September 12, 2005

The Honorable Mark R. Warner, Governor of Virginia
Executive Office Building, 3rd Floor
1111 East Broad Street
Richmond, Virginia 23219

Dear Governor Warner:

On April 9, 2005, the American Society of Crime Laboratory Directors, Laboratory Accreditation Board ("ASCLD/LAB"), issued a report detailing an interim audit of the Commonwealth of Virginia Division of Forensic Science Central Laboratory Biology/DNA Unit ("DFS"). The interim report focused on certain deviations from protocol that had occurred during the retesting of DNA evidence in connection with an executive clemency petition in a capital murder case. After reviewing the case file, the ASCLD/LAB inspectors found that, during the retesting, "there appear to have been deviations in protocol . . . that led to examination data that, in the ASCLD/LAB inspectors' opinion, should not have been relied upon by the DFS." In light of those deviations, the ASCLD/LAB interim report recommended, in pertinent part, as follows:

Ensure that the laboratory's Quality Manager determines whether the deficiencies revealed in this report are endemic to the DNA operations throughout the laboratory system in Virginia. This should be accomplished in part by a through examination of a minimum of 50 cases in the Virginia system dealing with low level DNA and/or [stained and heat-fixed histological slides] to determine whether process errors occurred and whether conclusions are scientifically supported.

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1 On July 1, 2005, DFS became an independent department within the Secretariat of Public Safety.
The Quality Manager should convene a suitable number of qualified DNA analysts, supervisors or technical leaders, internal and external to the laboratory or laboratory system, to **determine whether the selected cases have deficiencies that substantially affect the integrity of the results in those cases.** (Emphasis added.)

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Conduct a review of the analyst’s casework, using internal and external reviewers, from cases in an around 2000 and forward, particularly in cases in which there were low level DNA and/or mounted slides, to **determine if the conclusions are scientifically supported by the evidence.** (Emphasis added.)

On May 31, 2005, upon the recommendation of Chief Justice Leroy R. Hassell, Sr., and Senator Kenneth W. Stolle, the Chairman of the Virginia State Crime Commission, you appointed me as “special master” to form a team of independent scientists to carry out the recommendations embodied in the ASCLD/LAB interim report. As you know, I reluctantly agreed to my appointment as “special master” because I am convinced that in the 21st Century, a professional and credible forensic laboratory is absolutely essential to a reliable and effective criminal justice system for the Commonwealth.

Following discussions with Chief Justice Hassell concerning how I might balance an appointment as “special master” with my duties and responsibilities as a judge of the Court of Appeals of Virginia, I determined that, in an abundance of caution to avoid any appearance of impropriety or conflict with my judicial duties, I would not participate in or be otherwise personally involved in the review of individual cases in the event future litigation may be filed in any state or federal court in connection with any case selected for review, and I have not been so involved. In addition, I plan to recuse myself from involvement in any case reviewed by the scientific team that may later find itself before the Court of Appeals of Virginia. In other words, I have removed myself from personal involvement and potential involvement in all of the individual cases that have been

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2 Analyst Jeffrey D. Ban conducted the challenged testing in the case that was the subject of the ASCLD/LAB report.
reviewed, and I have made every effort to confine my role in this review to general
“activities designed to contribute to the improvement of the . . . legal system and
administration of justice” as permitted by Canon 4C(2) of the Virginia Canons of
Judicial Conduct. My role as “special master” has therefore been to select the members
of what I consider to be an outstanding scientific team, to charge them to conduct a
thorough and impartial review as outlined in the ASCLD/LAB report and as specifically
requested by you, to review the overall protocols, policies and procedures followed by
DFS; to request redundancy in the scientific review of all capital cases by more than one
scientist; to secure the full cooperation of DFS in providing logistical support to the
scientific team; and to convey the final report of the scientific team to you for any
further action you deem appropriate.

In selecting the members of the scientific review team, I sought and received
input from a number of sources, including DFS officials, the Chairman of ASCLD/LAB,
criminal defense attorneys with familiarity in DNA analysis issues, two Fellows of the
American Academy of Forensic Sciences, and the “special master” in charge of an
ongoing review of the Houston, Texas DNA laboratory. I used the input I received from
all of these sources in selecting the ultimate members of the scientific team. The five
independent scientists I chose as members of the review team are as follows: (1) Arthur
J. Eisenberg, Ph.D., Professor and Director of the DNA Identity Laboratory at the
University of North Texas Health Science Center, and former Chairman of the United
States DNA Advisory Board, (2) Stephen J. Lambert, Ph.D, Forensic DNA Analyst for
the South Carolina Law Enforcement Division, and Instructor of Forensic Analytical
Chemistry at the University of South Carolina, (3) Demris A. Lee, Technical Leader of
the Nuclear DNA Section at the Armed Forces DNA Identification Laboratory and a FBI
Certified DNA Auditor, (4) Carl A. Sobieralski, Jr., Statewide Technical Leader of the
DNA Unit for the Indiana State Police, ASCLD/LAB Inspector, DAB Document Auditor,
NFSTC Inspector and NFSTC ISO Assessor, and Instructor of Forensics at Concordia
University and Ivy Tech State College, and (5) Christine S. Tomsey, Forensic DNA
Manager of the DNA Laboratory for the Pennsylvania State Police, ASCLD/LAB
Inspector, and Fellow of the American Academy of Forensic Sciences. At my request, Dr. Eisenberg performed the function of team leader.

On June 13, 2005, the scientific team began their work following an orientation meeting chaired by me at the DFS headquarters in Richmond, during which I charged the members of the team with their task. Upon their own request, several members of the media attended this orientation meeting as well. Now, after substantively reviewing the files and supporting data of the selected cases, the scientific team has advised me that they have finished their work and have completed a joint report detailing their unanimous findings and recommendations. The joint report of the scientific team is attached as Appendix A.

Infallibility is too much to expect of any human endeavor certainly including scientific analysis. No matter how educated, experienced or well trained the scientist or technician, human frailty or equipment breakdown insure that mistakes can and eventually will occur. Given the increasing reliance by the Commonwealth’s criminal justice system on the DNA identification techniques used by DFS, those mistakes that inevitably do occur and which go undetected and uncorrected may result in the nightmare that all criminal justice professionals fear the most – an innocent person wrongfully convicted or a guilty person mistakenly exonerated. While on-going training and state-of the art equipment can minimize them, we cannot completely prevent the occasional human error or equipment malfunction from ever occurring. Within our ability to anticipate such things, credibility of laboratories is achieved and maintained by the creation and periodic revision of a system of internal checks, audits and peer review that are sufficiently comprehensive to detect such errors or malfunctions before a final scientific report is tendered to the criminal justice system.

DFS has long had a tradition of openness and a reputation for the unbiased reporting of scientific results and while it has had in place a comprehensive system of internal review which has detected and corrected analytical anomalies in the past, in the light of the findings of the ASCLD/LAB report and the attached report of the scientific

3 Copies of the scientific team members’ Curricula Vitae are attached as Appendix B.
team, these internal review procedures clearly leave room for improvement. To the credit of DFS, following their receipt of the ASCLD/LAB interim report and prior to my appointment as “special master”, the department had already begun a thorough re-examination of their internal review procedures. ⁴

In my view, the four global recommendations suggested by the scientific review team, if implemented and combined with the other remedial measures already proposed by DFS in response to the ASCLD/LAB interim report, would improve and further standardize the internal quality control where low-level DNA analyses are concerned, particularly where they involve multiple contributors of DNA material. I therefore endorse all of these recommendations unreservedly.

Finally, although I have already done so privately, I want to publicly express the profound gratitude of the citizens of Virginia and my personal thanks to the members of the scientific team for coming to Virginia’s aid on relatively short notice and donating their time to conduct this review.

Respectfully Submitted,

[Signature]

Robert J. Humphreys,
Special Master

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⁴ I have included the DFS responses to the remaining ASCLD/LAB recommendations as Appendix C for completeness and to provide a context for these additional recommendations.
September 1, 2005

The Honorable Robert J. Humphreys
477 Viking Drive, Suite 300
Virginia Beach, VA 23452

Dear Judge Humphreys:

The five members of the scientific team you selected first met on June 13, 2005. We began our work immediately following the orientation meeting during which you provided us with the parameters of our task. Over the course of the ensuing week and pursuant to the mission you gave us, we randomly selected 63 cases for review in addition to those cases already selected for review by virtue of their involvement with either Mr. Ban or the death penalty for a total of 123 cases ultimately reviewed by the scientific team. During the ensuing week together, we divided the cases among ourselves and reviewed the files and electronic data, made notes and gathered copies of any documentation contained in DFS files deemed pertinent for further review upon our return to our home jurisdictions. Virginia’s Division of Forensic Science was exceptionally helpful in producing all reports, notes, scientific data, making their protocols and policy manuals available for review and copying and in making analysts and supervisors available for interview by members of the scientific team.

We have now completed, to our satisfaction, a thorough review of more than double the number of DNA cases analyzed by DFS that were recommended for review by the ASCLD/LAB interim report including, as requested, a review of all of the DNA cases involving a charge of
Capital Murder from 1994 forward. The following is a summary of our findings and recommendations.

I. BACKGROUND

The Virginia DFS is comprised of four separate laboratories: the Eastern, Central, Northern, and Western Divisions. Currently, DFS uses the Fluorescent Detection PCR-Based STR DNA Protocol PowerPlex® 16 Bio System (“PowerPlex® 16”) at all four laboratories. DFS has been accredited by ASCLD/LAB since January 5, 1989.

II. MANDATE AND METHODOLOGY

The mandate of this scientific review team as stated in the ASCLD/LAB report of April 9, 2005 was to conduct “a thorough examination of a minimum of 50 cases in the Virginia system dealing with low level DNA . . . to determine whether process errors occurred and whether conclusions are scientifically supported.” Specifically, we were asked to “determine whether the selected cases have deficiencies that substantially affect the integrity of the results in those cases.” In addition to selecting some cases randomly, we were asked to implement the ASCLD/LAB interim report’s recommendation to “[c]onduct a review of [Jeffrey Ban’s] casework . . . from cases in and around 2000 and forward, particularly in cases in which there [was] low level DNA . . . to determine if the conclusions [in those cases] are scientifically supported by the data.”

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5 With the exception of the capital case examined in the ASCLD/LAB review, DFS has not conducted any other analyses of DNA material retrieved from a heat-fixed, stained histological slide of the type used in that capital case. Accordingly, this review focused on low-level DNA cases. Analyzing DNA material from such slides would be highly unusual and no member of the scientific review team has attempted to analyze DNA from a stained and heat-fixed histological slide of this type, and we note that the nature of such slides makes it extremely difficult to secure sufficient DNA tissue for analysis. Accordingly, we make no recommendation
In accordance with this mandate, we ultimately selected a total of 123 cases for thorough review from all four of the regional DFS laboratories. First, we reviewed twenty-eight capital cases, representing all of the capital cases involving levels of DNA that were analyzed by DFS on or after January 1, 1994. Second, we selected all thirty-three cases—including one capital case—that were analyzed by Jeffrey Ban on or after January 1, 1999. Third, we randomly selected fifty-three additional low-level DNA cases from among all examiners in all DFS divisional laboratories. Cases from all analysts system-wide were selected for review. Finally, we randomly selected ten additional cases for review. The selection of these ten additional cases was not made dependent upon the concentration of DNA in the sample that had been analyzed.

Each of the selected case files was reviewed by at least one of the five independent scientists comprising this review team. Each capital case was reviewed separately by at least two of the five independent scientists. During this review, the team members substantively reviewed each case file to evaluate whether the file reflected any deviations from protocol and/or conclusions that were not scientifically supported by the data.

III. FINDINGS

After substantively reviewing the 123 selected cases, we find as follows:

1. Of the twenty-eight capital cases reviewed, none of the cases contain a technical procedural error or deviation from accepted scientific protocol that “substantially affect[ed] the integrity of the results in those cases.”

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6 For purposes of this review, a “low-level DNA case” is defined as any case containing an evidence sample with an observed DNA concentration of less than or equal to 0.325 ng/5μL.

7 A complete list of the cases reviewed is attached.

8 The capital murder cases of Robin Lovitt and of Leon Jermain Winston, discussed in more detail in Part IV, were reviewed by all five members of the scientific team.
2. Of the twenty-eight capital cases reviewed, one of the cases contains an interpretive conclusion with respect to one of the items analyzed that we believe to be inappropriate. For further discussion of this case, see Part IV, infra. Of the remaining twenty-seven cases, none contain an interpretive conclusion that we consider to be inappropriate.

3. Of the thirty-three cases analyzed by Jeffrey Ban on or after January 1, 1999, none of the cases contain a technical procedural error or deviation from accepted scientific protocol that “substantially affect[ed] the integrity of the results in those cases.”

4. Of the thirty-three cases analyzed by Jeffrey Ban on or after January 1, 1999, none of the cases contain an interpretive conclusion that is not scientifically supported.

5. Of the fifty-three randomly selected low-level DNA cases, none of the cases contain a technical procedural error or deviation from accepted scientific protocol that “substantially affect[ed] the integrity of the results in those cases.”

6. Of the fifty-three randomly selected low-level DNA cases, none of the cases contain an interpretive conclusion that is not scientifically supported.

7. Of the ten additional randomly selected DNA cases, none of the cases contain a technical procedural error or deviation from accepted scientific protocol that “substantially affect[ed] the integrity of the results in those cases.”

8. Of the ten additional randomly selected DNA cases, none of the cases contain an interpretive conclusion that is not scientifically supported.

9. It appeared to all of us that, over the time period covered by our review, DFS analysts have been consistently conservative in making allele calls, tending to disregard faint bands that could arguably be reported as alleles.

10. However, of the 123 cases reviewed, none of the cases contain an allele call that resulted in an individual being excluded from consideration who should have been included.

11. Similarly, of the 123 cases reviewed, none of the cases contain an allele call that resulted in an individual being included for consideration who should have been excluded.

Based on the above-described findings, we further find as follows:

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9 The scientific team did not review the capital case analyzed by Mr. Ban that was the subject of the ASCLD/LAB interim report.
1. The testing methods employed by DFS contain no endemic deficiencies that may have substantially affected the results of low-level DNA cases analyzed by the laboratory in the past, or that may substantially affect the results of low-level DNA cases to be analyzed in the future.

2. Over the time period covered by this review, DFS has consistently conducted its low-level DNA testing within the boundaries of established and scientifically accepted practices.

IV. STATISTICAL INACCURACY: CASE NUMBER W02-3662

In one capital case, however, we find that the interpretive statistical conclusion reached by the analyst was inappropriate with respect to one of the items analyzed. In this case, numbered W02-3662, involving Leon Jermain Winston, DFS examined twenty-seven separate items for trace DNA evidence. One of the items found to contain human DNA, number 200, was "isolated from a sample from the inside of [a] right glove and sample from the inside of [a] left glove." The DNA mixture recovered from Item 200, along with DNA samples from seven other items of evidence, were amplified and typed using the PowerPlex® 16 Bio System.

The DFS analyst identified five possible contributors to the DNA recovered from the gloves: the two victims, Rhonda and Anthony Johnson, and three suspects, Leon Winston, Kevin Brown, and David Hardy. As reported by the analyst and reviewed by us, the PowerPlex® typing for the left glove and these five individuals yielded the following results:  

\[ \text{\footnote{Numbers typed in parentheses ( ) were reported as appearing less intense than types not in parentheses.}} \]
<table>
<thead>
<tr>
<th>Locus</th>
<th>Left Glove</th>
<th>Rhonda Robinson</th>
<th>Anthony Robinson</th>
<th>Leon Winston</th>
<th>Kevin Brown</th>
<th>David Hardy</th>
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<tr>
<td>FGA</td>
<td>19, 20, 21, (22), (23)</td>
<td>22, 24</td>
<td>23, 24</td>
<td>22, 22</td>
<td>19, 23</td>
<td>20, 21</td>
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<td>TPOX</td>
<td>11, 11</td>
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<tr>
<td>D8S1179</td>
<td>(13), 14</td>
<td>11, 13</td>
<td>13, 15</td>
<td>14, 14</td>
<td>14, 14</td>
<td>13, 14</td>
</tr>
<tr>
<td>VWA</td>
<td>(16), 17, (20)</td>
<td>14, 15</td>
<td>15, 16</td>
<td>16, 16</td>
<td>16, 20</td>
<td>17, 17</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>X Y</td>
<td>X X</td>
<td>X Y</td>
<td>X Y</td>
<td>X Y</td>
<td>X Y</td>
</tr>
<tr>
<td>Penta E</td>
<td>12, 14</td>
<td>12, 18</td>
<td>7, 8</td>
<td>12, 15</td>
<td>8, 14</td>
<td>11, 12</td>
</tr>
<tr>
<td>D18S51</td>
<td>(15), 16, (17)</td>
<td>13, 15</td>
<td>15, 17</td>
<td>15, 15</td>
<td>16, 16</td>
<td>16, 17</td>
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<tr>
<td>D21S11</td>
<td>30, 30</td>
<td>30, 32.2</td>
<td>28, 29</td>
<td>32.2, 32.2</td>
<td>27, 28</td>
<td>30, 30</td>
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<td>TH01</td>
<td>7, 8</td>
<td>7, 8</td>
<td>7, 9.3</td>
<td>7, 7</td>
<td>8, 8</td>
<td>6, 7</td>
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<tr>
<td>D3S1358</td>
<td>(14), 15, (16)</td>
<td>14, 16</td>
<td>16, 18</td>
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<td>14, 17</td>
<td>15, 16</td>
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<tr>
<td>Penta D</td>
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<tr>
<td>D5S818</td>
<td>11, (12) (13)</td>
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<td>11, 12</td>
<td>11, 12</td>
<td>12, 13</td>
<td>11, 11</td>
</tr>
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</table>

Based on these typing results, the analyst concluded as follows:

The DNA profile obtained from the sample from the left glove (Item 200) is consistent with a mixture. Leon Jermain Winston, Kevin Eugene Brown, and David Ralph Hardy cannot be eliminated as possible co-contributors to the genetic material obtained from the sample form the left glove. Rhonda Whitehead Robinson and Anthony McKinley Robinson are each eliminated as a possible contributor to this genetic material.
However, to attach statistical weight to this conclusion, the analyst employed a likelihood ratio,\textsuperscript{11} using the alleles reported at just five of the fifteen tested loci. Specifically, the analyst used the alleles reported at loci FGA, D8S1179, vWA, D18S51, and D5S818 to conclude that the DNA profile reported at those five loci is

1.8 billion times more likely if it originated from Leon Jermain Winston, Kevin Eugene Brown, and David Ralph Hardy than if it originated from (3) unknown individuals in the Caucasian population.

1.1 billion times more likely if it originated from Leon Jermain Winston, Kevin Eugene Brown, and David Ralph Hardy than if it originated from (3) unknown individuals in the Black population.

2.9 billion times more likely if it originated from Leon Jermain Winston, Kevin Eugene Brown, and David Ralph Hardy than if it originated from (3) unknown individuals in the Hispanic population.

It is evident that the five loci selected by the analyst were the only loci indicating the presence of all the alleles from each of the three suspected contributors. In other words, the analyst, using a likelihood ratio protocol, selected only those loci that “fit” her formulated hypothesis (specifically, that the DNA mixture isolated from the left glove contained the DNA of all three suspects), and disregarded the loci that did not necessarily “fit” that hypothesis. The analyst similarly failed to document why the reference samples for Rhonda Robinson, Anthony Robinson, Leon Winston, and Kevin Brown—each of which failed to yield interpretable results at one or more loci—were not re-run to obtain better results.

\textsuperscript{11} Generally, analysts working with a DNA mixture must choose between two statistical analyses when determining the weight to be given a particular DNA typing result. When using the likelihood ratio methodology, the analyst formulates two hypotheses, one of which includes the alleged suspect(s) and the other of which excludes the alleged suspect(s). The analyst then employs a calculation to determine which of the two hypotheses is more likely to have occurred in that given situation. The other statistical analysis, the combined probability of exclusion (or “CPE”) methodology, merely reports a statistic indicating what percentage of a given population would be included or excluded based on the typing results in that particular case.
Based on the PowerPlex® typing results, we agree with the analyst’s conclusion that the three suspects could not be eliminated as possible contributors to the DNA mixture and that the two victims could be eliminated as possible contributors. However, we also believe that, considering the complexity of this mixture and the high probability of allelic dropout at one or more of the tested loci, rendering any statistical interpretation involving all three men, collectively, was inappropriate. In our view, the weight she gave the inclusion based on the inappropriate use of likelihood ratios instead of CPE was problematic. Accordingly, we conclude that the statistical weight given to the typing results for this item of evidence was not appropriate.

We note that the analyst’s conclusions pertaining to the PowerPlex® typing results for the remaining seven items were scientifically supported. We further note that we reviewed other low-level DNA cases that had been analyzed by this individual, and none of those cases contained similar statistical inaccuracies.

V. RECOMMENDATIONS

Based upon our overall review and in particular in view of our findings detailed in part IV, supra, we believe that certain aspects of the protocol followed by the Virginia lab, although scientifically acceptable, could be improved. Accordingly, we advance the following recommended amendments to DFS protocols:12

1. Implement written guidelines to improve analyst documentation of the rationale behind disregarding bands assigned as alleles by the PowerPlex® typing instrument.

2. Implement a written policy to provide improved guidance for selecting the appropriate statistical analysis in low-level DNA cases, including, but not limited to, the following: If a low-level mixture is deemed interpretable, a likelihood

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12 These recommendations have already been conveyed informally to DFS supervisory personnel.
ratio is only appropriate if: (1) all of the suspected contributors' alleles have been accounted for at all of the tested loci, and (2) the reported alleles do not reflect more than two possible contributors to the mixture. If any of the loci fail to reflect interpretable results or indicate the possibility of missing alleles, do not eliminate the locus (or loci) and then use a likelihood ratio for the remaining loci. A likelihood ratio is inappropriate in that situation, and a combined probability of exclusion analysis should be implemented instead.

3. Implement a written policy providing that, if a probative reference standard fails to yield a complete profile, re-run the reference sample, if practical, until a full profile is obtained.

4. Implement a written policy providing that, when practical, separate technical and administrative reviewers should be assigned for each individual case.

VI. CONCLUSION

Based upon our review of the selected cases, we conclude that there is no evidence that the protocol implemented by DFS in low-level DNA cases contains scientific inaccuracies that would render the typing results in those cases scientifically unsound. If adopted and followed, we believe that the global recommendations we outline above will prevent any future inappropriate use of statistical likelihood ratios to interpret the scientific results as outlined in the single case discussed in Part IV, supra. Finally, we note with gratitude that DFS technical and supervisory personnel were very open and forthcoming during the course of this review, and we express our appreciation for their cooperation.

Respectfully submitted,

[Signature]
Arthur J. Eisenberg, Ph.D.
Director, DNA Identity Laboratory,
University of North Texas
Professor, Department of Pathology,
University of North Texas
Former Chairman, United States DNA Advisory Board
Stephen J. Lambert, Ph.D.
Forensic DNA Analyst/Serologist, South Carolina Law Enforcement Division
DAB Document Auditor
Instructor, Forensic Analytical Chemistry,
University of South Carolina

Demiris A. Lee
Technical Leader, Nuclear DNA Section,
Armed Forces DNA Identification Laboratory
FBI Certified DNA Auditor

Carl A. Sobieralski, Jr.
Statewide Technical Leader, Indiana State Police, DNA Unit
Forensic Scientist Supervisor, DNA South Zone Unit
ASCLD/LAB Inspector
DAB Document Auditor
NFSTC Inspector and NFSTC ISO Assessor
Instructor in Forensics, Concordia
University and Ivy Tech State College

Christine S. Tomsey
Forensic DNA Manager, Pennsylvania State Police, DNA Laboratory
ASCLD/LAB Inspector
Fellow, American Academy of Forensic Sciences
# LIST OF CASES REVIEWED

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<thead>
<tr>
<th>Case Number</th>
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<th>DNA Analyst</th>
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<td>T04-4403</td>
<td>Eastern</td>
<td>Betty J. Blankenship</td>
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<td>T04-805*</td>
<td>Eastern</td>
<td>Betty J. Blankenship</td>
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<td>Ruth C. Damaso</td>
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<td>Gloria D. Hill</td>
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<td>T02-10615</td>
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<td>T00-8416</td>
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<td>Anne M. Pollard</td>
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<td>David A. Pomposini</td>
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<td>Jerry W. Sellers</td>
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<td>Miriam S. Varty</td>
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<tr>
<td>T00-3797*</td>
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<td>Miriam S. Varty</td>
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</table>

* Capital murder case.
** Capital murder case, testing attempted, no interpretable results obtained.
<table>
<thead>
<tr>
<th>Code</th>
<th>Location</th>
<th>Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>T84-3787</td>
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<td>Jeffrey D. Ban</td>
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<tr>
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CURRICULUM VITAE

ARTHUR JAY EISENBERG

ADDRESS: UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER
DEPARTMENT OF PATHOLOGY AND ANATOMY
DNA/IDENTITY LABORATORY

EDUCATION:

1984
Ph.D. MOLECULAR BIOLOGY
Department of Biological Sciences
State University of New York at Albany
Research Advisor: Dr. Joseph P. Mascarenhas
Thesis Title: THE ROLE OF ABSCISIC ACID IN SOYBEAN EMBRYO DEVELOPMENT

1979
M.S. MOLECULAR BIOLOGY
Department of Biological Sciences
State University of New York at Albany
Research Advisor: Dr. Joseph P. Mascarenhas
Thesis Title: THE PRESENCE OF mRNAs IN THE MALE GAMETE OF THE AQUATIC FUNGUS ALLOMYCES AND THEIR POSSIBLE CONTRIBUTIONS TOWARDS EARLY ZYGOTE DEVELOPMENT

1977
B.S. BIOLOGY
State University of New York at Albany
Graduated Summa Cum Laude

PROFESSIONAL EXPERIENCE:

11/89 - Present
UNIVERSITY OF NORTH TEXAS HEALTH SCIENCE CENTER
POSITION: PROFESSOR, DEPARTMENT OF PATHOLOGY
DIRECTOR, DNA IDENTITY LAB
RESPONSIBILITIES: Develop and Administer a Clinical Reference Laboratory utilizing DNA Probe Technology for the determination of Paternity, forensic identification, cancer diagnostics, and other genetic diseases. Lecture on the application of molecular biological methodology in Pathology courses.

9/91 - Present
UNIVERSITY OF NORTH TEXAS
POSITION: ASSOCIATE MEMBER GRADUATE FACULTY,
DEPARTMENT OF BIOLOGY

22
PROFESSIONAL EXPERIENCE (CONT.)

RESPONSIBILITIES: Lecture in a variety of courses involving DNA methodology. Supervise the research activities of Graduate students in my Laboratory.

7/89 - 10/89
LIFECODES CORPORATION

DIVISION: DIAGNOSTICS
POSITION: ASSISTANT DIRECTOR OF TECHNOLOGY TRANSFER AND IDENTITY DEVELOPMENT

RESPONSIBILITIES: Direct the design and validation of new procedures and techniques which can be applied to the development of simple, cost effective products in the area of DNA identity testing. The transfer of new procedures and products to the manufacturing Division. Technical Training of the Sales and Marketing Division. Technical support for both the Reference Lab and the Identity Training Program.

3/87 - 6/89
LIFECODES CORPORATION

DIVISION: DIAGNOSTICS
POSITION: MANAGER OF TECHNOLOGY TRANSFER AND IDENTITY DEVELOPMENT

RESPONSIBILITIES: The design and validation of new procedures and techniques which can be applied to the development of simple, cost effective products in the area of DNA identity testing. The transfer of new procedures and products to the Manufacturing Division. Technical training of the Sales and Marketing Division. Technical support for both the Reference Lab and the Identity Training Program. Development of a QA/QC program for DNA Identity Testing.

3/86 - 2/87
GENCARE BIOMEDICAL CORPORATION

POSITION: SCIENTIFIC DIRECTOR
RESPONSIBILITIES: Creation of a Clinical Reference Laboratory to perform DNA based cancer diagnostic tests. Development and implementation of a variety of DNA diagnostic tests in the area of Haemopoetic Cancers. Training of Oncologists and Hematologists in the clinical utility of DNA based cancer diagnostic tests for leukemia's and lymphomas.

10/84 - 2/86
LIFECODES CORPORATION

DIVISION: CLINICAL LABORATORY
POSITION: MANAGER OF CLINICAL GENETICS
RESPONSIBILITIES: Development of a Clinical Reference Laboratory utilizing DNA probe technology to perform testing in the areas of cancer diagnostics, prenatal diagnostics, paternity, and forensics. Coordinate and supervise the activities of 6 technicians.
PROFESSIONAL EXPERIENCE (CONT.)

3/84 - 10/84

LIFECODES CORPORATION

DIVISION: RESEARCH AND DEVELOPMENT
POSITION: POST-DOCTORAL RESEARCH ASSOCIATE
RESPONSIBILITIES: Development of diagnostic assays for various genetic disorders.

1/86 - 10/89

SARAH LAWRENCE COLLEGE

DIVISION: GRADUATE PROGRAM HUMAN GENETICS
POSITION: FACULTY
RESPONSIBILITIES: Instruction in Human Genetics Laboratory; theory and application of molecular biology towards the diagnosis of genetic disorders.

1/82 - 9/83

STATE UNIVERSITY OF NEW YORK AT ALBANY

POSITION: LECTURER
RESPONSIBILITIES: Teach introductory Biology course.

PROFESSIONAL AFFILIATIONS:

Invited Member and former Chairman of the United States DNA Advisory Board

Invited Member of the Histocompatibility and Identity Committee of the College of American Pathology

Invited Member of the National Forensic DNA Review Panel

Invited Member of the Federal Bureau of Investigation’s Scientific Working Group on DNA Analysis Methods (SWGDAM) formerly known as TWGDAM

Member and Former Chairman of the Association of Forensic DNA Analysts and Administrators

Member of American Association of Blood Banks

Member of the American Association of Forensic Science

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A.J. EISENBERG Ph.D.
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PUBLICATIONS:


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PUBLICATIONS (CONT.)


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PUBLICATIONS (CONT.)


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PRESENTATIONS AND PUBLISHED ABSTRACTS


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PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


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PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


A.J. EISENBERG Ph.D.
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PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


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PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


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PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


A.J. EISENBERG Ph.D.
PAGE 17
PRESENTATIONS AND PUBLISHED ABSTRACTS (CONT.)


STEPHEN J. LAMBERT
Forensic DNA Analyst/ Serologist
South Carolina Law Enforcement Division
Forensic Science Building
4416 Broad River Road
Columbia, SC 29210

EDUCATION:

B.S., Mars Hill College, Mars Hill, North Carolina (1983)
Ph.D., University of South Carolina, Columbia, South Carolina (1990)
DNA Typing Methods, University of Virginia, FBI Academy, Quantico, Virginia (1990)
Basic Law Enforcement, South Carolina Criminal Justice Academy, Columbia, South Carolina
(1990): Advanced DNA Typing Methods, FBI Academy, Quantico, Virginia (1992)

PROFESSIONAL EXPERIENCE:

Forensic DNA Analyst/Serologist, South Carolina Law Enforcement Division, Columbia, South
Carolina (1990 – Present)
Visiting Scientist, FBI Forensic Science and Research Training Center (FSRTC), FBI Academy,
Quantico, Virginia (1990)
Scientific Working Group on DNA Analysis Methods (SWGDAM) – Member, 1999-2003;
Invited Guest, 2003-present.

TEACHING EXPERIENCE:

Forensic Analytical Chemistry (CHEM 622), Instructor, Department of Chemistry, University of
South Carolina, Columbia, South Carolina (1997-present)
Invited Faculty, National District Attorneys Association's National Advocacy Center, Columbia,
South Carolina (1998-present)
Invited Faculty, National Forensic Academy (NFA), Knoxville, TN (2002)

PUBLICATIONS:

B59
B60
DEMIRIS ANITA LEE
Curriculum Vitae

The Armed Forces DNA Identification Laboratory
1413 Research Boulevard, Bldg. 101
Rockville, MD 20850

EDUCATION:


Bachelor of Science, Virginia Commonwealth University, Richmond, Virginia 1990.

EMPLOYMENT:

Nov. 1999 - present
Technical Leader Nuclear DNA Section, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Rockville, MD

Dec. 1994 - Nov. 1999
Chief Nuclear DNA Analyst, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Rockville, MD

Senior Nuclear DNA Analyst, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Rockville, MD

Acting DNA Analyst, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Washington, D.C.
DNA Technician, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Washington, D. C.

Intern, Armed Forces DNA Identification Laboratory, Office of the Armed Forces Medical Examiner, The Armed Forces Institute of Pathology, Washington, DC

PROFESSIONAL AFFILIATIONS:

American Academy of Forensic Science, Full Member.
Mid-Atlantic Association of Forensic Sciences

CONTINUING EDUCATION:


"The CSI Effect", American Association for the Advancement of Sciences, Washington D.C., February 2005

"Seven Habits of Highly Effective Managers", Franklin Covey Workshop, Baltimore MD, January 2005


Casualty Conference, Honolulu, HI, February 2004

14th International Symposium on Human Identification, Promega, Phoenix, AZ, October 2003.
Parentage Minisymposium, Promega, Phoenix, AZ, October 2003.


Mid-Atlantic Association of Forensic Scientists Annual Meeting, Frederick, MD, April 2002


DNA Auditor Training, FBI Academy, Quantico, VA, June 2001.


STR DNA Workshop, First International Conference on Forensic Human Identification in the Millennium. October 1999

"How To Be A Highly Successful Team Leader", Rockhurst College, Gaithersburg, MD, June 1999.


Mid-Atlantic Association of Forensic Scientist, Ocean City, MD April 1999.
STR Interpretation Workshop, Mid-Atlantic Association of Forensic Scientist, Ocean City, MD April 1999.


Presenting Your Ideas and Work, Dun & Bradstreet Business and Education Services, Silver Spring, MD August 1998.


The Application of DNA STR Analysis in Forensic Science, Royal Canadian Mounted Police, Ottawa, ON, June 1998.

11th Annual Course Forensic Anthropology, Bethesda, MD, May 1997

Microsoft Excel 97 Introduction, CompUSA Computer Training, Gaithersburg, MD, March 1998.

Microsoft Word 97 Intermediate, CompUSA Computer Training, Gaithersburg, MD, March 1998.


Statistical Genetics for Forensic Scientists, Armed Forces DNA Identification Laboratory Education Series, September 1997

10th Annual Course Forensic Anthropology, Bethesda, MD, June 1997


The Seventh International Symposium on Human Identification, Scottsdale, AZ, September 1996.


The Sixth International Symposium on Human Identification, Scottsdale, AZ, October 1995.


Methods and Advanced Techniques In Human Identification, Bethesda, MD, June 1994.

Future Technologies For DNA Analysis, 4-5 October 1993.


DNA Databanks and Repositories, Rockville, MD, May 1993.


ABSTRACTS AND PRESENTATIONS (*-Presenting author):

“The Effect of Formalin Decontamination on STR Analysis Conducted on Human Remains Submitted for Identification”,

44
American Academy of Forensic Sciences, Dallas, TX, February 2004.

"Decontamination of Human Autopsy Specimens by $^{60}$Co Gamma Photon Irradiation and Human DNA Identification by Short Tandem Repeat Analysis of Irradiated Tissues", American Academy of Forensic Sciences, Dallas, TX, February 2004.


"Validation and Optimization of Reliagene’s Y-PlexTM 6 kit for Processing Nuclear DNA Case Samples at the Armed Forces DNA Identification", 14th International Symposium on Human Identification, Promega, Phoenix, AZ, October 2003.


"The DOD Registry’s Response to the Terrorists Attacks of 11 September, MVI, Lyon, France, May. 2002.*


"Forensic DNA Identification in Military Casework. MAAFS, Frederick, MD, April 2002.

"Matrix Files: Problems and Solutions (Obtaining Acceptable Data For Easy Interpretation)", 11th International Symposium on Human Identification, Promega, Biloxi, MS, October 2000.


"Validation of a Protocol to Extract DNA from Urine Samples for Short Tandem Repeat Analysis", AAFS, Reno, NV, February 2000.


"Mutation Rate for 13 Short Tandem Repeat Loci.", First International Conference on Forensic Human Identification in The Millennium. October 1999*


"The Use of DNA Analysis in Human Remains Identification", AFIP Anthropology Course, May 1999.*


"Don't Be A Doubting Thomas!", MAAFS, Ocean City MD, April 1999.*


"Mixed Sample Evaluation Using AmpFLSTR Profiler Plus on the ABI PRISM 310 Genetic Analyzer and ABI 377 DNA


"An Introduction to Forensic DNA", DeMatha Science Fair, Hyattsville, MD, May 1998.*

"DNA Analysis in Mass Disaster", Armed Forces Institute of Pathology Forensic Anthropology Course, Bethesda, MD, May 1998.*


"DNA Analysis of Pap Smear Slides, Biopsy Slides and Paraffin Blocks: Four Cases Studies, 49th Annual Meeting of


"Validation of the ABI Prism™ STR Primer Sets for Use in a Multiplex System on the 373 DNA Sequencer" The Sixth International Symposium on Human Identification, Scottsdale, AZ, October 1995.


"The Use of DNA Analysis to Identify Human Remains From the Waco, Texas Incident. American Academy of Forensic Scientist. February 1995. Seattle, WA.*

"Short Tandem Repeat Loci: Application to Forensic and Human Remains Identification", The American Academy of Forensic Sciences, Feb. 1993, Boston MA.*


PUBLICATIONS:


"Identification of Human Remains Using

CURRICULUM VITAE

CHRISTINE S. TOMSEY
PENNSYLVANIA STATE POLICE DNA LABORATORY
80 North Westmoreland Avenue
Greensburg, Pennsylvania 15601

EDUCATIONAL BACKGROUND:
Degree 1971 - BS in Chemistry, University of Pittsburgh
Degree 1972 - MS in Chemistry, University of Pittsburgh
Subsequent courses in Immunohematology, Genetics, Molecular Biology
and Statistics.

PROFESSIONAL ORGANIZATIONS:
Fellow -American Academy of Forensic Sciences
Member -Mid-Atlantic Association of Forensic Sciences

COMMITTEES:
Member - SWGDAM (Scientific Working Group on DNA Analysis Methods)
Member -SWGDAM Executive Board
Member -Potomac Region DNA Quality Assurance Audit Group
Member -National Institute of Justice Review Panel for Research Grants
Chairperson -MidAtlantic Association of Forensic Scientist DNA Auditors Training and
Certification Committee
Auditor -American Society of Crime Laboratory Directors Laboratory Accreditation
Board (ASCLD/LAB)

BOOK REVIEWS:
DNA IN THE COURTROOM, by Howard Coleman & Eric Swenson. Pub. by Genelex Corp.
1994

HANDBOOK OF CRIME SCENE INVESTIGATION, by Harrison Allison.
PUBLICATIONS:


EXPERIENCE:

January 1994 to present – Forensic DNA Manager, Pennsylvania State Police DNA Laboratory
Manages Pennsylvania State Police DNA laboratories. Manager and Coordinator of the Commonwealth of PA DNA Convicted Offenders Laboratory. DNA Technical Leader for Pennsylvania State Police DNA Laboratory.

October 1989 to January 1994 -- Forensic Scientist Supervisor, Pennsylvania State Police DNA Laboratory
Established and supervises the PSP DNA Unit. DNA Technical Leader for the Pennsylvania State Police DNA Laboratory.

May 1972 to September 1989 -- Forensic Scientist, Pennsylvania State Police Laboratory.
Primary field of analysis was Forensic Serology

1974 to 1977 (part-time) -- Instructor, Indiana University of Pennsylvania, Department of Criminology

1968 to 1972 (part-time) -- Research Assistant, University of Pittsburgh School of Medicine, Department of Physiology
Carl A. Sobieralski Jr.
8500 East 21st Street
Indianapolis, IN 46219

Education:
Indiana University at South Bend
Degree: Bachelor of Science
Major: Biology

Indiana University-Purdue University at Indianapolis
Degree: Masters of Science
Major: Biology


Career Enhancements:

- Currently DNA Supervisor of the DNA South Zone Unit
- Currently Statewide Technical Leader for the DNA unit.
- Currently performing DNA casework for the Indiana State Police Laboratory.
- ASCLD/ LAB Inspector.
- Diplomat of The American Board of Criminalists (D-ABC)
- DAB Document Auditor
- Attended and successfully completed ASCLD/ LAB Inspector School. (October 2001)
- Attended and successfully completed DNA Audit Document Auditor Training. (October 2001 and December 2004)
- Instructor in Forensics at Concordia University and Ivy Tech State College (Since 2002)
- Attended and successfully completed Instructor Development School (March 2002)
- Completed 40 hour course in Blood Spatter (April 2002)
- Performed Trace casework for the Indiana State Police Laboratory (ISP). (July 1999 – June 2001)
- Currently work as a Chemist on the ISP Clandestine Laboratory Team.
- Member of Clandestine Laboratory Investigating Chemists (CLIC)
- Member of International Association of Bloodstain Pattern Analysts (IABPA)
- Testified as an expert witness in Glass, DNA, Serology, Arson Clandestine Lab Recognition, Lamps and Hair.
- Statistics Workshop (November 2004)
- Capillary gel Analysis training (August 2004)
- Attended and successfully completed the DEA Clandestine Laboratory Investigation and Analysis Certification course. (August 2000).
- Attended and successfully completed Basic Mass Spectral Interpretation (May 2000).
- Attended and successfully completed Methamphetamine Clandestine Laboratory Investigation and Analysis (June 2000).
- Worked on the Internal Validation and population studies for PowerPlex 1.1, DQA1, PM, and DNA80 systems.
- Lectured numerous times of Forensic Science and DNA.
- Worked on the Internal Validation studies for PowerPlex 16
- Attended the Advanced DNA technology Workshop (May 03 and May 04)
- Attended the Megaplex STR Meetings (March 02 through March 04)
• Attended the Promega STR Meetings (1996, 2001 and 2003)
• Attended the Promega STR Educational Forum (September 2001 and 2003)
• Petroleum Refinery Process Workshop (April 2001)
• Chemistry of Color Workshop (April 2001)
• Attended and successfully completed Annual Clan Lab Recertification (2000-04)
• Attended the American Academy Meeting. (February 1997)
• Attended the Promega STR Workshop. (May 1997)
• Attended the Promega Statistics Workshop. (September 1996 and October 2001)
• Member of Member of Midwestern Association of Forensic Scientists (MAFS). Attended the 90, 94, 95, 96 and 99 meetings.
• Attended the SAFS 1999 meeting.
• Attended the MAAFS/SAFS 2001 meeting.
• Worked full time with the FBI on projects for the development of Amp-flps as a visiting scientist. (February 1992-June 1992)
• Attended and successfully completed the FBI Advanced aspects of forensic DNA analysis course. (March 1992)
• Attended and successfully completed the FBI Forensic Application of DNA Typing Methods Course (along with lab). (January 1992-February 1992)
• Attended and successfully completed the Roche Molecular Systems Forensic AmpliType PCR Amplification and Typing Workshop. (March 1993)
• Working serological and hair cases for the ISP Laboratory. (June 1992-present)
• The ISP has put me through an extensive 14-month training program in forensic serology and hair comparisons. I successfully completed
oral and written examinations, along with proficiency tests for each method of analysis.

- Attended and successfully completed the FBI Basic Forensic Serology Course. (February 1991)
- Attended and successfully completed the Analytical Genetic Testing Center’s Isoelectric Focusing course. (June 1991)
- The Indiana University Medical Center employed me as a Sr. Research Technician, which exposed me to DNA analysis. The work required the DNA analysis of leukemia patients for abnormalities in Chromosomes 9 and 22. This required the manipulation of DNA with restriction enzymes. The ability to get vectors in and out of plasmids which are contained in bacteria. The ability to perform Southern Blots and the subsequent development of autoradiograms. To label probes with $^{32}$P. An understanding of biochemistry, molecular biology, tissue culture, restriction enzymes, and radioactive probes as it pertains to DNA isolation, purification, and examination. Learned the techniques, application, and uses for PCR as it pertains to DNA and RNA. (November 1989-May 1990)
- Successfully completed radiation safety course and was lab room radiation safety officer. (December 1989)
APPENDIX C

DFS PROPOSED RESPONSES TO THE REMAINING RECOMMENDATIONS IN THE ASCLD/LAB INTERIM REPORT

1. *Conduct validation studies on the extraction procedures of DNA from mounted slides.*

The validation study to extract DNA from mounted smears will be performed in three phases. Phase I is designed as a control study to demonstrate that DNA can be successfully isolated from heat-fixed vaginal smears mounted in a permanent mounting media containing a mixture of vaginal and sperm cells. Phase II is designed to evaluate the procedure outlined below to demonstrate that the DNA can be isolated from actual non-probative vaginal smears of varying ages. Once the procedure for isolating DNA from heat-fixed, stained, and mounted smears has been shown to work effectively, Phase III will to transfer the technology to one of the Division’s Regional Laboratories to demonstrate that the procedure can be reproduced successfully.

In addition, a survey of other forensic DNA testing laboratories will be conducted to determine how stained, heat-fixed, and mounted slides containing a mixture of vaginal and sperm cells consisting of a low level of sperm are processed.

The procedure DFS will follow is detailed further below:

**VALIDATION STUDY ON THE EXTRACTION PROCEDURES OF DNA FROM MOUNTED SLIDES**

Phase I (Control Set) - This portion of the study will be conducted in the Forensic Biology Section of the Central Laboratory of the Virginia Division of Forensic Science:

- Ten saliva swabs, used as known standards, and 5 postcoital swabs will be collected to prepare vaginal smears. The vaginal smears will be dried at room temperature for a minimum of 1 week and the cellular material heat fixed to the smears, stained and mounted in permanent mounting media.
- The cellular material will be scraped from the smear using a clean razor blade and transferred into a 1.5 mL microcentrifuge tube.
- The samples will be processed as described in the procedure outlined below.

Phase II (Experimental Set) - This portion of the study will be conducted in the Forensic Biology Section of the Central Laboratory of the Virginia Division of Forensic Science:

- Fifteen saliva swabs or bloodstains, used as a known standard from the vaginal cell donor and 15 heat fixed, stained and mounted vaginal smears of varying ages will be examined during this study.
- The procedure outlined below will be used during this validation study.

Phase III (Extension of Experimental Set) - This portion of the study will be conducted in the Forensic Biology Section of one of the Regional Laboratories of the Virginia Division
of Forensic Science:

- Ten saliva swabs or bloodstains, used as a known standard from the vaginal cell donor and 10 heat fixed, stained and mounted vaginal smears of varying ages will be examined during this study.
- The procedure outlined below will be used during this validation study.

Procedure:

1. While examining the fixed, stained and mounted vaginal smear under the microscope, photograph the smear as a permanent record. Also document the number of spermatozoa that are observed on the smear.

2. Soak the vaginal smear in xylene overnight to remove the cover slip and mounting medium. Slides that contain an excess of mounting media may need to soak in xylene for a prolonged period of time.

3. Scrape the cells from the slide with a clean razor blade and transfer the cellular material into a 1.5 mL microcentrifuge tube.

   **NOTE:** After scraping the material from the slide, examine the slide microscopically to determine if cellular material (in particular sperm cells), is still attached to the slide. If the sperm cells are still attached to the slide, process the slide using the contingency procedure outlined in steps 5B – 9B below.

4. Add 1.0 mL of 95% Ethanol to the microcentrifuge tube to wash the xylene from the cellular material. Briefly vortex the microcentrifuge tube at room temperature.

5. Place the tube into a microcentrifuge and spin for 1-2 minutes at ~10,000 rpm to force the cellular material to the bottom of the tube.

6. Using a P1000 pipette remove the supernatant and discard.

7. Repeat steps 4 through 6 an additional time.

8. Place the tube in DNA concentrator/evaporator for approximately 5 minutes to remove the excess 95% Ethanol and to dry the sample.

9. Add:

   - 400 μL TNE
   - 25 μL 20% Sarkosyl
   - 75 μL Sterile Type I Water
   - 5 μL Proteinase K
10. Mix by hand or light vortex

11. Place the tube into a 37°C incubator or heat block for a minimum of 2 hours. **NOTE:** If the slide was processed further to remove additional cellular material (i.e. sperm cells), set the tube aside until the digest on the slide has been completed.

12. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm to pellet the sperm cells. **NOTE:** If the slide was processed further to remove additional cellular material (i.e. sperm cells), combine the samples into one tube before spinning the tube in the microcentrifuge.

13. Using a pipette, carefully transfer all but approximately 50 μL of the supernatant into a new 1.5 mL labeled tube with a lid. Be careful not to dislodge or disturb the pellet on the bottom of the tube. The supernatant removed from the pellet is the NONSPERM FRACTION. The remaining 50 μL of supernatant contains the SPERM FRACTION/SPERM PELLET.

14. At this stage set the non-sperm fraction tube aside and wait until the sperm fraction is ready (step 21), and then proceed to Section III, Chapter 2, Step 2.6, Microcon Purification Procedure, of the section's procedures manual.

15. Wash the pellet as follows: Resuspend the pellet in 500 μL of PCR digestion buffer by vortexing briefly. Spin the tube for 5 minutes in a microcentrifuge at a minimum of 10,000 rpm. Using a pipette with a sterile 1 mL pipette tip, remove all but 50 μL of the supernatant and discard.

16. Repeat the wash in step 15 an additional 2 times. If a low sperm count has been determined during the microscopic examination of the slide, the sperm pellet may be washed up to 5 times. After the final wash, remove all but 50 μL of the wash buffer and discard. The sperm cells are not lysed at this point.

17. Remove 3 μL of the sperm pellet and place onto a slide. Perform the Christmas Tree staining procedure as described in the Section’s procedures manual.

18. Proceed to Chapter 1, Isolation of DNA, 1.7 Organic/DNA IQ™ Extraction Method for Mixed Body Fluid Stains, starting at 1.7.11 for the sperm digest utilizing the organic procedure and then following with the Microcon® purification procedure. Add to each sperm fraction or reagent blank tube:

- 150 μL TNE
- 50 μL 20% Sarkosyl
- 40 μL 0.39M DTT
- 150 μL Sterile Type I Water
- 10 μL Proteinase K
19. Mix by hand or light vortexing then place the tube into a 56°C incubator or heat block for 2-3 hours.

20. Pulse spin the tube.

21. Proceed to Section III, Chapter 2, Step 2.6, Microcon Purification Procedure, of the section’s procedures manual 2.6, Microcon® Purification Procedure, with the sperm fraction.

**NOTE:** As a result of recent information obtained through the survey conducted thus far regarding this procedure, the following contingency procedure, steps 5B-9B, has been developed.

**Contingency experiments:**

5B Take the slide with sperm cells still attached and place on top of a clean, moistened paper towel in an enclosed Petri dish, plastic or glass, with a lid.

6B Circle the smear heavily with a wax pencil. Place the digest buffer directly on the slide over the smear within the circle of wax pencil. If the wax pencil creates problems, then cut the smear out of the remainder of the slide using a diamond tipped pencil and proceed to 6B1, otherwise proceed to 7B.

6B1 Prior to placing the cut slide into a small conical tube, clean the back of the slide with 95% ethanol to remove any contaminating cellular material from technicians who may have handled the aged slides without gloves.

6B2 Add sufficient digest buffer to submerge the smear and incubate for a minimum of 2 hours at 37°C.

6B3 Remove the glass from the conical tube and transfer the entire sample to a 1.5 mL microcentrifuge tube; then complete steps 12-21 above.

7B Place slide in the covered dish into a 37°C incubator and incubate for 2 hours. Check frequently to ensure that the liquid has not dried up. Add more digest buffer if necessary.

8B Aspirate the liquid off the slide using a P1000 pipette and place the entire sample into a 1.5 mL tube; then complete steps 12-21 above.

9B Re-examine the slide after the digest and if the sperm are still attached and no DNA results obtained, then alternative procedures will be investigated.
References:


2. Define a process to insulate the examiners from pressures that may be applied from inside and outside of the laboratory in situations similar to this case.

Legislation passed in 2001 established formal procedures for post-conviction testing to be ordered through the courts. Therefore the likelihood of such a unique request for DNA testing through a Governor’s office as in this case will be rare. In the unlikely event such a situation arises again, a panel of senior scientists shall review any such requests, make recommendations to the Division administration and provide assistance in language and/or formatting of the results of the analysis and conclusions. Such an issue may also be referred to the Department of Forensic Science’s Scientific Advisory Board for consideration.

Language formalizing this process will be incorporated into the Division’s Quality Manual.

3. Refine the technical review process to ensure that policies and protocols are followed and that conclusions are scientifically supported by the data in the case.

In September 2002, Chapter 1, Section 1.12 of the Forensic Biology Section Quality Assurance Program Manual was revised to include a specific reference to the Division’s Quality Manual, Section 14, which addresses monitoring analytical results and the associated case file documentation through the technical peer review process. Subsequently in February 2005 this chapter was revised again to include a “Guide for Review of DNA Data” to aid the Forensic Biology Section examiner during the review of the case file documentation. This review form was developed with input from the Forensic Biology Section examiners in all four to the Division’s laboratories. Since February 2005, this review form has been used routinely in conjunction with the Division’s Technical Review Form when reviewing Forensic Biology case files. A copy of Chapter I of the Forensic Biology Section’s Quality Assurance Program Manual and the referenced sections of the Division’s Quality Manual are reproduced below:
1 QUALITY ASSURANCE PROGRAM

The Forensic Biology quality system is directed by the Commonwealth of Virginia Department of Criminal Justice Services Division of Forensic Science Quality Manual, hereafter referenced as the Quality Manual.

1.1 GOALS AND OBJECTIVES

1.1.1 Goals

1.1.1.1 To support criminal justice agencies in the Commonwealth with DNA typing of selected biological materials associated with criminal investigations using polymerase chain reaction (PCR) based testing.

1.1.1.2 To maintain a DNA Data Bank of convicted offenders and arrestees.

1.1.1.3 To ensure the quality, integrity, and accuracy of the DNA typing data through the implementation of a detailed quality control program.

1.1.2 Objectives

1.1.2.1 Establish and monitor quality requirements for reagents, supplies, equipment, and analytical procedures.

1.1.2.2 Ensure that the entire DNA typing procedure is operating within the established performance criteria and that the quality and validity of the analytical data is maintained.

1.1.2.3 Ensure that problems are noted and that corrective action is taken and documented.

1.2 ORGANIZATION AND MANAGEMENT STRUCTURE

The organization and management of the Virginia Division of Forensic Science is addressed in the Quality Manual, Section 3, Organization and Management.

The management structure for the Virginia Division of Forensic Science is set forth in the Department of Criminal Justice Services Division of Forensic Science Organizational Chart.

1.3 PERSONNEL QUALIFICATIONS AND TRAINING

The procedures for the qualification and training of personnel is addressed in the Quality Manual, Section 15, Personnel and Training.

The policy that addresses attendance meeting and seminars is addressed in the Quality Manual, Section 15.9. Continuing Education and Training.

Training reports, transcripts, and position descriptions for all Forensic Biology personnel conducting DNA casework or Data Bank analysis are maintained by the Forensic Biology Program Manager, Section Chief, and/or the Section Supervisor.
<table>
<thead>
<tr>
<th>1 QUALITY ASSURANCE PROGRAM DNA TYPING OF BIOLOGICAL MATERIALS - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL SECTION VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4 VALIDATION</td>
</tr>
<tr>
<td>The validation of new DNA technologies/methodologies utilized by the Virginia Division of Forensic Science, Forensic Biology Section is addressed in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 13, Quality Assurance Program DNA Typing of Biological Materials. In addition, a summary of each validation study is maintained with the data. This summary and data are maintained by the Section Chief of the Forensic Biology Section as directed by Section 17 of the Quality Manual, Technical Procedures and Manuals.</td>
</tr>
<tr>
<td>1.7 ANALYTICAL PROCEDURES</td>
</tr>
<tr>
<td>The DNA analytical procedures used by the Virginia Division of Forensic Science, Forensic Biology Section are set forth in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 13, Technical Procedures DNA Typing of Biological Materials. Establishment of these procedures is guided by the Quality Manual, Section 8, Quality System Manuals and Council, and Section 17, Technical Procedures and Manuals.</td>
</tr>
<tr>
<td>1.8 CALIBRATION AND MAINTENANCE</td>
</tr>
<tr>
<td>Procedures for the calibration and maintenance of laboratory equipment are set forth in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 17, Quality Assurance Program DNA Typing of Biological Materials. Establishment of these procedures is guided by the Quality Manual, Section 17, Technical Procedures and Manuals, Section 18, Supplies and Services, and Section 19, Equipment.</td>
</tr>
<tr>
<td>1.9 PROFICIENCY TESTING</td>
</tr>
<tr>
<td>The proficiency testing program for the Virginia Division of Forensic Science is referenced in Section 14.5 of the Quality Manual, Proficiency Testing, as well as Section 8 of the Quality Manual, Discrepancies and Corrective Actions.</td>
</tr>
<tr>
<td>1.10 CORRECTIVE ACTION</td>
</tr>
<tr>
<td>The procedures for corrective action is set forth in the Quality Manual, Section 8, Discrepancies and Corrective Actions.</td>
</tr>
<tr>
<td>1.11 REPORTS</td>
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<tr>
<td>---</td>
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<tr>
<td>The procedure for generating a Certificate of Analysis is addressed in the Quality Manual, Section 1.2, Reporting Results. The procedure for the preparation, storage and disposal of case file records is addressed in the Quality Manual, Section 1.3, Case Files and File Administration. The procedure for the management of records is addressed in the Quality Manual, Section 1.11, Management of Records.</td>
</tr>
<tr>
<td>1.12 REVIEW</td>
</tr>
<tr>
<td>The procedure for the peer review of case files is addressed in the Quality Manual, Section 1.2, Reviewing Results. To aid the Forensic Biology Section examiner during the review of the documentation found in the case file, the &quot;Guide for Review of DNA Data&quot; form found at the end of this chapter is used as a guide. The procedure for the assessment of the accuracy of casework standards is addressed in the Quality Manual, Section 1.4.6, Technical Monitoring.</td>
</tr>
<tr>
<td>1.13 SAFETY</td>
</tr>
<tr>
<td>The policies and procedures for safety in the laboratory are addressed in the Division of Forensic Science Safety Manual.</td>
</tr>
<tr>
<td>1.14 AUDITS</td>
</tr>
<tr>
<td>The procedure for audits of the Virginia Division of Forensic Science is addressed in the Quality Manual, Section 1.15, Audits. A FBI Quality Assurance Audit of the Virginia Division of Forensic Science, Forensic Biology Section is conducted by an external agency at a minimum of every other year. The official record and response to prior audits is maintained by the Virginia Division of Forensic Science Quality Assurance Coordinator in the Division's headquarters laboratory.</td>
</tr>
<tr>
<td>Procedure</td>
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<tr>
<td>-----------</td>
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<tr>
<td>Quality Assurance Program</td>
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</tbody>
</table>

**Specific Tasks:**
- Sample extraction from tissue or body fluid samples
- DNA extraction performed in a clean area
- Appropriate range limit used
- Extraction method qualified

**Editing Worksheet:**
- Lot # recorded
- Sample & controls in proper location
- Test control results documented if data not included
- Operator actions initialed

**Aliquot Worksheet:**
- Operator actions initialed
- Cytocentrifuge quantities accurate
- Aliquotated values recorded for aliquots & samples
- Proper documentation present if SDS not included
- Lot # & Expiration date included

**Report Amplification Worksheet:**
- Lot # & expiration date included
- Dilution amplification data recorded
- IC & OC data recorded

**Product of Amplification Worksheet:**
- Lot # recorded
- Interpretation results recorded
- Photographs present and appropriately labeled
- Loading volumes appropriate, if recorded
- Dilutions recorded, if not documented elsewhere

**Gel Data Worksheet:**
- Lot # recorded
- Gel type and run parameters
- Proper controls
- Gel banding for documentation present
- Reticulate labeled, if applicable

**Scanned Images:**
- Scanning parameters printed, disposable
- Gel documented

**Print Images:**
- Scanning parameters printed, disposable
- Gel documented
- Laser labeled

**Logging Notes:**
- FT band achieved, if not done so in SDS
- Control evaluated, proper action taken if expected values not obtained

**Landscape:**
- Gel documented
- Up-to-date record
- Microfilm documented, if appropriate

**Analysis:**
- Gel documented
- Linear designation accurate
- IC & OC, if designated accurate
- Out of range values acknowledged, if applicable

**Note:**
- Documented, FT3004, Landscape
- Gel documented
- Documentation
- Reference with examiner data
- Landscape

**CONTROLS:**
- Control material present
- Retain and return sample aliquots, if applicable
- Proper aliquots labeled
- Infection properly evaluated and documented
- Appropriate slide value used

**Specimen detail report present:**
- SF appropriate sample entered into COGIS
- Appropriate specimen information entered
- Appropriate specimen category assigned
- Source SS appropriately assigned, if applicable
- Control slide entered for specimen
- Reagent SDS available, customers requested

**Notes:**
- Appropriate calculation made
- For IC, appropriate acceptability made
- Control slide entered
- Sample description/identification obtained
- Reported value listed, if applicable
## 14 MONITORING RESULTS

### 14.1 Administrative Review of Certificates of Analysis

14.1.1 Administrative review is the proofreading examination of the Certificates of Analysis. The administrative reviewer will look for such things as typographical or grammatical errors, misspellings and incorrect addresses on the certificate.

14.1.2 All completed and signed CoAs will be administratively reviewed prior to release.

14.1.3 The administrative reviewer will be an FLDV6 or higher preferably from the same section as the examiner of record.

14.1.4 If there are no errors, the reviewer's initials will appear on the file copy of the CoA in the space underneath the report's initials near the left margin.

14.1.5 The technical reviewer for those files subject to technical review should also be the administrative reviewer.

14.1.6 Corrective action will be handled in accordance with Section 6, "Discrepancies and Corrective Actions".

### 14.2 Technical Review of Case Files

14.2.1 Prior to reaching the CoA, a portion of the case file, particularly the examination documentation in the file, will be reviewed on 2 cases (or all cases) completed, which even in decency, for each examiner, not more than once. The reviewer will be an individual who has experience gained through detailed reading and experience in the discipline reviewed. The recent, current or previous experience must conduct at least two of the reviews (either present progress or retesting). If the Section Chief/Supervisor does not possess the necessary experience, he will designate the formal reviewer to an appropriate examiner, but must still perform a detailed review. To meet the PCR DNA Quality Assurance Standards for Forensic DNA Testing Laboratories requirements, a technical reviewer of all completed PCR DNA case files will be performed.

14.2.2 Review will be performed after the CoA has been signed by the examiner of record and within three (3) working days of receipt by the reviewer.

14.2.3 The Technical Review Form (Appendix C-1) must be used to document discrepancy.

14.2.4 Cross which notify all technical review comments may be documented in the Technical Review Summary Form (Appendix C-7). The Technical Review Form may also be used to document compliance in terms of involving no more than a small number of cases.

14.2.5 Any discrepancy or other deficiency must be disclosed by the appropriate Laboratory Director when a technical case is ready to provide a technical review before release of the CoA. In this situation, the technical reviewer will be conducted as soon as possible after the release of the CoA.

14.2.6 Technical Review Summary Form will be forwarded to the appropriate Laboratory Director for review. These forms will be placed in and become part of the Technical Review File, which will be maintained by each laboratory director as documentation of compliance with this section.

14.2.7 Any Technical Review Form that documents a discrepancy will be discussed with the examiner. Corrective action will be handled in accordance with Section 6, "Discrepancies and Corrective Actions".
14.3 Technical Review of Convicted Offender Sample Analysis

14.3.1 Prior to uploading any DNA profile from a convicted offender sample into the convicted offender DNA database, a review of all supporting documentation will be performed. The technical aspects of the review will be conducted by an individual who has extensive training through documented training and experience.

14.3.2 The review of the data generated by DFRs will be performed using the DNA Database STS Peer Review Form (Appendix C-6). The review of the data generated by a chemical laboratory will be performed using the Chemistry Review Form (Appendix C-7).

14.3.3 The DNA Database STS Peer Review Form will be placed in and become part of the convicted offender sample analysis review file, which will be maintained as documentation of compliance with this section.

14.4 Testimony Monitoring

14.4.1 To ensure that examiners' testimony is effective and does not compromise or require scientifically defensible and legally admissible C&RNA and examinations, the Division has established a program of testimony monitoring.

14.4.2 Each examiner's testimony will be monitored at least once each calendar year in which they testify. The monitoring may be performed in one of three ways:

14.4.2.1 In-Court and Deposition Observations

The preferred method for testimony monitoring is personal observation of an examiner's testimony by another examiner. A Director, the Forensic Attorney, the QAC, Section Chief, Supervisors, and examiners may observe the actual testimony of an examiner, even if the examiner is not in their Section. However, no examiner at the level position can observe the testimony of a supervisor at level position. Forensic scientists I and II and non-supervisory Forensic Scientists IV, V, and VI positions are considered equivalent for this purpose. During or at the conclusion of the testimony, the observer will complete an Expert Testimony Evaluation (ETE) form (Appendix C-8), and review it with the examinee in a timely manner.

14.4.2.2 Review of Transcript

If an examiner testifies but is not observed, the appropriate Laboratory Director will obtain a transcript of the examiner's testimony. A Director, Section Chief, or Supervisor, as appropriate, will review the transcript and complete an ETE form. The ETE form and the transcript will be reviewed with the examinee in a timely manner.

14.4.2.3 Input from Officers of the Court

If an examiner testifies but cannot be observed, or if the transcript cannot be obtained, a Director, Section Chief, or Supervisor, as appropriate, may complete an ETE in conjunction with the applicable judge or attorney. This method may only be used because of overwhelming circumstances such as last minute litigation of a scheduled observer or unexpectedly closed court proceedings, and only with the approval of a Director. The ETE form will be reviewed with the examinee in a timely manner.

14.4.3 Supervisors and Section Chiefs will consider the following types of testaments in determining the need and frequency for personally observing testimony:

- Complaints from attorneys or judges (Section 7, "Complaints")
- Examiners who are newly qualified or require improvement in this aspect of their work.

14.4.4 If an examiner does not testify in a given calendar year, their immediate supervisor will document the reason on an ETE form by January 31st of the following year.
### 14 MONITORING RESULTS

#### QUALITY MANUAL

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</table>

14.4.5 EME forms will be forwarded to the appropriate Laboratory Director who will review the forms and forward a copy to the QAC. The original will be maintained in the laboratory. The QAC will maintain a database to aid in the documentation of compliance with these requirements. The QAC will provide a quarterly summary of the appropriate database entries to each Laboratory Director. The QAC will also provide a report on the Division Ternary Monitoring Program in memorandum format at least quarterly to the Deputy Director.

#### 14.5 Proficiency Testing

14.5.1 The primary purpose of the Division's proficiency testing program is to conduct regular, objective assessments of the staff's ability to perform examinations in a scientifically defensible and legally admissible manner and to follow Division and federal policies and procedures. This is generally accomplished by using items with previously verified test results against which individual's test results are assessed. Also, because proficiency tests are performed, as much as possible, in the same manner as examinations on casework, the program assesses compliance with administrative policies and procedures as well.

14.5.2 The Division will adhere to the proficiency testing criteria outlined in the current editions of the following documents, as applicable:

- ASCLD/LAB: Accreditation Manual
- FBI Quality Assurance Standards for Forensic DNA Testing Laboratories
- FBI Quality Assurance Standards for Convicted Offender DNA Database Laboratories

14.5.3 Personnel who perform analyses on casework in multiple subdisciplines will be proficiency tested annually in each of the subdisciplines as identified by ASCLD/LAB.

14.5.4 There are three types of proficiency tests (PTs) presently available for use by the Division:

#### 14.5.4.1 External Tests

14.5.4.1.1 An external test is one received from outside the Division, known by the examiner being tested to be a test, and in which the expected results, at least initially, are unknown to anyone in the Division.

14.5.4.1.2 Some external tests, e.g., those for Fingerprint, Toolmarks and Latent Fingerprints, are taken independently by multiple examiners in successive. One or more of those examiners will have completed the test by the time the expected results become known to the Division.

#### 14.5.4.2 Internal Tests

14.5.4.2.1 An internal test is one produced by the Division, known by the examiner being tested to be a test, and in which the expected results are unknown to that examiner.

#### 14.5.4.3 Blind Tests

14.5.4.3.1 A blind test is one received from outside the Division, not known by the Division to be a test, and in which the expected results are unknown to anyone in the Division. The Division does not conduct blind tests at this time.

14.5.4.3.2 Evaluation of evidence is a form of blind test in which the original examiner does not initially know the results being sought. This practice has the advantage of minimizing all aspects of the examination being peer reviewed, front to end, as to accuracy. The disadvantage, however, is not having known, or well-defined, expected results.
## 14 MONITORING RESULTS

### QUALITY MANUAL

**Page 3 of 6**

**Division of Forensic Science**

**Amendment No.: C**

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24.4.5 ETE forms will be forwarded to the appropriate Laboratory Director who will review the forms and forward a copy to the QAC. The original will be maintained by the laboratory. The QAC will maintain a database to aid in the documentation of compliance with these requirements. The QAC shall provide a quarterly summary of the appropriate database entries to each Laboratory Director. The QAC will also provide a report on the Division Testimony Monitoring Program in memorandum format at least quarterly to the Deputy Director.

### 14.5 Proficiency Testing

14.5.1 The primary purpose of the Division's proficiency testing program is to conduct regular, objective measurements of the staff's ability to perform examinations in a scientifically defensible and legally admissible manner and to follow Division and Section policies and procedures. This is generally accomplished by using test kits with previously verified test results against which an individual's test results are assessed. Also, because proficiency tests are performed, as much as possible, in the same manner as examinations on casework, the program assists compliance with administrative policies and procedures as well.

14.5.2 The Division will adhere to the proficiency testing criteria contained in the current editions of the following documents, as applicable:

- ASCLD/LAB® Accreditation Manual
- FBI Quality Assurance Standards for Forensic DNA Testing Laboratories
- FBI Quality Assurance Standards for Combined Fingerprint/DNA Database Laboratories

14.5.3 Personnel who perform analyses on casework in multiple subdisciplines will be proficiency tested manually in each of the subdisciplines as identified by ASCLD/LAB®.

14.5.4 There are three types of proficiency tests (PTs) presently available for use by the Division:

#### 14.5.4.1 External Tests

- **14.5.4.1.1** An external test is one received from outside the Division, known by the examiner being tested to be a test, and upon which the expected results are unknown to anyone in the Division.

- **14.5.4.1.2** Some external tests, e.g., those for Fingerprint & Toolmarks and Latent Fingerprints, are taken independently by multiple examiners in succession. One or more of these examiners will have completed the test by the time the expected results become known to the Division.

#### 14.5.4.2 Internal Tests

- **14.5.4.2.1** An internal test is one produced by the Division, known by the examiner being tested to be a test, and in which the expected results are unknown to that examiner.

#### 14.5.4.3 Blind Tests

- **14.5.4.3.1** A blind test is one received from outside the Division, not known by the Division to be a test, and in which the expected results are unknown to anyone in the Division. The Division does not conduct blind tests at this time.

- **14.5.4.3.2** Examiners of evidence is a form of blind test in which the original examiner does not initially know he/she is being tested. This practice has the advantage of testing all aspects of the examination being repeated from sample receipt through return. The blind testing, however, is not having known, or well-unknowned expected, results.

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### 14.5.5 Program Management and Process

14.5.5.1 The QAC will track and communicate proficiency test information both in files and in a database to document compliance with those requirements. The QAC shall provide a quarterly progress of the appropriate database entries to each Laboratory Director.

14.5.5.2 The QAC will meet with each Section Chief twice each calendar year to address the Section’s work for the upcoming year. They will discuss and decide on test samples to be obtained and/or proposed, and develop a sample distribution plan and schedule. Copies of the sample distribution plan will be furnished to the Laboratory Directors.

14.5.5.3 External Tests

14.5.5.3.1 On receipt of an external test, the QAC will open and inspect the test, then initiate a tracking chain, test file, and database record to document the test's passage through the Division.

14.5.5.3.2 The QAC will reference the test distribution plan and schedule of the appropriate Section and assign the test accordingly.

14.5.5.3.3 The QAC will forward the test and accompanying paperwork to the appropriate individual, along with an assignment memo specifying the date by which the test must be completed and the disposition of the test sample.

14.5.5.3.4 The assigned individual will perform the test and forward the results in the prescribed format, together with all supporting documentation, to the QAC on or before the due date.

14.5.5.3.5 The QAC will review the individual's results and supporting documentation as necessary, and release forwarded items to the test provider.

14.5.5.3.6 When the test provider supplies the expected results to the QAC, that information shall be forwarded to the appropriate Supervisor (or branch independent) Section Chief and/or Laboratory Director.

14.5.5.3.7 The QAC shall compare the individual's results to the expected results, review the supporting documentation, and forward the results and documentation to the Section Chief, as necessary, for review.

14.5.5.3.8 The QAC shall discuss the outcome of the review(s) with the Section Chief.

14.5.5.3.9 The QAC shall notify the individual in writing of his/her performance on the test.

14.5.5.4 Internal Tests

14.5.5.4.1 Near the beginning of a month in which an individual is scheduled to receive an internal test, the QAC will ensure that the appropriate Section Chief forward a test, with the expected results, to the QAC.

14.5.5.4.2 On receipt of the test, the QAC will initiate a tracking chain, test file, and database record, and forward the test, accompanying paperwork, and assignment memo to the individual.

14.5.5.4.3 The assigned individual will perform the test and forward the results and supporting documentation to the QAC on or before the due date.
### 14 MONITORING RESULTS

**QUALITY MANUAL**

<table>
<thead>
<tr>
<th>Division of Forensic Science</th>
<th>Amendment No.: C</th>
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<tr>
<td></td>
<td>Effective Date: 29 July 2004</td>
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</table>

#### 14.5.5.6

The QAC will review the individual's results and supporting documentation, compare the results to the expected results, and forward the results and documentation to the Section Chief, as necessary, for review.

#### 14.5.5.7

The QAC shall discuss the outcome of the review(s) with the Section Chief.

#### 14.5.5.8

The QAC shall notify the individual in writing of his/her performance on the test.

### Blind Tests

**14.5.5.3.1**

A reexamination will require coordination between the QAC, the appropriate Section Chief, and the appropriate Laboratory Director(s). They will determine the individual whose case will be reexamined, the case to be reexamined, and the individual who will perform the reexamination.

**14.5.5.3.2**

All documentation relating to the reexamination will be placed in the case file noting that the evidence was subjected to a reexamination as a proficiency test. The QAC will maintain a copy of the examination documentation in the proficiency test files.

**14.5.5.3.3**

The Laboratory Director in possession of the evidence shall arrange for delivery of the evidence to the assigned individual. The QAC will initiate the test's mailing sheet, test file, and database record, and forward the appropriate paperwork and assignment memo to the examiner.

**14.5.5.3.4**

The reexamination will be performed as though it were the initial examination of the evidence. Information about the initial examination and its results will not be made available to the individual performing the reexamination.

**14.5.5.3.5**

The assigned individual will perform the test and forward the results and supporting documentation to the QAC as soon as before the due date.

**14.5.5.3.6**

The QAC will review and compare both original and reexamination results and corresponding supporting documentation, and forward the results and documentation to the Section Chief, as necessary, for review.

**14.5.5.3.7**

The QAC shall discuss the outcome of the review(s) with the Section Chief.

**14.5.5.3.8**

The QAC shall notify both examiners in writing of the test outcome.

### Practices

**14.5.6.1**

Proficiency tests will be performed at the same manner as casework. This includes use of the appropriate procedures, presentation of examination documentation, and, when prescribed, the involvement of other personnel, such as a second team in DNA analysis, a second examiner's verification of latent print matches, or the assistance of scientific support staff. Also, all proficiency test files are to undergo administrative and technical review.

**14.5.6.2**

Involvement of other personnel, when not prescribed, is not prohibited, but must be done in such a manner as to not compromise the primary aim of the test, which is to assess the performance of the individual examiner. This does not preclude an examiner from submitting opinions concerning the test samples as when examining actual evidence. However, proficiency tests are generally straightforward, and any test on which an examiner requires more than minor input from another examiner should be brought to the attention of the QAC. Also, because of the requirements in \( 14.5.5.3.6 \), below, much input may not be obtained from another examiner who has taken, is taking, or may take the same test.
### 14 MONITORING RESULTS

<table>
<thead>
<tr>
<th>Division of Forensic Science</th>
<th>Quality Manual</th>
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<tbody>
<tr>
<td>Amendment No.: F</td>
<td>Effective Date: 29-July-2004</td>
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</table>

14.5.6.3 Any individual involved in the performance of another’s test, e.g., as a second analyst, verifier or reviewer, generally may not take the same test. Such an individual may be involved in multiple points in the performance of a test, e.g., may verify and review a single test, and may second stage, verify, review, etc., the same test from multiple individuals taking the test.

14.5.6.4 One obvious difference between a test and a case is the absence of a CnA for a test. For external tests, the CnA is largely replaced by the test provider’s reporting forms. For internal tests, a reporting form to be completed by the examiner collecting the test will be prepared by the appropriate Section Chief in conjunction with the QAC.

14.5.6.5 Internal tests shall be prepared by the appropriate Section Chief or his/her designee in conjunction with the QAC. The preparation shall be documented in sufficient detail to allow for preparation of an identical test, if necessary. An examiner other than the one who prepared the test shall perform the validation.

14.5.6.6 A toxicology test will be generally assigned to the Toxicologist at the laboratory responsible for collecting the test to best utilize the resources already managed. If an individual in Toxicology does not perform a particular type of test during the mandatory performance period because of the nature of the samples, then another test would be assigned directly to that individual.

<table>
<thead>
<tr>
<th>14.5.7 Documentation</th>
</tr>
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<tbody>
<tr>
<td>14.5.7.1 The QAC will maintain all original proficiency test files.</td>
</tr>
<tr>
<td>14.5.7.2 Proficiency test files shall contain the following:</td>
</tr>
<tr>
<td>- Tracking sheets</td>
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<td>- Appropriate original or copy of documentation received from external or providers, and records of distribution of such documentation</td>
</tr>
<tr>
<td>- Preparation and validation records for internal tests</td>
</tr>
<tr>
<td>- Copies of assignment memos</td>
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<tr>
<td>- Copies of results forwarded to external test providers (or original if results were forwarded by fax)</td>
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<tr>
<td>- Proof of delivery to external providers</td>
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<tr>
<td>- Completed Division reporting forms for internal tests</td>
</tr>
<tr>
<td>- Supporting documentation</td>
</tr>
<tr>
<td>- Records of notification of individuals of the test outcome</td>
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<tr>
<td>- Corrective action records, as appropriate.</td>
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</table>

14.5.7.3 Each regional laboratory is limited to maintaining only the following documentation:

- Copy of the QAC’s assignment memorandum
- Copy of each individual’s memorandum to each test result to the QAC
- Copy of the QAC’s memorandum to an individual reporting the outcome of his/her performance
- Copy of any corrective action documentation

14.5.8 Corrective Action

- Discrepancies identified at any point of testing will be handled in accordance with Section 8, “Discrepancies and Corrective Action,” of this manual.
4. Institute a policy by which deviations from standard operating procedures are approved in advance and documented in the case file.

In September 2002, Chapter 1, Section 1.7 of the Forensic Biology Section Quality Assurance Program Manual was revised to include a specific reference to the Division’s Quality Manual, Section 4, Quality System Manuals and Controls, and Section 17, Technical Procedures and Manuals, which address the process for protocol deviations. In accordance with Standard 8.1.4 of the FBI Quality Assurance Standards, where methods are not specified, the laboratory shall, wherever possible, select methods that have been published by reputable technical organizations or in relevant scientific texts or journals or have been appropriately evaluated for a specific or unique application. This approach is routinely applied when specific or unique applications are required. In accordance with
the Division's Quality Manual, when deviations from the protocol are necessary the issue is discussed with the examiner's supervisor and Section Chief and approval from the Section Chief is documented in writing in the case file. A copy of Chapter 1 of the Forensic Biology Section's Quality Assurance Program Manual, FBI Quality Assurance Standard 8.1.4, and the referenced sections of the Division's Quality Manual are reproduced below:
### 1 QUALITY ASSURANCE PROGRAM

#### PROTOCOL MANUAL SECTION VI

<table>
<thead>
<tr>
<th>1.4 FACILITIES</th>
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<tbody>
<tr>
<td>The facilities of the Virginia Division of Forensic Science are addressed in the Quality Manual, Section 16, Facilities and Security.</td>
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</tbody>
</table>

<table>
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<tr>
<th>1.5 EVIDENCE CONTROL</th>
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<tbody>
<tr>
<td>The procedures for the management of laboratory numbers in submission of physical evidence, and item [lab] numbers to evidence containers in the submission of physical evidence is addressed in the Quality Manual, Section 15, Case Files and File Administration.</td>
</tr>
<tr>
<td>The procedures for the handling of physical evidence is addressed in the Quality Manual, Section 20, Evidence Handling</td>
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</tbody>
</table>

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<tr>
<th>1.6 VALIDATION</th>
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<tbody>
<tr>
<td>The validation of any DNA methodology/microscope utilized by the Virginia Division of Forensic Science, Forensic Biology Section is addressed in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 14.3, Quality Assurance Program. DNA Typing of Biological Materials. In addition, a summary of each validation study is contained within the data. The summary and data are maintained by the Section Chief of the Forensic Biology Section as directed by Section 17 of the Quality Manual, Technical Procedures and Manuals.</td>
</tr>
</tbody>
</table>

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<tr>
<th>5.1 ANALYTICAL PROCEDURES</th>
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<tbody>
<tr>
<td>The DNA analytical procedures used by the Virginia Division of Forensic Science, Forensic Biology Section, are set forth in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 14.3, Quality Assurance Program. DNA Typing of Biological Materials. Establishment of these procedures is guided by the Quality Manual, Section 17, Technical Procedures and Manuals.</td>
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</table>

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<tr>
<th>5.3 CALIBRATION AND MAINTENANCE</th>
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<tbody>
<tr>
<td>Procedures for the calibration and maintenance of laboratory equipment are set forth in the Commonwealth of Virginia Division of Forensic Science Forensic Biology Section Procedure Manual, Section 14.5, Quality Assurance Program. Testing of Biological Materials. Establishment of these procedures is guided by the Quality Manual, Section 17, Technical Procedures and Manuals. Section 18, Supplies and Services, and Section 19, Equipment.</td>
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</table>

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<tr>
<th>1.9 PROFICIENCY TESTING</th>
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<tbody>
<tr>
<td>The proficiency testing program for the Virginia Division of Forensic Science is referenced in Section 14.5 of the Quality Manual, Proficiency Testing, as well as Section 8 of the Quality Manual, Discrepancies and Corrective Action.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.10 CORRECTIVE ACTION</th>
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</thead>
<tbody>
<tr>
<td>The procedure for corrective action is set forth in the Quality Manual, Section 8, Discrepancies and Corrective Action.</td>
</tr>
</tbody>
</table>
Quality Assurance Standards for Forensic DNA Testing Laboratories
DNA Advisory Board
July 1998

8.1.4 Where methods are not specified, the laboratory shall wherever possible, select methods that have been published by reputable technical organizations or in relevant journals, such as journals or have been appropriately evaluated for a specific or unique application.
### Appendix A

#### 4 QUALITY SYSTEM MANUALS AND CONTROL

**QUALITY MANUAL**

**Division of Forensic Science**

**Effective Date:** 25 April 2003

<table>
<thead>
<tr>
<th>4.1 Quality System Manuals</th>
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<tbody>
<tr>
<td>4.1.1 The only two official copies of any Quality System (QS) manual will be the signed hard copy held by the Manual Coordinator and the electronic copy that is published on the Division's intranet.</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>4.1.2 QS manuals include but are not limited to</th>
</tr>
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<tbody>
<tr>
<td>The Manual</td>
</tr>
<tr>
<td>Division Safety Manual</td>
</tr>
<tr>
<td>Standard Operating Procedures (SOPs); Technical Procedure Manuals</td>
</tr>
<tr>
<td>Training Manuals</td>
</tr>
<tr>
<td>Administrative Operating Procedures (AOPs)</td>
</tr>
<tr>
<td>Regional Operating Procedures (ROPs)</td>
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</table>

<table>
<thead>
<tr>
<th>4.1.3 Every page of a QS manual will have the same format as this manual. All QS manuals will follow the format of this manual:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Page 1: Title page of the manual</td>
</tr>
<tr>
<td>Page 2: Acknowledgement page</td>
</tr>
<tr>
<td>Page 3: etc. Table of Contents</td>
</tr>
<tr>
<td>Page 4: etc. Body of the manual</td>
</tr>
</tbody>
</table>

| 4.1.4 Each QS manual will have a unique title and will be signed on its cover page by the Issuer. All pages in QS manuals will follow the page format of this manual. The end of a section will be designated by "End" justified on the right margin. |

<table>
<thead>
<tr>
<th>4.2 The Manual Coordinator and the Issuer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2.1 The Quality Assurance Coordinator (QAC) is the Manual Coordinator for all manuals issued under the Quality System. In the responsibility of the Manual Coordinator to ensure the following:</td>
</tr>
<tr>
<td>The format of the manual is correct</td>
</tr>
<tr>
<td>New issues or amendments are promptly placed on the Intranet and appropriate staff notified</td>
</tr>
<tr>
<td>The serial file of official manuals and the sections 5a are maintained (4.1).</td>
</tr>
</tbody>
</table>

| 4.2.2 The Issuer of a manual is the person who has responsibility for the content of the manual. The Issuer of the Quality Manual is the Division Director. The Issuer of the technical manual is the Section Chief of that Section. The Issuer of the Regional Operating Procedures is the Regional Laboratory Director. It is the responsibility of the Issuer of the manual to ensure the following: |
| The manual is prepared, correct, and coordinated (4.4.3). |
| Amendments to the manual are prepared and coordinated in a timely manner |
| A hard copy and a disk of the manual or amendments are forwarded to the Manual Coordinator |
| All amendments are promptly notified to appropriate staff |
| A documented review of the manual is completed at least annually. |

<table>
<thead>
<tr>
<th>4.3 Master File of QS Manuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.3.1 The Manual Coordinator will maintain the hard copy of QS manuals.</td>
</tr>
</tbody>
</table>

| 4.3.2 The Manual Coordinator will maintain electronic versions of QS manuals on the Division's intranet for staff access. |
5. Formulate a process to be used to develop an analytical approach when working with DNA samples having a low level of genetic material and for evaluating allelic dropout.

Allelic dropout is a common occurrence when analyzing biological samples collected from crime scenes. The likelihood of observing allelic dropout is significantly increased when analyzing biological samples consisting of a mixture of biological fluids (e.g., vaginal and seminal fluids or saliva and vaginal fluid) from two or more individuals. A survey of 10 to 15 forensic DNA testing laboratories will be conducted to determine how other laboratories approach working with DNA samples containing a low level of genetic material and the evaluation of allelic dropout. The survey will include laboratories that use the Applied Biosystems instrumentation, the Hitachi/MiraiBio FMBIO Fluorescence Image Analysis System instrumentation, or both. The laboratories will be requested to provide a copy of sections of the procedures manual that address these questions and/or respond to the list of questions below. This information will be evaluated and modifications will be made accordingly to the Virginia Division of Forensic Science’s procedures.
SURVEY QUESTIONS AND RESPONSES

A. What is the analytical approach used by the laboratory when working with DNA samples which contain a low level of genetic material?

i. Does the laboratory use the quantitation results to decide if the sample will be amplified; if so what is lowest concentration used?

ii. If no DNA is detected during the quantitation step, do the laboratory procedures permit the examiner to go forward with amplification of the sample?

iii. Do the laboratory procedures require that a complete DNA profile is obtained at a specified number of loci in order to interpret the results?

B. How does your laboratory evaluate allelic dropout?

i. If allele dropout is suspected at a particular locus do the laboratory procedures require the results at the locus to be recorded as inconclusive or is the locus still used in the overall interpretation and considered inconclusive for statistical purposes?

ii. Do the laboratory procedures specify a measure used to determine when allelic dropout has occurred or is allelic dropout considered to be a training issue and evaluated by the examiner based upon his/her training and experience?

NOTE: The responses from the laboratories reproduced below have not been altered by rephrasing. The number in parenthesis () represents the laboratory’s response to the question.

A. What is the analytical approach used by the laboratory when working with DNA samples which contain a low level of genetic material?

(3) In the future we are planning to use real time PCR to determine Low copy number versus high copy number samples. The cut off will be 100pg, everything below is low, above high. Then we are going to amplify down to 25pg (identifier 31 cycles) but do each amplification in triplicate. The triplicate amplifications really help to verify allele calls and detect allelic
drop out. In our experience a true heterozygote will have two alleles in at least one of the three aliquots. We did not succeed in determining a ratio based false homozygote value. We will not be using a higher RFU for homozygotes. This approach still has to be approved by the NYS DNA Subcommittee.

i. Does the laboratory use the quantitation results to decide if the sample will be amplified; if so what is lowest concentration used?

(1) Samples are amplified even if they do not show up on a slot blot. Profiler Plus and COfiler are more sensitive then the detection limits of the slot blot.

(2) No – we routinely amplify samples that do not have a QB result.

(4) When we were using Quantiblot we did not use the Quantiblot results to decide if we would amp because we obtained full profiles from samples that did not yield results on the Quantiblot. We recently validated the QB Quantifier and it is significantly better and we have been using it as a guide (especially with the IPC controls). If we do not obtain a result via Quantifier we are not amping with STRs. Our cutoff is 0.025ng. Based on our validation with PP16 we were able to obtain results with .25ng DNA. Since we can amp up to 10 microliters of extract we anticipate we will obtain some results.

(5) WHEN WE WERE USING QUANTIBLOT, ANALYSTS COULD AMP WITH THE TECH. LEADERS PERMISSION SAMPLES THAT WERE NEGATIVE OR BELOW 0.5NG. NOW THAT WE'RE USING RT-PCR, NEGATIVE QUANTS. DON'T GETMOVED FORWARD AS THE QUANT. SYSTEM GIVES YOU A DIRECT INDICATION OF THE AMOUNT OF AMPLIFIABLE DNA IN THE SAMPLE, AND IN OUR VALIDATION STUDIES, WE NEVER ENCOUNTERED (OR HEARD OF ANY INSTANCE FROM ANOTHER LAB) WHERE A NEGATIVE RT-PCR QUANT. RESULTED IN STR PROFILES DOWNSTREAM.

(6) Response provided under question ii.

(7) We use QB results to estimate the amount of DNA. If QB is negative we concentrate the sample and try amplification. STR analysis is more sensitive than QB. As we move to rePCR with Quantifier we will be looking into stopping after rtPCR. Our initial data indicated that depending on the volume the sample was
in, a negative rtPCR result can give an STR profile. So currently for rtPCR, if the sample is negative we will still concentrate and amp. We are looking into this more and are collecting more data.

(8) See response under question ii.

(9) If so what is your cut off? Yes, we use Real Time PCR as our method of Quantification. After 40 cycles of amplifications, if the Quantifiler results are undetermined analysis will stop there (usually). If the Quantitation results yield a cycle threshold of 36 or greater, analyst discretion will determine if analysis will continue.

(10) We use a RT-PCR that is sensitive enough to allow us to not amp samples if there is not DNA, we have begun to do that, somewhat at analyst discretion.

(11) Not currently however we will be considering a move towards a limit (ie. somewhere in the 100 to 250pg range) when our Quantifiler assay work is complete.

(12) Yes, Samples are only amplified if they are above 500 pg using the ABI Prism 7900.

(13) No. We currently use the slot blot (Quantiblot) method. The analysts routinely run samples that were blank on the slot blot. They often get full profiles. They do however also get partial profiles that have dropout. We hope this issue will improve with the real time instruments.

**ii. If no DNA is detected during the quantitation step, do the laboratory procedures permit the examiner to go forward with amplification of the sample?**

(1) Same as above.

(2) Absolutely – and have had great success in doing so.

You can use the ATF serial arsonist case (that will be making headlines tonight and tomorrow) as an example of how your approach to LCN testing provides tangible results. Not a single case that was linked to this arsonist by our lab yielded a QB result – and, we used the VA approach of mixture and LCN data interpretation taught to me by the DFS to intelligently and carefully obtain useful (and in my opinion, scientifically reliable) results.
(4) See above

(5) SEE ABOVE.

(6) We continue to amplify even if we have no results on the Quantiblot but we do indicate in the manual to interpret with caution due to potential stochastic effects. We, too, have had many cases where the results are excellent even when there is nothing on the QB.

From Section Manual:

1.6.1.3 If the sample concentration is less than 0.03 ng/ul, the sample may be amplified. Samples below 0.05 ng/ul may exhibit stochastic effects and must be interpreted with caution.

(7) Yes

(8) Currently we use the ABI 7000 and Quantifiler. We amplify everything despite a "negative" result. We just want some data and a track record that everything that tests negative does not amplify. My only concern is this.....if someone fails to add a sample to the plate (by omission or pipetting error), then a sample may be "negative" that actually has amplifiable amts of DNA. I know training should be adequate and this should NOT happen, but to me it's just a safety step. We will assess in a couple of months and see what happens. My bet is we will stop amplifying negative samples.

(9) For real time PCR see above answer. When we were using QuantiBlot yes, we would go forward with amplification.

(10) Sometimes

(11) Based on the current Quantiblot procedure, yes.

(12) See Above

(13) Yes, if that is the only sample from an item. The analyst may choose (based on experience) not to amp a sample if there were other probative stains that gave a Quantitation result on that item.

iii. Do the laboratory procedures require that a complete DNA profile is obtained at a specified number of loci in order to interpret the results?
(1) No

(2) No – it is more a matter of looking at the results as a whole – for example, if we have results at 7 loci but 6 of them are homozygous, we will inconclusive the whole profile (giving our “reason” as being due to LCN). It comes down to examiner discretion, along with agreement by the technical reviewer.

(3) For regular casework we are doing this right now. We are still doing 28 cycles with Co and Pro Plus. 10% of the extract are submitted to Quantiblot. 0 values are not amplified routinely. If the sample is very probative the analyst will concentrate the sample, qblot again and amplify if at least 150pg are present. There is no minimum number of loci that must be obtained for comparison. Fewer loci will create a weaker match as evident by the lower stats.

(4) Unofficially yes. We would not report anything with just one or two loci.

(5) NO

(6) No, but we do not generally report out alleles if there is a presence of only a few isolated alleles, especially if it shows only one allele per locus. However, we do not give specifics on this in our manual. We extensively discussed putting specifics but every analyst felt that this was a case by case basis and did not want a defined limit. It all depends on what you see and how rare, etc.

(7) No

(8) No.....but we are very careful when we do this. We have given statistics when just a few loci work (and this is after re-extracting, reamplifying the maximum amount, etc). Many times we will state in our report that the individual "cannot be excluded".

(9) No. If we obtain a good/reasonable profile (i.e. strong homozygote or heterozygote) at only one genetic marker we will call the alleles and use it to include or exclude individuals. If the profile is very weak, partial, indicative of a possible mixture, we would call the allele(s) and use to exclude only.

(10) No, it may be reported as limited, but of value for exclusion.

(11) No
(12) No, the interpretation is based on the quality of the data and analyst discretion.

(13) Yes. One loci must work without dropout. This rarely occurs and typically we have at least half of the loci that give results on weak samples.

B. How does your laboratory evaluate allelic dropout?

i. If allele dropout is suspected at a particular locus do the laboratory procedures require the results at the locus to be recorded as inconclusive or is the locus still used in the overall interpretation and considered inconclusive for statistical purposes?

(1) The locus or is the locus still used in the overall interpretation and considered inconclusive for statistical purposes.

(2) We do not inconclusive the locus -- we report the findings and will put a notation that we have apparent allelic dropout. We then do not use the locus for statistical purposes.

(3) We do have a reporting option that we use, were we say "another allele might be present at this locus". We also use this for mixture deductions were there is only one definite foreign allele at a locus and allele sharing and or homozygosity or drop out are possible.

We also are allowed to use a 2p formula to determine the frequency for a locus with suspected drop out. If sufficient other loci are present the locus will be INC for statistical purposes.

(4) We report the locus as insufficient data to render a conclusion so basically inconclusive. We do not consider the results for statistical purposes.

(5) THE ANALYST MAY INC. THE LOCUS AND NOT USE IT IN STATS. HOWEVER, WHEN WE WERE DOING FMBIO/POWERPLEX WE RAN OUR Q-STAINS IN DUPLICATE, 2 AMPS FROM THE SAME EXTRACT, AND RUN ON A GEL, AND FOR A LOCUS TO GET CALLED AND INCLUDED IN STATS., RESULTS FROM BOTH SAMPLES WERE PREFERRED. THAT MEANT THAT NOT
ONLY MUST THE ANALYST SEE A BAND AND MAKE
THE VISUAL CALL, BUT IT WAS ALSO PREFERRED FOR
THE BAND TO BE CALLED IN STRCALL AS WELL, IN
BOTH SAMPLES, ALTHOUGH THIS WASN'T ALWAYS
THE CASE. NOW THAT WE'RE USING IDENTIFILER AND
CE, OUR SENSITIVITY IS GREATLY INCREASED,
SAMPLES ARE AMPED AND ELECTROPHORESED
ONCE, AND IF DROPOUT IS SUSPECTED, THE
ANALYST HAS THE AUTHORITY TO INC. THE LOCUS
AND NOT USE IT IN THE STATS., RATHER THAN 'MIS-
CALL' AN EXCLUSION.

(6) We put the allele detected in the table with a dash next to it to
indicate potential drop out. The locus is inconclusive and no stats
given for that locus.

(7) We prepare an excel table with the results, so it would be in that
table; but we would not use the results for statistical purposes.

(8) The locus still used in the overall interpretation and considered
inconclusive for statistical purposes.

(9) If the profile appears to be single source we will inconclusive the
locus. However, if a mixture profile is evident we will call the
locus and it would be considered inconclusive for statistical
purposes.

(10) It would be used for exclusion/inclusion but not for stats.

(11) Loci with suspected dropout signals below our reporting threshold are
still used for overall interpretation (eg possible exclusion) but the locus is
not used in the statistical estimates.

(12) We use 150 rfu as the reporting threshold. Allelic dropout must be
considered for any locus with alleles below 300 rfu. Generally, the
locus can be used for interpretation; the possibility of allelic
dropout is taken into consideration for statistical purposes.

(13) The locus would be used for exclusion, but not used for inclusion
nor used in the statistical calculation.

ii. Do the laboratory procedures specify a measure used to determine
when allelic dropout has occurred or is allelic dropout considered to be a
training issue and evaluated by the examiner based upon his/her training
and experience?
Our interpretation threshold for inclusion and statistics is 150 rfu. Peaks between 50 and 150 rfu may be used for exclusion.

Determination of whether or not there is allelic dropout is considered a training issue and is evaluated by the examiner based upon his/her training and experience. The training and experience of the technical case reviewer is also key, since both examiners must agree.

Drop out detection is based on experience and we do not have separate hetero / homo thresholds or any other quantitative rules.

We have defined our heterozygotes must have peak heights of at least 100 relative fluorescent units (RFUs) and our homozygotes must have peak heights of at least 200 RFUs. We also evaluate the peak heterozygote peak height ratio. Ultimately, it does come down to training and experience especially when you start analyzing challenged specimens.

EXTENSIVELY COVERED IN TRAINING, BUT IT DOES LARGELY OCCUR WITH THE LARGER STR LOCI, FGA, ETC., AND MUST NOT ONLY BE EVALUATED BY THE PRIMARY EXAMINER, BUT ALSO CONCURRED WITH BY THE SECOIND READER AND THE TECH. REVIEWER (POTENTIALLY 3 INDIVIDUALS). OUR STANDARD PRACTICE IS TO RE-AMP Q-SAMPLES THAT MAY HAVE DROPOUT AND RE-INJECT FOR 9 SECS. TO SEE IF THERE IS ANYTHING THERE THAT WAS PREVIOUSLY THERE BELOW THRESHOLD.

Again, no specific measures and dropout is evaluated by the analyst and the case reviewer since they need to agree.

This protocol is to serve as a general guideline for the interpretation of STR profiles. However, it is not an exhaustive list of all casework scenarios. Therefore, the scientist’s experience and discretion is always taken into account before reporting STR profiles. The purpose of these guidelines is to establish a general framework and outline minimum standards to ensure that:

From Section Manual -

2.1.4 Partial profiles: When only partial profiles are present due to degradation, inhibited or limited DNA or to stochastic effects occurring during amplification, the locus should be deemed inconclusive.
2.1.4.1 Highly degraded samples - Highly degraded samples shall be interpreted with caution. Highly degraded samples may show differences in stutter peaks, unbalanced heterozygous alleles, and off-ladder artifacts. Stutter peaks are generally higher in intensity than those observed on high quality DNA. There is also a greater amount of unbalanced heterozygous alleles. Typically, the smaller heterozygous peak will be no less than 50% of the height of the larger allele. Finally, “off-ladder alleles” can be detected at peak heights greater than stutter percentage heights. Samples suspected of being highly degraded may be repeated, when feasible, to confirm the alleles.

2.1.4.2 Inhibited samples – Inhibited samples are those samples which contain an impurity that inhibits the polymerase enzyme activity. Inhibited samples may show differences in stutter peaks, off-ladder artifacts, allele or locus drop-out, and unbalanced heterozygous alleles. Inhibited samples differ from degraded samples in that the drop-out of loci is not related to the size of the template DNA of the loci. Inhibited samples may be diluted to reduce the effect of inhibitors.

2.1.4.3 Stochastic effects – Stochastic effects can alter peak height ratios between the known and question sample. This is not a basis for an exclusion. At sample concentrations of less than 0.25 ng, the stochastic effects may cause allele drop out. Where this occurs, that locus is deemed inconclusive.

There are a good deal of discussion when cases like this occur. We tend to be conservative but do not want to restrict ourselves with cut-off and too many rules. Sometimes your “gut” feeling is best especially with many years of experience behind the interpretations.

(7) In our stochastic study (as well as in our training) we evaluated allelic dropout by setting up multiple tubes of the same sample and amplifying approx. 62pg (based on slot blot and serial dilution). We then analyzed the 310 data at 50 rfu. We did observe allelic dropout at this level of input DNA; but when analyzed at 150rfu
there were very few instances where one peak was above the threshold and the second peak was totally gone. So we feel our 150 interpretation threshold is sufficient. We use data below our threshold for exclusionary purposes only.

(8) We run a lot of mixture samples and dilutions during training...so they have a lot of experience before they go into their internship. There is nothing specific in our Interp. Guidelines about any sort of measurement...it's based on training and experience. Of course, there has to be signs of low level DNA or the typical "sloping" of the allele rfu's from small to larger fragments.

(9) It is based on training and analyst experience/discretion.

(10) We use 150 as the reporting threshold but can use peaks lower than that to exclude.

(11) Data from our validation studies (eg what happens to peak height ratios in a dilution series) indicates a safe guideline for deducing a homozygote is a single peak over 350RFU. This also provides another assessment parameter for when to suspect allele dropout versus an additional contributor(s) being present near background or trace levels. The data was presented in a reduced amp volume validation study summary report for one of our state functional area meetings but it is not in an SOP. You can consider it as a training/continuing education issue for us.

(12) The possibility of undetected alleles will be considered for weak or degraded single contributor profiles and mixed DNA profiles when possible stochastic effects are a concern.

(13) The occurrence of allelic dropout is considered a training issue and evaluated by the examiner; only experienced examiners may tech review cases and need to agree with the original examiner.