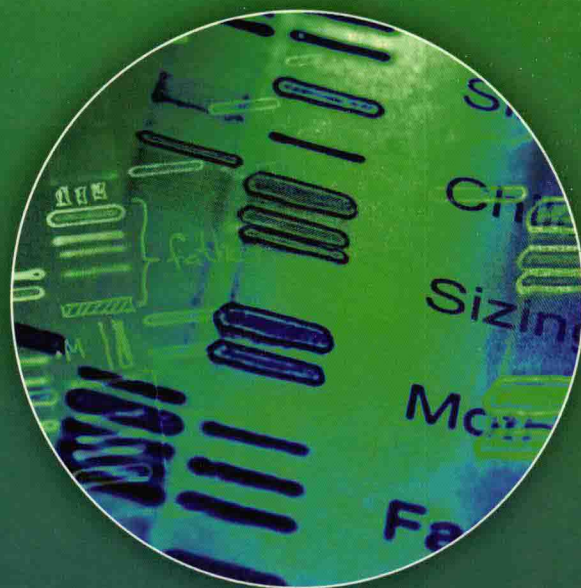




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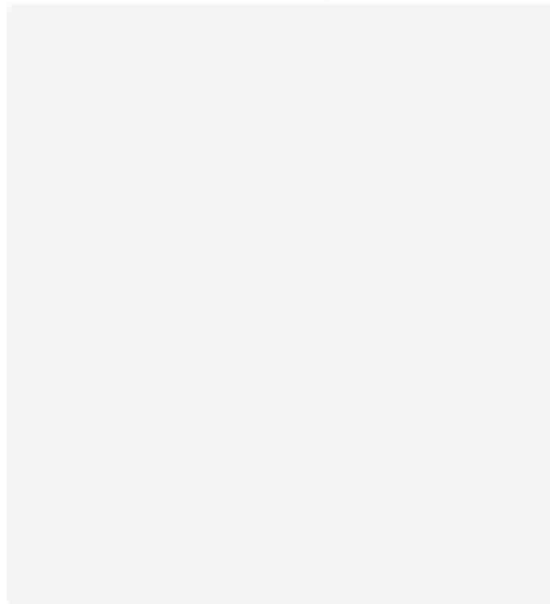
FORENSIC ANALYSIS OF BIOLOGICAL EVIDENCE

A Laboratory Guide for
Serological and DNA Typing

FORENSIC ANALYSIS OF BIOLOGICAL EVIDENCE

**A Laboratory Guide
for Serological and DNA Typing**

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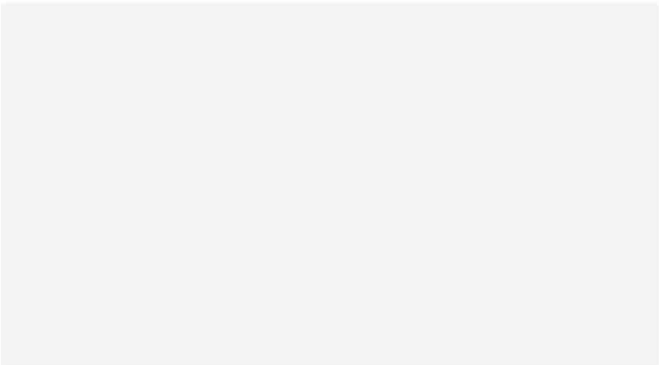
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for Serological and DNA Typing**

Preface

DNA typing has revolutionized criminal investigations and has become a powerful tool in the identification of individuals in criminal and paternity cases. In the past few years, the general public has become familiar with forensic DNA typing based on exposure from media coverage (e.g., the O.J. Simpson trial, the President Clinton and Monica Lewinsky scandal, and identification of individuals killed in the September 11, 2001 attack on the World Trade Center (WTC) in New York City and the Pentagon in Arlington, Virginia) and television (e.g., *Forensic Files*, *CSI: Miami*). Although these cases have generated widespread media attention, they represent only a small fraction of the thousands of forensic DNA and paternity cases that are conducted by public and private laboratories in the United States and abroad.

The purpose of this laboratory manual is to introduce the student to the science of serological analysis and DNA typing methods by focusing on basic techniques used in forensic DNA laboratories. This laboratory manual is designed to provide the student with a fundamental understanding of serological and forensic DNA analysis as well as a thorough background of the molecular techniques used to determine an individual's identity or parental lineage. This manual is intended to challenge the student with the methodology of the investigation in DNA typing, develop an understanding of the scientific principles involved in serology and DNA analysis, and be able to analyze and interpret the data that are generated in each exercise with clarity and confidence.

The exercises in this laboratory manual have been organized to first provide an overview of forensic DNA analysis, the sources or types of biological material used in DNA analysis, and then the background principles and practical methodology for a specific serological analysis and DNA typing technique. In some exercises, the protocols have been adapted from methods and protocols used in federal, state, and private forensic laboratories. Each exercise is designed to simulate human forensic testing but can also be used to simulate a wide range of applications for genetic analysis. The actual scenario employed in each exercise is up to the discretion of the course instructor. Lastly, an extensive glossary has been included to assist students with terminology used in the forensic analysis of biological evidence, as well as basic terms used in molecular biology.

Compiled below is a brief history of forensic DNA typing. Since DNA testing was first introduced in the United States in 1986, it has been used in thousands of cases. However, the list below highlights specific events or developments in forensic DNA analysis as well as those cases brought to the attention of the general public by media exposure.

Brief History of Forensic DNA Typing

- 1980: Ray White describes first polymorphic restriction fragment length polymorphism (RFLP) markers.
- 1985: Alec Jeffreys discovers multilocus variable number tandem repeat (VNTR) probes.
- 1985: First paper on polymerase chain reaction (PCR).

- 1986: DNA testing goes public (Cellmark and Lifecodes).
- 1986: First RFLP case in the United States (*Florida v. Tommy Lee Andrews*).
- 1988: FBI starts DNA casework (RFLP).
- 1989: The Technical Working Group on DNA Analysis Methods (TWGDAM) was established.
- 1991: First short tandem repeat (STR) paper.
- 1992: National Research Council I report *DNA Technology in Forensic Science*.
- 1993: First STR kit available.
- 1995: Forensic Science Service (FSS) starts UK DNA database.
- 1995: O.J. Simpson trial; public becomes aware of DNA.
- 1996: National Research Council (NRC) II report *The Evaluation of Forensic DNA Evidence*.
- 1996: First use of mitochondrial DNA test in a U.S. criminal trial (*Tennessee v. Ware*).
- 1998: FBI launches Combined DNA Index System (CODIS) database.
- 1998: Establishment of quality assurance standards for forensic DNA testing laboratories through the DNA Advisory Board.
- 1998: Kenneth Starr investigates allegations of President Clinton's sexual relationship with a White House intern, Monica Lewinsky.
- 1999: Multiplex STRs are validated.
- 1999: The decision in *State v. Ware* (1996) was upheld by an appellate court.
- 2002: Division of Forensic Science Laboratory in the Commonwealth of Virginia became the first state laboratory to mark 1,000 "cold hits" from its DNA database.
- 2003: A field DNA test was completed to provide preliminary confirmation of the identification of Saddam Hussein less than 24 hours after his capture. A full test performed in the laboratory provided confirmation.
- 2003: The National Institute of Standards and Technology develops a "mini-STR assay" to allow the remains from 16 additional victims from the September 11, 2001 attacks on the World Trade Center to be positively identified.
- 2006: Members of the Duke University men's lacrosse team are arrested and accused of raping a female exotic dancer. Samples are collected from the dancer and the men's lacrosse team for DNA analysis.
- 2006: DNA testing failed to connect any members of the Duke University men's lacrosse team to the alleged sexual assault of an exotic dancer.
- 2007: Prosecutor handling the Duke case is forced to recuse himself. North Carolina's attorney general declared three former Duke University lacrosse players who had been accused of gang raping a stripper innocent of all charges, ending a prosecution that provoked bitter debate over race, class, and the tactics of the Durham County district attorney.
- 2007: Applied Biosystems introduced the first DNA testing kit for analyzing degraded or limited DNA.
- 2007: FSS in the UK uses laser microdissection (LMD), which enables single cells to be extracted from a microscope slide, with fluorescence *in situ* hybridization (FISH), a method to highlight chromosomes, to distinguish between male (XY chromosomes) and female (XX chromosomes) cells.
- 2008: Idaho National Laboratory develops a method for identifying people through antibody analysis, a technique reported to be inexpensive, faster, and simpler than DNA analysis.
- 2009: Standards for forensic DNA testing laboratories and standards for DNA databasing laboratories were updated by the DNA Advisory Board and implemented by the FBI.
- 2010: Applied Biosystems' AmpFISTR MiniFiler, a forensic DNA kit designed to obtain DNA results from compromised or degraded samples, receives approval for inclusion in the National DNA Index System (NDIS).
- 2010: Two new Applied Biosystems forensic DNA test kits (Identifiler Direct and Identifiler Plus) receive approval for use by the National DNA Index System (NDIS).

- 2011: The Commonwealth of Virginia becomes the third state to approve familial DNA searches in the DNA database for individuals who might be closely related to people whose DNA is found at a crime scene.
- 2011: Osama bin Laden killed by U.S. forces. DNA tests confirmed that the body recovered from the 1-acre compound in Abbottabad, Pakistan was bin Laden.
- 2012: A forensic scientist linked DNA on medical waste to Roger Clemens in the perjury trial of major league baseball's seven-time Cy Young Award winner. The "Rocket" was ultimately acquitted on all charges by a jury that decided he did not lie to Congress when he denied using performance-enhancing drugs.
- 2012: Scientists develop a forensic test that can predict both the hair and eye color of a possible suspect using DNA left at a crime scene.
- 2013: Life Technologies introduces a portable DNA testing system that can identify whether a specimen contains human DNA that can be analyzed, and the person's gender, in 75 minutes.

It is hoped that this laboratory manual will develop the student's curiosity and confidence to further explore questions and issues involving forensic science investigations. I look forward to teaching you the techniques and applications in forensic analysis of biological evidence.

J. Thomas McClintock, PhD

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List of Acronyms/Abbreviations

ABO	Blood group system
AK	Adenylate kinase
ALS	Alternate light source
AP	Acid phosphatase
CE	Capillary electrophoresis
CHCl ₃	Chloroform
CODIS	Combined DNA Index System
CSF1PO	Locus or site on chromosome 5
DIS80	Locus or site on chromosome 1
D7S80	Locus or site on chromosome 7
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EAP	Erythrocyte acid phosphatase
EDTA	Ethylenediamine tetraacetic acid
EtOH	Ethanol
FBI	Federal Bureau of Investigation
FISH	Fluorescence <i>in situ</i> hybridization
FSS	Forensic Science Service
FTA	Collection card for biological samples
GC	Group-specific component; locus on chromosome 4
GYPA	Glycophorin A; locus on chromosome 4
HaeIII	Restriction endonuclease
HBGG	Hemoglobin G gammaglobin
HCL	Hydrochloric acid
HeLa	Human cell line
HepG	Human cell line
HindIII	Restriction endonuclease
HLA DQA1	Human leukocyte antigen; locus on chromosome 6
HVI	Hypervariable region 1 in mtDNA
HVII	Hypervariable region 2 in mtDNA

INC	Inconclusive
K562	Human cell line
KS	Kernechtrot staining solution
LCG	Leucomalachite green
LDLR	Low-density lipoprotein receptor; locus on chromosome 19
LMD	Laser microdissection
mtDNA	Mitochondrial DNA
NaCl	Sodium chloride
NDIS	National DNA Index System
ng	Nanogram; one billionth of a gram
NRC	National Research Council
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PGM	Phosphoglucomutase
PIC	Picroindigocarmine
PM	Polymarker
RBC	Red blood cell
REN	Restriction endonuclease
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RSID	Rapid stain identification test
SDS	Sodium dodecyl sulfate
SNP	Single-nucleotide polymorphism
STR	Short tandem repeat
SWGDM	Scientific Working Group on DNA Analysis Methods
TAE	Tris-acetate buffer
TE	Tris-EDTA buffer
THO1	Locus or site on chromosome 11
TNE	Tris-sodium chloride-EDTA buffer
TPOX	Locus or site on chromosome 2
TWGDAM	Technical Working Group of DNA Analysis Methods
VNTR	Variable number tandem repeat
WBC	White blood cell
Y-STR	Y chromosome short tandem repeat

Safety and Other Considerations in the Laboratory



1. There will be no eating, drinking, smoking, applying cosmetics, or handling contacts in the laboratory at *any* time.
2. There will be no pipetting by mouth. Use a pipettor at all times.
3. Minimize splashing and production of aerosols.
4. Store all books, backpacks, cell phones and other electronic devices, purses, and coats in the cabinet of your laboratory bench (or designated area). Only your laboratory notebook should be on the bench.
5. Do not place pencils, pens, or any other object into your mouth while in the laboratory.
6. *Never* take any reagents, samples, or cell cultures out of the laboratory.
7. Notify the laboratory instructor immediately of any spills, of any accidents, or if you cut or injure yourself.
8. In most instances you will be wearing disposable gloves. Be extra careful when handling reagents or chemicals to eliminate skin contact. If working with samples thought to contain biological material (i.e., blood) wear goggles with a particle mask as well as gloves.
9. Wash your hands at the beginning and at the end of the laboratory exercises.
10. Clean your laboratory bench with dilute alcohol (70%) or with a 10% bleach solution before you begin work and when you have completed the laboratory exercise.
11. Laboratory coats are not required. However, in forensic laboratories, laboratory coats must be worn as well as disposable gloves.
12. Children should not be allowed in the laboratory.
13. Familiarize yourself with the location of the eye wash station, the fire extinguisher, and the fire blanket.
14. Dispose of all materials potentially contaminated with biological material into a disposable biohazard container.

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