and "suspect", sexual battery evidence collection kit with semen, clothing with blood stains, and a bed sheet with a semen stain. The analyst must submit a final report and a casefile with all pertinent information. The case is then adjudicated at a mock trial at the Palm Beach County Courthouse in front of a judge, prosecutor, defense attorney, and court clerk. A final serology/DNA certificate of completion is presented to the qualifying analyst.

Summary

The implementation of a forensic DNA program must follow an organized process in order to assure that only quality DNA analysis is conducted within the laboratory. There are many resources available such as publications for DNA QA/QC procedures, validation of DNA genetic markers for forensic casework, training manuals and examination questions, Internet forensic courses, population data and instrumentation expertise.

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Correspondence to:
Cecilia A. Crouse
Palm Beach County Sheriff's Office Crime Laboratory
3228 Gun Club Road
West Palm Beach, FL 33406, USA
CrouseC@pbso.org