Award Number: 2009-DN-BX-K034

Authors: Vincent J. Anderson and Jeff Thompson

Abstract

Traditionally, the Scientific Investigation Division (SID) of the Los Angeles Police Department receives requests to examine about one-half of all of the Sexual Assault Evidence Kits (SAEKs) which were booked into evidence each year. While all of these requested SAEKs were examined, the remaining SAEKs that were submitted over the last 12 years (approximately 6,132), remained unexamined. In order to obtain the valuable investigative information, potentially contained within the unexamined SAEKs, the City of Los Angeles devoted significant financial resources towards the goal of examining all of these kits by June 2011. This consisted of the outsourcing of the unexamined kits to four different laboratories for screening and DNA profiling. In addition, the Department's second goal was to put in place the personnel and infrastructure to allow all SAEKs collected in the future to be routinely tested. While all of these personnel have now been hired (and played a large part on the team of criminalists who were sending the SAEKs to the outsourcing labs), training all of them to perform DNA analysis is still years away.

While outsourcing was the most efficient short-term solution to the problem, it created an issue that could not be addressed by existing Department personnel without further delaying the training of new personnel and the implementation of efficiency measures. In order to effectively gain useful investigative information from any evidence present in these kits, the DNA profiles generated needed to be uploaded into the state and national CODIS DNA databases. For this upload and comparison to occur, the profiles typed by the private outsourcing laboratories had to be extensively reviewed by a qualified public agency laboratory DNA analyst. An estimated 3,679 DNA profiles would need to be reviewed prior to being uploaded to CODIS. The time required to perform a review would take approximately 2 hours per case. As a result, there would be a very significant time commitment: 3,679

SAEKs x 2 hours = 7,358 hours in order to review all of these profiles. At the start of this grant period, the LAPD employed 16 qualified DNA analysts, and could not afford to commit one-fourth of its DNA staff to this project, nor could public safety concerns allow significant delay in reviewing and uploading these profiles.

In order to deal with the necessity of uploading subcontractor profiles into the CODIS database in a timely and efficient manner (while still allowing us time to develop and implement DNA analysis efficiency measures), LAPD was encouraged to apply for this 2009 Forensic DNA Unit Efficiency Improvement grant (Efficiency Grant) in order to supplement our limited resources. As a result, the LAPD was awarded this grant, funded as a critical need.

Originally, LAPD planned to utilize the services of other governmental agencies to review and enter the DNA profiles obtained from subcontractors, and re-imburse said agencies with funds from this grant. Due to contractual difficulties and a shift in the Department's focus, it was decided that all of the subcontractor reviews would be performed on grant funded overtime by DNA analysts employed at the LAPD. This became one of our objectives for this grant, **Objective 2: Decrease the number of contract laboratory DNA cases awaiting data review**. As of September 30, 2011, LAPD has reviewed 2,865 reports from outside vendors under the Efficiency Grant. Those reviews have lead to 1,705 cases with at least one CODIS upload and 895 cases with at least one CODIS Hit Notification. These cases were reviewed on grant funded overtime in the amount of $238,061.64.

Another of our grant objectives was **Objective 1: To improve DNA analysis efficiency**. As of September 30, 2011, case turn-around time increased from 71 days to 108 days for delivery of final report, due to the assigning of older cases in the backlog and a change in reporting dates. The samples per analyst per month increased 82% from a baseline of 15.9 to 29.0. Also, the backlog of requests for DNA analysis decreased approximately 52% from a baseline of 3,107 to 1,493.

Although no funds from this grant were directly used to fund the validation of new analytical platforms, because of funds utilized from this grant for overtime to perform subcontractor reviews, LAPD personnel were able to utilize regular working hours to validate two new analytical platforms, the Qiagen Investigator Kit and ABI's Quant Duo®. Additionally, LAPD personnel were able to conduct research into developing a method for spermatozoa identification and extraction utilizing Laser Micro dissection.

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Executive Summary

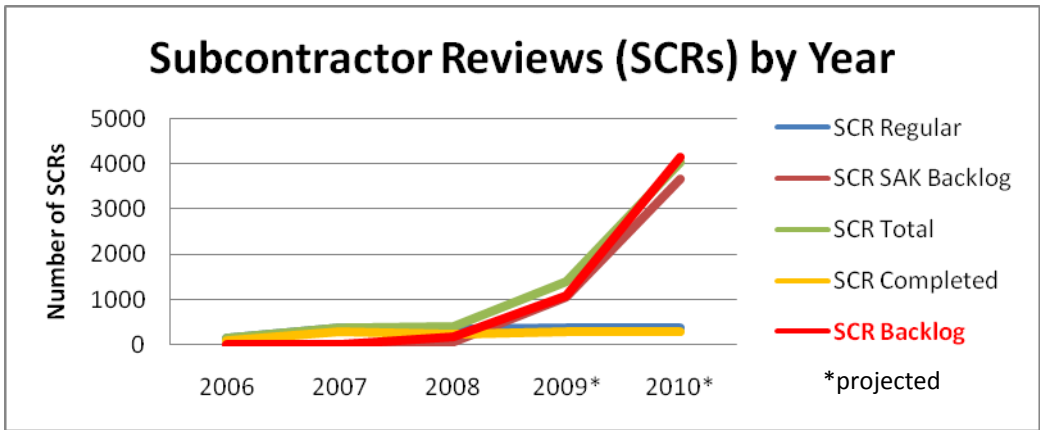
Scientific Investigation Division (SID), Los Angeles Police Department, has historically examined about one-half of all of the Sexual Assault Evidence Kits (SAEKs) which are booked into evidence each year. Investigators did not appreciate that the remaining, unexamined SAEKs (if tested), could provide them with useful investigative information, above and beyond their relevance to the specific case under which they were collected. Investigative information could include leads on cases that were not thought to be related, or information on a previously unknown suspect or suspects who are victimizing some of the more at-risk members of society. As a result, the Department did not commit sufficient resources to allow the testing of each of these kits. Over the past 12 years, this backlog of unexamined SAEKs had been accumulating in the Department's evidence freezers and had reached approximately 6,132 in number (based on the most current inventory). The City of Los Angeles has recently devoted significant financial resources towards the goal of examining all of these kits by June 2011. This consisted of the outsourcing of the unexamined kits to four different laboratories for screening and DNA profiling (LAPD criminalists at their peak were sending approximately 450 SAEKs per month to outsourcing laboratories). In addition, the Department's second goal was to put in place the personnel and infrastructure to allow all SAEKs collected in the future to be routinely tested. While all of these personnel have now been hired (not utilizing any grant funds), and were part of the team of criminalists who were sending the SAEKs to the outsourcing labs, they have not yet been trained to perform DNA typing.

While outsourcing was the most efficient short-term solution to the problem, it created an issue that could not be addressed in a timely manner by existing department personnel. In order to effectively gain useful investigative information from any evidence present in these kits, the DNA profiles generated needed to be uploaded into the state and national CODIS DNA databases. This is where the DNA profiles are compared to known offenders and unsolved cases. For this upload and comparison to

occur, the profiles typed by the private outsourcing laboratories must be extensively reviewed by a qualified public agency laboratory DNA analyst (see Federal Bureau of Investigation, "Quality Assurance Standards for Forensic DNA Testing Laboratories," taking effect July 1, 2009). Approximately 60% of the kits sent for testing resulted in a possible suspect DNA profile. That meant that approximately 6,132 x .60 = 3,679 DNA profiles had to be reviewed prior to being uploaded to CODIS. These reviews (while substantially less than the time required to perform the actual testing in-house) took an approximate 2 hours per case. As a result, a very significant time commitment: 3,679 SAEKs x 2 hours = 7,358 hours in order to review all of these profiles, would be required by laboratory personnel. This translates to one criminalist working full time for three and one-half (3.5) years, or four (4) criminalists working full time for almost one year each. Originally, at the onset of this grant, the LAPD employed 16 qualified DNA analysts, and could not afford to commit one-fourth of its DNA staff to this project, nor could public safety concerns allow significant delay in reviewing and uploading these profiles (a suspect who would otherwise have been identified could continue to offend). During the period of this grant, five new DNA analysts were qualified, allowing SDU to utilize these new analysts (not hired using any grant funds) to review subcontractor reports allowing for the profiles to be uploaded ahead of specific statutes in order to provide useful information that could be acted on by an investigator.

In conjunction with the outsourcing of the backlog of previously unexamined SAEKs, and due to the City's commitment to address the issue, the SID added an additional 14 criminalist positions. When coupled with the 12 criminalists added in the previous year, and the loss of the criminalist who was running the DNA training program, the capacity of the SDU to effectively train that many people as DNA analysts was greatly exceeded. As a result, the SDU trained these personnel in the fundamental task of screening evidence (SAEKs, clothing, weapons, etc.) for biological evidence. This is a fundamental skill that an analyst must master and gain experience with prior to moving on to DNA training. As these people were added, and as the SDU moved personnel hired earlier into DNA training, the capacity of the

screening personnel exceeded the capacity of the DNA typing personnel. Further, demand for the use of DNA on property crimes and on more "touch" types of evidence (lacking blood, saliva or semen) continued to increase dramatically. This put pressure on the SDU to maintain screening capacity and DNA typing capacity. In the short term, this increase in screening capacity and demand for DNA typing was addressed by sending samples identified by a screening criminalist to contract (outsourced) laboratories. The below chart indicates the increase in outsourced laboratory data reviews (Sub-Contractor Reviews, or "SCRs") over the previous three years, and projections for 2009 and 2010, and indicates the SCR backlog which would have developed:



In order to assist in addressing this issue, the LAPD/SID originally solicited the cooperation of other public agency forensic DNA laboratories to review the profiles and upload them into the CODIS DNA database. This sharing of the load between LAPD DNA analysts and DNA analysts from other public agency laboratories would minimize the impact of the review of these profiles on individual laboratories, while maximizing the potential benefit to public safety. Requiring LAPD DNA analysts to work enough overtime to complete this task on their own was originally not an option, due to the concurrent commitments the LAPD had to implement several efficiency measures which were in various stages of development, as well as provide training to numerous newly hired employees. Funds utilized from this grant relieved some of the burden on the LAPD DNA analysts, and allowed them to better focus on

completing the training of the newly hired personnel (not hired using any grant funds) and implementing the numerous efficiency measures which were in development. In utilizing this grant, LAPD had two main objectives. **Objective 1: To improve DNA analysis efficiency**. An important aspect of improving our efficiency was to develop a team approach, allowing groups of DNA analysts to analyze cases together and batch samples in order to eliminate duplication of work. The Efficiency Grant has allowed us to utilize overtime to perform the influx of SCR's that we experienced. This in turn allowed DNA Analysts to develop the team approach during regular working hours and institute it into casework. As a direct result of being able to utilize funds from this grant for overtime to perform SCR's, LAPD criminalists were able to validate Qiagen's Investigator Kit to be used for the robotic extraction of DNA from case samples, put the method on-line, train and qualify LAPD personnel in its proper use. LAPD was also able to validate ABI's Quant Duo® Real Time Quantitation Kit for use in quantifying total as well as male DNA. The majority of LAPD's DNA Analysts are now trained and qualified in its use. SDU DNA analysts are currently working on the second phase of Quant Duo®'s use, namely the detection of male DNA in sexual assault case samples, which will potentially take the place of traditional microscopic detection of sperm, as well as other components of semen or saliva.

A third new efficiency method under development is the use of a laser micro dissection scope for the detection and removal of individual sperm cells from prepared microscopic slides. Originally, we had planned on using this for the rapid identification and removal of individual cells from glass slides related to sexual assault evidence, for the purpose of obtaining DNA without having to go through the tedious process of differential extractions. The use of the laser micro dissection scope has not progressed as we had planned. Unfortunately, although we have been able to remove and type nucleated epithelial cells from glass slides, after many attempts by different analysts, we have not yet been able to successfully remove and type spermatozoa cells from glass slides. In contrast, when the samples are deposited on

non-glass substrates (slides), we have had success in obtaining complete or nearly-complete, single-donor DNA profiles from both epithelial cells and spermatozoa collected via LMD.

As a result of overtime funds provided by this grant (allowing the LAPD to focus on measures to increase efficiency), the samples per analyst per month increased 82% from a baseline of 15.9 to 29.0. Also, the backlog of requests for DNA analysis decreased approximately 52% from a baseline of 3107 to 1493. Although case turn-around increased from 71 days to 108 days for delivery of final report, this statistic is skewed due to the assigning of older cases in the backlog and the method for computing turn-around time (date requested to date delivered to the requesting agency, for cases completed during the reporting period).

Our second objective was **Objective 2: Decrease the number of contract laboratory DNA cases awaiting data review**. Because the LAPD was able to qualify five additional DNA analysts during the period of this grant (who were not hired using any grant funds), due to changing departmental needs, the original plan to utilize other governmental laboratories to assist in the review of subcontractor profiles was abandoned and a new approach was developed. LAPD DNA analysts would now review all of the subcontractor DNA profiles for CODIS upload suitability, utilizing funds from this grant to pay for overtime, as well as performing a portion during regular working hours.

As of September 30, 2011, as reported in our Semi-Annual Progress Report No. 5, DNA Analysts from LAPD were able to utilize overtime granted from this Efficiency Grant to perform subcontractor reviews (SCR) on 2,865 reports returned from outside vendors. Of these 2,865 subcontractor reviews (SCR), 1,705 cases had at least one CODIS upload and 895 of these uploaded profiles resulted in at least one CODIS Hit Notification.

I. Introduction

A. Statement of Problem

One problem facing LAPD was a large number of backlogged sexual assault cases which needed to be analyzed quickly. Lacking the manpower to perform the DNA analysis in-house, LAPD chose to outsource the majority of 6,132 backlogged sexual assault cases to contract laboratories. The resulting DNA profiles then presented LAPD with another hurdle, getting these profiles uploaded to CODIS. In order to accomplish this, LAPD was awarded the 2009 Forensic DNA Unit Efficiency Improvement grant on a critical needs basis to be utilized in either paying overtime to LAPD DNA Analysts to perform the profile reviews or entering into contracts with other governmental agencies to do the reviews. Utilizing no funds from this grant, five new DNA Analysts were qualified during the lifetime of the grant, allowing LAPD to use their own personnel, significantly supplemented by overtime hours from the grant, to do the profile reviews and uploads.

By performing the profile reviews on overtime, LAPD Criminalists were able to work on another problem area: improving their DNA analysis efficiency. One of the ways this was accomplished was by improving their team and batching approach to casework. To improve sample throughput, two new analytical platforms were validated for the extraction and quantitation of DNA, Qiagen's EZ1 Advanced XL using their Investigator Kit for extraction of DNA and ABI's Quant Duo® Kit for the quantitation of total DNA and male DNA present in an extracted sample.

In a further attempt to improve their efficiency, LAPD began the process of developing two new approaches intended to speed up analysis of sexual assault cases. One of these processes was the use of ABI's Quant Duo® kit for identifying male DNA in a female victim's case sample, and continuing on to

DNA analysis if a significant amount of male DNA was detected. This would eliminate the time consuming step of microscopic detection of spermatozoa. A second approach was the development of a method utilizing a laser micro dissection scope to physically remove the cellular component of interest from the microscope slide, eliminating the need for liquid extraction. Both of these new techniques are still in the developmental stage.

II. Methods

Objective 1: To improve DNA analysis efficiency

The validations for the efficiency projects were assigned to a minimum of two DNA criminalists each and each of the projects were overseen by the DNA Technical Lead (who is also a supervisor). Non-DNA qualified personnel (newer criminalists and laboratory technicians) were utilized to assist wherever possible, although much of the newer criminalists' time was committed to either screening evidence, training in how to screen evidence, or sending unexamined SAEKs to outsourcing (contract) laboratories. Each team was required to report their current status and progress to the DNA Technical Lead and the Assistant Laboratory Director on a regular basis. Following completion of the validations and the appropriate reviews and approvals (if warranted), the DNA Technical Lead or his designee coordinated training for all DNA analysts or other relevant personnel in each efficiency technique. Once the training was completed, the techniques were implemented in the Unit.

QIAGEN EZ1 Advanced XL/ Investigator Kit Validation

The EZ1 Advanced XL is a self-contained, robotic platform designed for the extraction of DNA from up to 14 forensic samples. In order to accomplish this, the robot uses magnetized silica beads that bind genomic DNA in whole cell lysates under conditions of low pH and high ionic strength. The beads are immobilized with a magnet, both during the wash steps to remove RNA, proteins, and other cellular

components, and following the elution of DNA. All of this is achieved by utilizing the DNA Investigator Kit. Validation of the EZ1 Advanced XL/ DNA Investigator Kit has demonstrated its suitability as a substitute for organic extraction for several types of forensic samples (dried blood, dried saliva, cigarette butts, semen and epithelial cell mixed stains, hair and "touch" samples). For the analytical procedure developed for the EZ1 Advanced XL/ DNA Investigator Kit, see Appendix A. For a Summary of the EZ1 Advanced XL/ DNA Investigator Kit validation performed at LAPD, see Appendix D.

ABI Quantifiler Duo® Quantification Kit

The Quantifiler Duo® Quantification kit simultaneously quantifies the total amount of amplifiable human DNA and male DNA as well as the presence of PCR inhibitors in a sample. The primers used for this real-time assay are the ribonuclease P RNA component H1 (RPPH1) for human detection, sex determining region Y (SRY) for human male detection and an internal PCR control (IPC). When validated using Applied Biosystems 7500 Real-Time PCR instrument, the Quantifiler Duo® Kit was found to be a reliable and accurate assay for degraded, inhibited and male/female mixed DNA samples. For the analytical procedure developed for the Quantfiler Duo® Kit, see Appendix B. For a Summary of the Quantfiler Duo® Kit validation performed at LAPD, see Appendix E.

Zeiss PALM Laser Microdissection System (LMD)

The Zeiss PALM Laser Microdissection System (LMD) uses a laser to detach single cells from glass slides or other substrates. After detachment, the isolated cells are collected into the cap of a microcentrifuge tube to process for DNA analysis. Although DNA typing following collection from glass slides has not proven successful for spermatozoa cells, methods for collection and analysis from other substrates are still in the developmental stage (and have shown some promise). Since we are still evaluating this technique for possible applications, once reliable, reproducible protocols are established, validation will ensue.

Objective 2: Decrease the number of contract laboratory DNA cases awaiting data review

Mandatory data reviews generated from contract laboratories (during analysis of the backlogged SAEKs) were handled by LAPD criminalists, utilizing overtime. The LAPD CODIS administrator or designee verified that each profile that met the qualifying criteria was uploaded and LAPD was the entity notified when a match to a suspect or other case occurred.

III. Results

A. Statement of Results

OBJECTIVE 1: To improve DNA analysis efficiency

Goal: Reduction in the average number of days between the submission of a request for DNA analysis to the laboratory and the delivery of the test results.

Average number of days between the submission of a request for DNA analysis to the laboratory and the delivery of the test results at the beginning of the grant period.
30 days for results, 71 days for final report as of 10/1/09

Average number of days between the submission of a request for DNA analysis to the laboratory and the delivery of the test results at the end of the reporting period.
108 days

Goal: Increase in DNA analysis throughput for the laboratory.

Average number of DNA samples analyzed per analyst at the beginning of the grant period.
15.9 samples per analyst per month as of 10/1/09

Average number of DNA samples analyzed per analyst at the end of the reporting period.
29 samples per analyst per month 7/1/11 to 9/30/11

Goal: Reduction in the backlog of requests for DNA analysis.

Number of backlogged requests for DNA analysis at the beginning of the grant period.
131 DNA requests, 730 requests to screen for material, 2246 rape kits as of 10/1/09 – Total 3107

Number of backlogged requests for DNA analysis at the end of the reporting period.
482 DNA requests, 887 requests to screen for material, 124 rape kits as of 9/30/2011 – Total 1493

OBJECTIVE 2: Decrease the number of contract laboratory DNA cases awaiting data review

Goal: Elimination or reduction in the backlog of subcontract laboratory cases awaiting data review and CODIS upload

Number of backlogged contract laboratory cases awaiting data review at the beginning of the award period.
1,778 from the Sexual Assault Evidence Kit (SAEK) project; 692 from routine cases (not part of the SAEK Project) = 2470 total

Number of backlogged contract laboratory cases awaiting data review at the end of the reporting period
376 total (from all categories) as of 9/30/2011.

Validations

EZ1 + Inv. Kit:

- Internal validation study was completed **9/7/10**

- On-line on **9/24/10**

- All personnel trained **1/12/11**

Quant Duo®:

- Part I internal validation study was completed 9**/7/10**

- Majority of personnel trained by **5/30/11**

- On-line by **6/15/11**

- Part II (using it to screen SAEKs) in progress, anticipated completion date mid to late 2012.

Laser Micro Dissection Microscope

Evaluation of the Zeiss PALM Laser Microdissection System (LMD) is currently in progress. When evaluation is complete, and if reliable protocols appear possible, the LAPD Serology/DNA Unit (SDU) will perform the internal validation study.

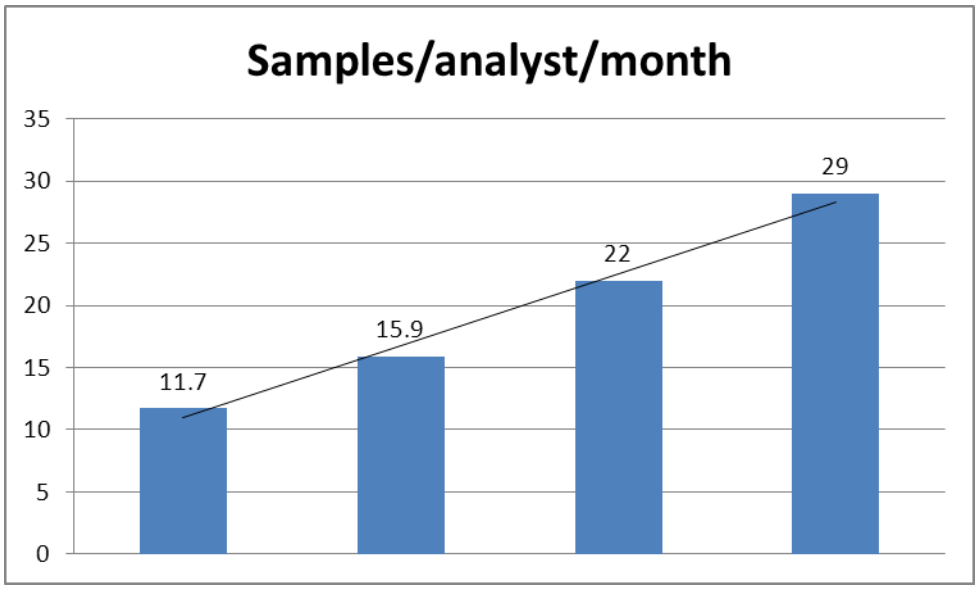
IV. Conclusions

A. Discussion of Findings

OBJECTIVE 1: To improve DNA analysis efficiency

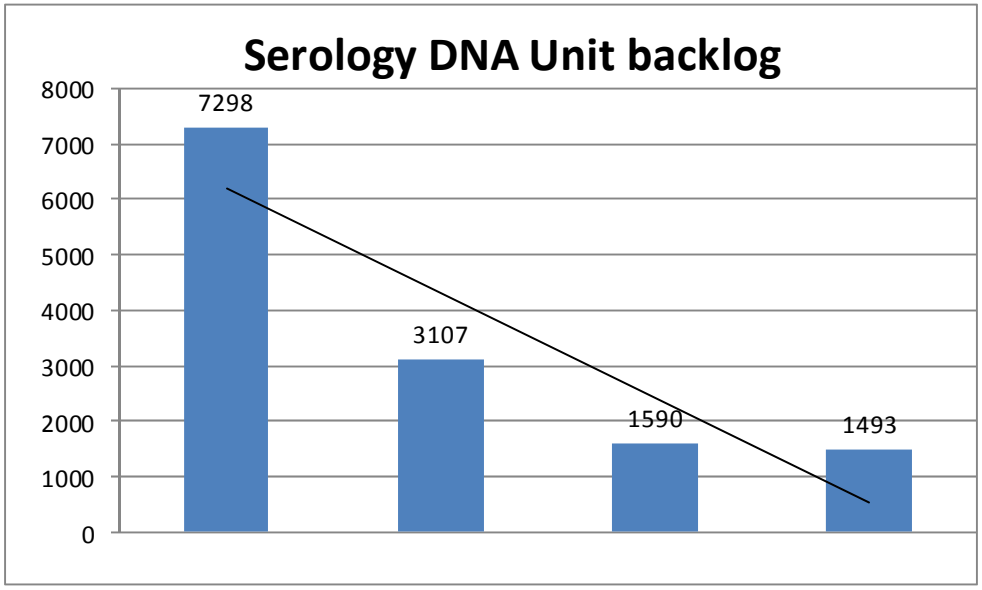
The turn-around time for requests for DNA analysis increased from 71 days to 108 days. This is due to the assigning of older cases in the backlog and the method for computing turn-around time (date requested to date delivered to the requesting agency, for cases completed during the reporting period).

The samples per analyst per month increased 82% from a baseline of 15.9 to 29.0.



Date	10/1/08	10/1/09	12/31/10	9/30/2011
Samples/Analyst/mo.	11.7	15.9	22.0	29.0

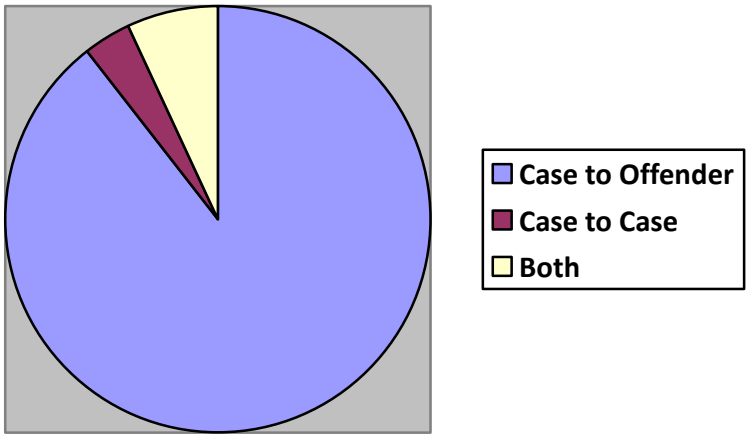
The backlog of requests for DNA analysis decreased approximately 52% from a baseline of 3107 to 1493.



Date	10/1/08	10/1/09	12/31/10	9/30/11
Total Cases	7298	3107	1590	1493

OBJECTIVE 2: Decrease the number of contract laboratory DNA cases awaiting data review

The backlog of contract laboratory cases awaiting data review decreased approximately 85% from a baseline of 2,470 (1778+692) to 376. As of September 30, 2011, LAPD has reviewed 2,865 reports returned from outside vendors under the 2009 Efficiency Grant. Those reviews have led to 1,705 cases with at least one CODIS upload and 895 cases with at least one CODIS Hit Notification. The Cases with Hit Notifications can be broken down as follows: 800 cases with Case to Offender Hits, 32 cases with Case to Case Hits, and 63 cases with both Case to Offender and Case to Case Hits. These cases were reviewed on overtime, billed to the grant, in the amount of $238,061.64.



CODIS Hits	Case to Offender	Case to Case	Both
895	800	32	63

B. Implication for Policy and Practice

OBJECTIVE 1: To improve DNA analysis efficiency

By utilizing funds from the Efficiency Grant, LAPD DNA Analysts were able to use grant overtime to perform reviews of DNA reports received from contract vendors and upload the profiles to CODIS. This freed up DNA Analysts to significantly improve our Laboratory's DNA analysis efficiency due to the additional time acquired during regular working hours. LAPD was able to use the additional time to become more efficient by improving our teams approach which relied on batching of like cases to avoid duplication of work. Additionally, we were able to validate and implement two new analytical platforms, Qiagen's Investigator Kit for DNA robotic extraction on Qiagen's EZ1 Advanced XL and ABI's Quant Duo® Kit for quantitation of extracted total and male DNA.

The EZ1 Advanced XL robotic extraction instrument from QIAGEN, utilizing QIAGEN's Investigator kit extraction chemistry, improved our ability to extract DNA rapidly using robotic techniques instead of extracting with chemicals by hand. Building upon our experience with an earlier model of QIAGEN's

extraction robot, the EZ1, the EZ1 Advanced XL offered the distinct advantage of being able to handle 14 samples at once, instead of the six sample capacity of the EZ1. The Investigator Kit was an improvement to QIAGEN's extraction chemistry, allowing LAPD to extract samples with smaller quantities of DNA, avoiding the time consuming step of organic extraction. Higher sample throughput together with application to samples with diminished amounts of DNA, have made LAPD more efficient in the time consuming step of DNA extraction.

The first step of the Quant Duo® Kit validation was to validate the method as a replacement for the current method used to quantify DNA, which yielded only information on total DNA quantity. Quant DUO® has helped make the LAPD more efficient in DNA analysis by giving us additional information on the presence or absence of male DNA, male DNA quantity vs. female DNA, and whether or not an inhibitor is present. All of this new information has aided the DNA Analyst in determining the next step in the analysis, potentially helping them to avoid unnecessary steps. The first step of the Quant Duo® validation has been completed and Quant Duo® is in use as our primary method for quantifying DNA.

The second step of the Quant Duo® validation is to validate a method that will allow us to utilize Quant Duo® not only as a quantifier of DNA, but also as a screening tool for the presence of male DNA, replacing microscopic identification of spermatozoa or epithelial cells as our primary sexual assault screening tool. Instead of extracting samples, preparing microscope slides, determining if spermatozoa or nucleated epithelial cells are present and then performing a second extraction of DNA from the same sample, the Quant Duo® kit can detect and quantify total, male and female DNA in one step. The DNA analyst can then decide if enough male DNA is present to attempt full STR profiling, perform Y STR profiling, or adjust their protocol to counteract the presence of an inhibitor. This will be a great time saver, making us more efficient by eliminating the time consuming step of microscopic identification of

cellular components and informing us on the nature of the sample, aiding the analyst in deciding upon the next step in analysis. The use of the Quant Duo® Kit to determine if male DNA is present has the potential to significantly speed up the analysis of sexual assault cases, making us more efficient and improving our sample throughput. The LAPD estimates that the second phase will be completed mid to late 2012. We anticipate to be done with the sample analysis sooner. It is believed that developing a SAEK "flow chart" for the manual will take the most time.

Validating a method for the Zeiss Palm Laser Microdissection (LMD) Scope has been challenging to say the least. The LAPD has had some success in developing the LMD for identifying and typing spermatozoa and nucleated epithelial cells removed from specialized non-glass, plastic (PEN) microscope slides. If it is decided to incorporate the LMD into our analysis protocols, the procedures will be refined, and we will then perform an internal validation study using the Zeiss PALM Laser Microdissection System (LMD). We are still in the early stages of preparation, but at this point we have been consistently able to obtain complete or nearly-complete, single-donor DNA profiles from both epithelial cells and spermatozoa collected via LMD when the sample is deposited on specialized PEN microscope slides. We have refined our method of mock evidence slide preparation, and analyzed yields using several collection and extraction protocols, in order to optimize LMD for use in casework. This includes evaluation of collection from glass versus PEN slides, collection into adhesive caps versus regular microcentrifuge caps, and extraction using our trace protocol versus Qiagen's Microextraction Kit. From what we've been able to accomplish so far, it appears that this instrument will be better suited for use on specialized cases with limited amount of spermatozoa and/or nucleated epithelial cells, and not suited for high throughput use. One possible application would be to isolate cellular material from old or archived slides, such as those found in old Coroner's sexual assault kits. Further work is

currently being performed with this instrument and we will continue to evaluate its use for efficiently extracting cellular material from slides that can yield useful DNA profiles.

Overall, the LAPD was able to improve its efficiency for samples per analyst per month by 82% and decrease its backlog of DNA analysis requests by 52%. Although it appears that our turn-around time increased from 71 to 108 days, this was skewed due to many older cases being analyzed during the latter phases of our contractor review process and a change in reporting parameters. Overtime from this grant allowed the LAPD to keep resources focused on the preceding areas, while still meeting the public safety necessity of completing the data reviews in a timely manner.

OBJECTIVE 2: Decrease the number of contract laboratory DNA cases awaiting data review

In order to prevent a large scale backlog from occurring again, the LAPD has devised and to a great extent has implemented a large scale hiring plan, with the goal of being able to perform the DNA analysis on virtually all types of DNA cases submitted to the laboratory for analysis. Because circumstances will always exist where specialized types of analysis will be required, a small portion of cases will in all likelihood continue to require the use of an outside contract laboratory for certain specialized cases. The hiring plan was implemented in 2008 and completed in June of 2011. However, positions which opened due to personnel lost to normal attrition continue to remain unfilled as Los Angeles City is currently experiencing large budget shortfalls, forcing the City to freeze hiring and promotions in most personnel classes, particularly civilians. Recognizing an extreme need, the Los Angeles City Council did allow Criminalists to be hired with the express intent of increasing the staff of DNA Analysts, which would allow for the reduction of our backlog. The proposed hiring plan is listed in Appendix C. None of the positions hired were paid for with funds from this grant.

Although we were able to hire personnel at an accelerated pace, it did not help with the immediate backlog that we faced, since hiring a large number of untrained personnel at a pace that had never been experienced before, created an enormous training problem that slowed down our case production, as experienced personnel were required to train new hires while away from casework. Faced with an inability to process an additional 6,132 sexual assault cases that were added to our backlog virtually overnight, the LAPD was forced to turn to contract laboratories and relied solely upon them to process the historical (6,132 old cases) backlog. Sending the sexual assault cases out to contract laboratories may have solved the analysis problem, but any resulting DNA profiles required a DNA analyst from a governmental laboratory to review the profiles before they could be submitted to CODIS.

In order to solve the problem of Subcontractor reviews (SCR), LAPD considered either utilizing the services of other governmental laboratories to perform the SCR's, or allow LAPD personnel to perform the SCR's on overtime. The LAPD opted to use the Efficiency Grant to pay for overtime utilized by its own personnel to complete the SCR's. By performing SCR's on overtime, experienced analysts were freed up to train new personnel, do casework, and complete validation projects on regular duty.

The use of the Efficiency Grant to fund overtime for the data review of DNA cases received from contract laboratories allowed the LAPD to greatly decrease its backlog of cases awaiting data review, going from 2,470 cases at the beginning of this grant period to 376 as of September 30, 2011, an 85% decrease. This led to 1,705 cases with at least one CODIS upload and 895 cases with at least one CODIS Hit Notification. The Cases with Hit Notifications can be broken down as follows: 800 cases with Case to Offender Hits, 32 cases with Case to Case Hits, and 63 cases with both Case to Offender and Case to Case Hits.

For the multitude of issues surrounding sexual assault backlogs at both LAPD and the Los Angeles County Sheriff's Department (LASD), refer to the Final Report Draft of the SEXUAL ASSAULT KIT BACKLOG STUDY, for the NATIONAL INSTITUTE OF JUSTICE's # 2006-DN-BX-0094 Grant. This report, authored by Joseph Peterson, Donald Johnson, Denise Herz, Lisa Graziano, and Taly Oehler of the California State University, Los Angeles, School of Criminal Justice & Criminalistics, provides an in-depth analysis of the history, approach, and resolution of issues surrounding the sexual assault kit backlogs that developed at both LAPD and LASD.

Without the award of this critical needs grant, only a small portion of the goals described herein would have been accomplished. What cannot be calculated yet are how many successful arrests and prosecutions that will occur because of the assistance that this grant has provided, but from the numbers that we have, it appears that this grant has been of great assistance to LAPD, law enforcement in general and ultimately to public safety.

Appendices

Appendix A

Extractions Using the EZ1 Advanced XL and Investigator Kit

The analyst shall refer to the instructions below that pertain to the particular type of item being extracted to ascertain the quantity of item to sample and lysis conditions/procedure. Following lysis, the sample is placed into the 2-mL tubes provided in the EZ1 DNA Investigator Kit. These tubes will be placed in row four of the EZ1 Advanced XL. Row 3 is not used. Row 2 holds the pipette tips/tip holders and the 1.5-mL elution tubes are placed in row 1. Select the desired protocol and the elution volume according to the anticipated sample yield. After the extraction is complete, the analyst may cap/collect the eluted DNA samples. Mix eluted samples by vortexing and proceed to quantification.

Extraction Procedures by Sample Type

Dried Blood, Dried Saliva, Fabric Stains, Cigarette Butts

1. Prepare the sample for extraction by cutting the stain and substrate. (For extracting a cigarette butt, cut approximately half of the filter paper into several smaller pieces.) Place the forensic sample to be extracted in a 2-mL sample tube.
2. Add 190-µL diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 or LAPD Digest buffer to the sample tube until the sample

volume is 190-µL.

Note: Prepare diluted Buffer G2 as described: Dilute Buffer G2 in sterile water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of sterile water)

3. Add 10-µL proteinase K, and mix thoroughly by vortexing for 10 seconds.

4. Incubate at 56 °C for 15 minutes. Vortex the tube once or twice during the incubation.

5. Optional: Incubate at 95 °C for 5 minutes.

Incubating the sample at 95 °C may increase the yield of DNA.

6. If necessary, centrifuge the tube briefly to remove drops from inside the lid.

7. Remove any solid material from the tube. Piggyback spin this substrate in a *Spin-Ease* tube and return the eluate to the original tube, along with any cellular material. The sample volume should be approximately 200-µL.

8. Optional: Carrier RNA (cRNA) may be added to each sample tube immediately prior to beginning the DNA Purification Trace Protocol. Carrier RNA can enhance the binding of DNA to the silica surface of the magnetic particles, especially if the sample contains less than 100-ng of DNA. Add 1-µL of reconstituted* cRNA to each of the sample tubes immediately prior to beginning the DNA Purification Trace Protocol.

* Reconstitute the cRNA with 310-µL of sterile, RNAse-free water

and aliquot 50-µL into sterile or autoclaved 0.5-mL tubes. Store in freezer.

9. Continue with Protocol: DNA Purification Trace Protocol.

Semen and Epithelial Cell

1. Place the forensic sample in a 1.5-mL or 2-mL sample tube.

2. Add 190-µL Buffer G2 to the sample.

3. Add 10-µL proteinase K, and mix thoroughly by vortexing for 10 seconds.

4. Incubate at 56 °C for 15 minutes. Vortex the tube once or twice during the incubation.

5. If necessary, centrifuge the tube briefly to remove drops from inside the lid.

6. Remove any solid material from the tube. Piggyback spin this substrate in a *Spin-Ease* tube and return the eluate to the original tube, along with any cellular material. The sample volume should be approximately 200-µL.

7. Centrifuge the tube at 15,000 x g for 5 minutes. Carefully transfer the supernatant to a new tube without disturbing the sperm cell pellet. DNA from epithelial cells can be purified from the tube containing the supernatant.

Note: The cell pellet may not be visible.

8. Wash the sperm cell pellet by resuspending the pellet in 500-µL diluted Buffer G2 or LAPD Digest Buffer. Centrifuge the tube at

15,000 x g for 5 minutes and discard the supernatant.

9. Repeat step 8 two or three times.

10. Add 180-µL Buffer G2 to the pellet and resuspend the pellet.

11. Add 10-µL proteinase K and 10-µL 1M DTT, and mix thoroughly by vortexing for 10 seconds.

12. Incubate at 56 °C overnight.

13. If necessary, centrifuge the tube briefly to remove drops from inside the lid. DNA from sperm cells can now be purified from this tube.

14. Optional: Carrier RNA (cRNA) may be added to each sample tube immediately prior to beginning the DNA Purification Trace Protocol. Carrier RNA can enhance the binding of DNA to the silica surface of the magnetic particles, especially if the sample contains less than 100-ng of DNA. Add 1-µL of reconstituted* cRNA to each of the sample tubes immediately prior to beginning the DNA Purification Trace Protocol.

* Reconstitute the cRNA with 310-µL of sterile, RNAse-free water and aliquot 50-µL into sterile or autoclaved 0.5-mL tubes. Store in freezer.

15. Continue with Protocol: DNA Purification Trace Protocol. The two tubes in which the epithelial and sperm cell fractions have been separated are ready for DNA purification.

Hair

1. Examine the hair under dissecting and or compound microscope; note presence of possible body fluids on hair. Wash hair to reduce surface dirt and contaminants as follows;

2. Loose hairs; cut 5- to 10-mm off the proximal (root) end of the hair and transfer to a 2-mL sample tube. Save the remaining hair.

3. Add about 1000-µL of sterile water to the tube containing the hair root, cap and wash by shaking or vortexing for 30 seconds.

4. Let stand for 10 minutes and shake or vortex again for 30 seconds.

5. Remove and discard the wash liquid with a sterile pipette leaving the hair section in the tube.

6. Repeat steps 3 through 5, if needed.

7. Add 180-µL Buffer G2 to the sample.

8. Add 10-µL proteinase K and 10-µL DTT solution, and mix thoroughly by vortexing for 10 seconds.

9. Incubate at 56 °C for at least 6 hours. Digestion may continue overnight. Vortex the tube once or twice during the incubation.

10. Add another 10-µL proteinase K and 10-µL DTT solution, and mix thoroughly by vortexing for 10 seconds.

11. Incubate at 56 °C for at least 2 hours or until the hair samples are completely dissolved.

12. If necessary, centrifuge the tube briefly to remove drops from inside the lid.

13. Optional: Carrier RNA (cRNA) may be added to each sample tube immediately prior to beginning the DNA Purification Trace Protocol. Carrier RNA can enhance the binding of DNA to the silica surface of the magnetic particles, especially if the sample contains less than 100-ng of DNA. Add 1-µL of reconstituted* cRNA to each of the sample tubes immediately prior to beginning the DNA Purification Trace Protocol.

* Reconstitute the cRNA with 310-µL of sterile, RNAse-free water and aliquot 50-µL into sterile or autoclaved 0.5-mL tubes. Store in freezer.

14. Continue with Protocol: DNA Purification Trace Protocol

Other Substrates

1. Place the forensic sample in a 2-mL sample tube.

2. Depending on the type of sample, follow either step 2a. (for nonabsorbent samples) or step 2b. (for absorbent samples).

2a. Non-absorbent samples: Add 190-µL Buffer G2 to the sample.

2b. Absorbent samples: Add 190-µL diluted Buffer G2 to the sample. Check if the sample has absorbed some or all of the buffer, and if necessary add more diluted Buffer G2 or LAPD Digest Buffer to the sample tube until the sample volume is 190-µL.

Note: Prepare diluted Buffer G2 as described: Dilute Buffer G2 in sterile water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of sterile water)

3. Add 10-µL proteinase K, and mix thoroughly by vortexing for 10 seconds.

4. Incubate at 56 °C for 15 minutes. Vortex the tube once or twice during the incubation.

5. If necessary, centrifuge the tube briefly to remove drops from inside the lid.

6. Remove any solid material from the tube. Piggyback spin this substrate in a *Spin-Ease* tube and return the eluate to the original tube, along with any cellular material. The sample volume should be approximately 200-µL.

7. Optional: Carrier RNA (cRNA) may be added to each sample tube immediately prior to beginning the DNA Purification Trace Protocol. Carrier RNA can enhance the binding of DNA to the silica surface of the magnetic particles, especially if the sample contains less than 100-ng of DNA. Add 1-µL of reconstituted* cRNA to each of the sample tubes immediately prior to beginning the DNA Purification Trace Protocol.

* Reconstitute the cRNA with 310-µL of sterile, RNAse-free water and aliquot 50-µL into sterile or autoclaved 0.5-mL tubes. Store in freezer.

8. Continue with Protocol: DNA Purification Trace Protocol.

Forensic Surface and Contact Swabs

1. Cut and place the forensic sample in a 2-mL sample tube.

2. Add 290-µL of diluted Buffer G2 to the sample.

Note: Prepare diluted Buffer G2 as described: Dilute Buffer G2 in sterile water using a ratio of 1:1 (i.e., one volume of Buffer G2 to one volume of sterile water)

3. Add 10-µL proteinase K, and mix thoroughly by vortexing for 10 seconds.

4. Incubate at 56 °C for 25-30 minutes. Vortex the tube once or twice during the incubation.

5. Recommended: Incubate at 95 °C for 5 minutes. Incubating the sample at 95 °C may increase the yield of DNA.

6. If necessary, centrifuge the tube briefly to remove drops from inside the lid.

7. Remove any solid material from the tube. Piggyback spin this substrate in a *Spin-Ease* tube and return the eluate to the original tube, along with any cellular material. The sample volume should be approximately 290-µL.

8. Add 1-µL of reconstituted* cRNA to each of the sample tubes immediately prior to beginning the DNA Purification Trace Protocol.

* Reconstitute the cRNA with 310-µL of sterile, RNAse-free water and aliquot 50-µL into sterile or autoclaved 0.5-mL tubes.
Store in freezer.

9. Continue with Protocol: DNA Purification Trace Protocol.

Positive and Negative Extraction Controls EZ1 Advanced XL

1. The positive extraction control (PEC) shall be processed with the samples being extracted. Ideally, the positive extraction control should be the same type of sample as the samples being extracted. For example, a blood swatch would be used along with a blood extraction; hair for a hair extraction; and semen for semen / epithelial cell extraction. In other situations it is not possible to have the same type of PEC sample. The analyst must choose an appropriate positive extraction control for these situations. A negative extraction control shall also be processed with the samples being extracted and shall consist of the reagents used to perform the lysis and extraction of forensic or reference samples.

2. A positive extraction control must be included for each subsequent run of forensic or reference samples being extracted in a large batch.

Appendix B

Directions for Manual Set-up of Real-time PCR Reactions for the Quantification of Human DNA using Quantifiler® Duo® Quantification Kit

Introduction

The Quantifiler Duo® Quantification kit simultaneously quantifies the total amount of amplifiable human DNA and male DNA as well as the presence of PCR inhibitors in a sample. The primers used for this real-time assay are the ribonuclease P RNA component H1 (RPPH1) for human detection, sex determining region Y (SRY) for human detection and an internal PCR control (IPC). When validated using Applied Biosystems 7500 Real-Time PCR instrument, the Quantifiler Duo® Kit was found to be a reliable and accurate assay for degraded, inhibited and male/female mixed DNA samples.

Sample Sheet Set-Up

1. Locate the "Quant Duo® set-up sheet" on the P:/DNA ANALYSIS FORMS and input the sample names as per sheet instructions.
2. Print the second sheet, Quantifiler Duo® Sample Sheet & Plate Set-up sheet. This page is used to record reagent lot numbers during plate set up.
3. Save the last sheet as a text (tab delimited) file using your run name (based on date and your initials i.e. 011510spr). A message may be displayed that says you cannot save the file in this format. Disregard these messages by clicking on "OK" and "YES".
This file will be used to import your plate on the instrument.

Standard and Sample Preparation

1. Obtain the following reagents and thaw to room temperature if necessary: Quantifiler Duo® DNA standard, Quantifiler Duo® Primer Mix and Quantifiler Duo® PCR Reaction Mix and TE-4 buffer.

2. Prepare DNA quantification dilution series of eight standards. These can be used up to 1 month from date of preparation. The volume of standards can be prepared in different volumes (2X, 3X, etc) depending on analyst usage. The minimum volume of standards should be made as follows:

a. Label eight microcentrifuge tubes 1-8 with the date of preparation

b. Pipette 30-µL of TE-4 buffer to the tube labeled 1.

c. Pipette 20-µL of TE-4 buffer to the tubes labeled 2-8.

d. Vortex the Quantifiler Duo® DNA standard and spin down briefly.

e. Pipette 10-µL of the Quantifiler Duo® DNA standard to tube labeled 1, vortex to mix and spin down briefly.

f. Pipette 10-µL from standard 1 into the tube labeled 2, vortex to mix and spin down briefly.

g. Pipette 10-µL from standard 2 into the tube labeled 3, vortex to mix and spin down briefly.

h. Continue the dilution series for standards 4-8.

i. The eight standards are now in the following concentrations:

STANDARD	DNA Concentration **[ng/µL]**
1	50.0
2	16.7
3	5.56
4	1.85
5	0.620
6	0.210
7	0.068
8	0.023

3. Prepare the master mix: obtain the Quantifiler Duo® Primer Mix and Quantifiler Duo® PCR Reaction Mix. Vortex the Primer Mix briefly and swirl the PCR Reaction Mix prior to use.

4. Obtain the DNA dilution series standards, positive DNA control (made in-house for qPCR) and negative control (TE-4 buffer). Vortex before use.

5. Pipette the required volumes of Quantifiler Duo® Reaction Mix and Quantifiler Duo® Primer Mix into a 1.5-mL or 2-mL tube to make the PCR master mix as follows (add 3 additional reactions to provide excess volume for the loss that occurs during reagent transfers):

Component	**Volume per Reaction (µL)**
Quantifiler Duo®	Reaction Mix 12.5
Quantifiler Duo®	Primer Mix 10.5

6. Dispense 23-µL of the PCR master mix to each well being used.

7. Add 2-µL of sample, standard or control to the applicable wells.

8. Seal the reaction plate with the Optical Adhesive Cover. Smooth out the cover with the gold-colored squeegee tool.

9. Centrifuge the plate at 3,000 RPM for about 20 seconds in a tabletop centrifuge to remove any bubbles.

Plate and Instrument Set-Up

1. Turn on the laptop computer. After the computer has booted up, turn on the ABI 7500 by pressing the power button on the lower right front of the instrument. Double-click the ABI Prism 7000 or 7500 SDS SOFTWARE icon on the desktop. Under the FILE menu heading, select "NEW". Click "FINISH," then select "IMPORT SAMPLE SET UP" under the FILE menu heading and select the previously saved text (tab delimited) file.

2. Check the configuration for accuracy once the 96-well sample layout opens by doubleclicking a well or by selecting "Well Inspector" under the VIEW heading.

a. Verify the standards have the correct concentrations listed and that "Duo® Human" and "Duo® Male" are checked and Task is listed as "standard". The "Duo® IPC" should be checked and the Task listed as "unknown".

b. Verify the negative control has "Duo® Human" and "Duo® Male" checked and Task is listed as "NTC". The "Duo® IPC" should be checked and the Task listed as "unknown".

c. Verify the samples have "Duo® Human", "Duo® Male" and "Duo® IPC" checked and Task is listed as "unknown" for all three detectors.

3. Press on the tray door of the instrument to open it.

4. Load the 96-well sample plate into the plate holder in the instrument. Ensure that that notched A12 position is aligned at the top-right of the tray. Close the tray door.

5. Before starting the run, select the "INSTRUMENT" tab of the program and perform the following:

a. change the sample volume to 25-µL

b. ensure that the 9600 emulation box is selected

c. check the following cycling parameters

Stage 1	1 Rep of 50.0 for 2 minutes
Stage 2	1 Rep of 95.0 for 10 minutes
Stage 3	40 Reps of 95.0 for 15 seconds, then 60.0 for 1 minute

6. Save the plate document (as an SDS Document (*.sds) file) in your personal folder located within the "Casework" Folder using the date and your initials (i.e. 011510spr).

7. Click on the start button. Wait until the program calculates the completion time, then the instrument can be left unattended.

8. Upon completion of the run, the arrow on the system software tool bar turns green.

9. Remove the sample plate from the instrument and discard.

10. Turn off the ABI 7500 instrument and close out of the SDS SOFTWARE, leave the laptop on.

Data Analysis

1. To analyze the plate document, either select the green arrow on the tool bar or select "ANALYZE" under the Analysis header.

2. Check your standard curve as follows:

a. Click on the “RESULTS” tab, and select the “STANDARD CURVE” tab.

b. In the detector drop-down list select each detector individually: Duo® Human and Duo® Male. Both standard curves can be viewed at the same time if you select “ALL” in the Detector drop-down list.

c. View the CT values for the quantification standard reactions and the calculated regression line slope and R2 values. The values should be:

R2 (linear correlation coefficient)	> 0.98
Slope	between -3.0 and -3.6

Note: If the slope or R2 value falls outside this range, the analyst may omit one standard data point that appears to be a statistical outlier. If the R2 value and slope continue to not meet the above criteria, consult the DNA Technical Leader or their designee to troubleshoot the issue.

3. Check your y-intercept and positive DNA control as follows:

a. Compare the y-intercept of the calculated human and male regression line with the section’s current range located on the P:/drive > Instrument Sign up > 7500

b. Compare the quantitation of the positive control with the section’s current range located on the P:/drive > Instrument Sign up > 7500

c. For large deviations with either the y-intercept or positive DNA control, consult with the DNA Technical Leader or their designee to troubleshoot the issue to troubleshoot the issue.

4. Check your negative controls as follows:

a. Confirm the NTC well(s) and negative extraction controls contain no DNA or

amounts low enough that these may not produce STR profiles

b. For DNA amounts in these wells high enough to produce STR profile, the analyst may repeat the Quant Duo® assay or consult with the DNA Technical Leader or their designee to troubleshoot the issue to troubleshoot the issue.

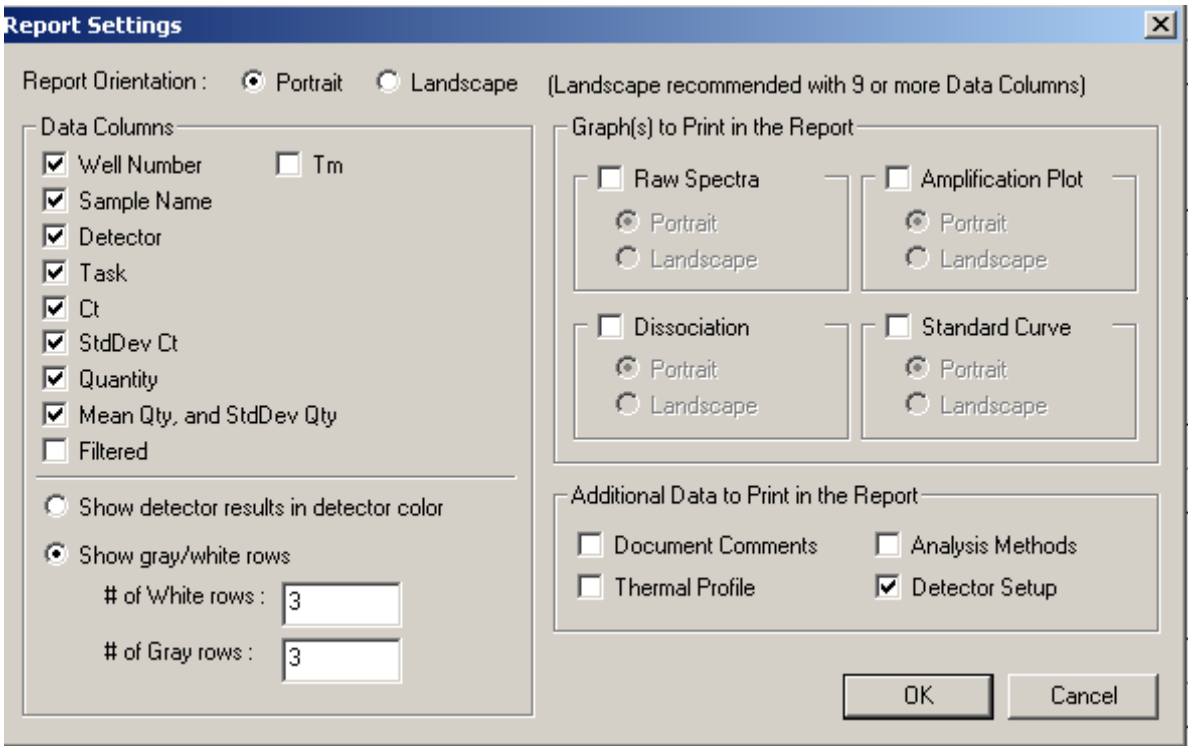
5. Fill out the standard curve information (slope, y-intercept, and R2 value) on the Quantifiler Duo® Sample Sheet & Plate Set-up sheet.

6. Archive the analyzed run by saving it on the U:/drive under the folder LAPD DNA Quant Duo® Runs.

Printing and Saving Results

1. Print the standard curves individually (for both the Human and Male detectors) by clicking "PRINT" under the FILE heading while viewing them if desired.

2. Go to "REPORT SETTINGS" under the TOOLS heading to format the report for printing:



3. Highlight all the samples, controls and standards under the “Report” heading. Select “PRINT” under the FILE heading to print the quantitation report if desired.

4. Next print out the plate results by selecting the “PLATE” heading under the RESULTS tab.

5. Save your analyzed project and place a copy of the run on the U:/drive for electronic storage.

6. Only the Quantifiler Duo® Sample Sheet & Plate Set-up sheet and Plate results sheet is needed for your case file.

Sample Interpretation and Evaluation

1. IPC amplification

The purpose of the IPC system is to distinguish between true negative sample results and reactions affected by: PCR inhibitors, assay setup and chemistry or instrument failure.

a. Check the IPC Ct value of a sample against the IPC Ct value of the standards. Place a check mark on the Quantifiler Duo® Sample Sheet & Plate Set-up sheet that the IPC has been reviewed.

b. If the IPC Ct value of the sample is greater than the average value of the standards, your sample may be inhibited. Manually write this Ct value to the Plate results sheet.

c. Verify amplification in the Duo® Human and/or Duo® Male to interpret your sample as below:

Duo® Human(VIC)®and/or Duo® Male (FAM™)	**Duo® IPC (NED™)**	**Interpretation**
No amplification	Amplification	Negative result (no human DNA detected)
No amplification	No amplification	*Invalid result (Possible PCR inhibition)
Amplification	No amplification or CT higher than ~31	Possible PCR inhibition

*Invalid result- If the human or male-specific target and the IPC target fail to amplify, then it is not possible to distinguish between PCR failure and PCR inhibition.

d. If the sample has a potential inhibitor, the analyst may prepare a dilution of the sample (1: 10 dilution is recommended) and quantify the dilution and/or amplify the dilution for STR typing. If the degree of inhibition detected is not considered great enough to affect STR amplification the analyst may continue without sample dilution.

e. The sample may also be cleaned up using a filter device such as the Microcon Ultracel YM-100 Centrifugal Filter Device.

2. Human and Male sensitivity

Quantifiler Duo® was shown to detect male DNA, reproducibly, at quantities down to 10-pg/µL and human DNA down to 15-pg/µL. For samples that are negative for human DNA and male DNA, analysts may want to concentrate and re-quantify.

3. Male to Female Ratios

The Quant Duo® kit provides the quantity of human and male DNA in a single sample.

The ratio of male and female DNA can be calculated using the following equation:

Male DNA: Female DNA Ratio =

Male DNA/Male DNA: (Human DNA - Male DNA)/Male DNA

For example, if the [Male DNA] = 2-ng/µL and [Human DNA] = 8-ng/µL

Then the Male DNA: Female DNA ratio is 2/2: (8-2)/2 = 1: 3

The Male: Female DNA ratio may be calculated and considered when determining the best course of action for a given sample in a case.

4. After assessing the quantity and quality of DNA in a sample, proceed to STR amplification using the human DNA value or dilute/concentrate if necessary.

Appendix C

The SID multi-phased DNA backlog elimination and real time testing plan, currently being implemented, was established to maximize both the rapid elimination of the rape kit backlog and to increase in-house capacity to meet current and future demands for DNA services while eventually minimizing reliance on outsourcing in the future. The plan incorporates both outsourcing and in-house analysis of DNA cases as additional in-house staff are hired and trained.

The initial phases of the plan relied heavily on outsourcing to rapidly reduce the large backlog of unanalyzed sexual assault cases. This was necessary because the City did not possess enough trained in-house staff to meet the need in a timely manner and avoid case statute issues. Criminalists and support personnel hired under the plan, once trained, will ensure the Department meets the City's DNA analysis needs into the foreseeable future without relying on private companies to perform standard DNA analysis. Limited outsourcing will always be required to perform unique or specialized analysis. For the limited number of instances when specialized types of analysis are required, it is fiscally prudent to outsource the work as opposed to hiring and training criminalists, providing specialized equipment, and maintaining those procedures in house.

	Staff Increase Fiscal Year	Goal	Backlog Impact
Phase 1	14 – FY 07/08	Start building infrastructure. Outsource cases at a rate to exceed new cases being added to the backlog	Negligible FY 07/08
Phase 2	16 – FY 08/09	Increase infrastructure. Outsource cases at a rate anticipated to eliminate the historical backlog of cases in a timely manner	Sexual Assault kit backlog reduced by ~65% by Nov. 2009 utilizing phase 1, phase 2 and previously existing staff. Negligible impact on other category backlogs.
Phase 3	26 – FY 09/10	Complete the hiring of analytical staff needed to meet the DNA needs of the Department for real time testing of all sexual assault kits and the current level of all other requests for DNA analysis on violent crimes	The analysts in this phase will ensure the backlogs in other categories are reduced and the backlog of sexual assault cases does not return.
Phase 4	10	Complete the hiring of support staff, including supervisors,	This phase provides proper span of control of supervisors and

		leads and technicians.	technical leads over analysts hired in Phase 3. Which is essential to ensure quality results. Some of the positions provide technical support for the analysts hired in Phase 3 to ensure trained DNA analysts are not performing clerical and/or information technology tasks

Breakdown by Phase including Civil Service Class

	Includes Phase 1	Phase 2		Phase 3				Phase 4	
Rank	Staffing Levels on 6-30-08	Approved in 08/09 Budget	Total Staff	Needed to Meet Current Requests and all SAK's	Total Staff	Needed to Process Outsourced SAK Backlog	Total Staff	Needed for proper span of control and support	Total Staff
CFCI	1	1	2	0	2	0	2	0	2
Supervising Criminalist	3	1	4	0	4	0	4	4	8
Criminalist III	5	1	6	1	7	0	7	3	10
Criminalist	30	10	40	17	57	3	60	0	60
Laboratory Technician	6	2	8	2	10	2	12	1	13
Clerk Typist	1	0	1	1	2	0	2	0	2
Management Analyst II	1	0	1	0	1	0	1	0	1
Systems Analyst II	0	1	1	0	1	0	1	1	2
Systems Analyst	0	0	0	0	0	0	0	1	1
Total	47	16	63	21	84	5	89	10	99

Appendix D

EZ1 Advanced XL Robotic Workstation and EZ1 DNA Investigator Kit Internal Validation Study

FBI Quality Assurance Standard 8.3.1 states that internal validation studies conducted on or after July 1, 2009, shall include as applicable: known and non probative evidence samples or mock evidence samples, reproducibility and precision, sensitivity and stochastic studies, mixture studies, and contamination assessment. This validation addresses each of these areas for use of the EZ1 DNA Investigator Kit on the EZ1 Advanced XL robotic workstation. The protocols provided by the manufacturer (Qiagen) were used with minor modifications when necessary. As with any validation, all possible scenarios cannot be tested. However, a wide range of samples was used to show that the platform and kit function as intended. Ultimately, the usefulness and performance capabilities of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit will be determined by the DNA analysts.

Internal Validation Studies

Reproducibility and precision

Objective:
A blood dilution series was analyzed in duplicate to establish reproducibility of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit.

Methods:
Two sample sets of five swabs with different amounts of whole blood were prepared. Each swab was spotted with one of the following predetermined amounts of whole blood: 10-µL, 1-µL, 0.1-µL, 0.01-µL and 0.001-µL. The samples were extracted using Qiagen Protocol: Pretreatment for Dried Blood. Samples were then quantified using the Laboratory's current procedure for real-time PCR (CFS-HUMTH01 assay). Amplification was performed on the Applied Biosystems (AB) 9700 thermal cycler using the AB AmpFℓSTR Identifiler PCR Amplification Kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GeneMapper ID (GMID) software version 3.2.1.

Results and discussion:
Full profiles were obtained from the 10-µL and 1-µL samples. At 0.1-µL, a full profile was obtained from one sample while dropout in the FGA locus was observed in the second sample. No results were obtained from the 0.01-µL and 0.001-µL samples. Comparable amounts of DNA were recovered in the replicate samples from each group. Additionally, there was a direct correlation between the sample size and the amount of DNA recovered.

Sample	Amount	Quant	Total DNA	Sample	Amount	Quant	Total DNA
1x-1	10-µL	2.23E-01	22.30-ng	2x-1	10-µL	2.95E-01	29.50-ng
1x-3	1-µL	8.47E-02	8.47-ng	2x-3	1-µL	1.36E-01	13.60-ng
1x-5	0.1-µL	2.15E-02	2.15-ng	2x-5	0.1-µL	2.55E-02	2.55-ng
1x-7	0.01-µL	2.42E-03	0.24-ng	2x-7	0.01-µL	9.53E-04	0.10-ng
1x-9	0.001-µL	0.00E+00	0.00-ng	2x-9	0.001-µL	0.00E+00	0.00-ng

Sensitivity and stochastic effects

Objective:

To assess the ability of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit to extract low-level samples using blood, and mixed epithelial cell and semen samples. These low-level samples were also evaluated for allelic dropout and stochastic effects by taking the samples through to STR typing.

Methods:

Blood dilution series

Four sample sets of five swabs with different amounts of whole blood were prepared. Each swab was spotted with one of the following predetermined amounts of whole blood: 10-µL, 1-µL, 0.1-µL, 0.01-µL and 0.001-µL. The samples were extracted using Qiagen Protocol: Pretreatment for Dried Blood. Two of the sample sets were treated with carrier RNA (cRNA) and two were not. Samples were then quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Mock vaginal swabs

Two sets of mixed epithelial cell and semen samples were prepared. Mock vaginal swabs were prepared by collecting buccal swabs from a female. The semen dilution series consisted of the following: neat, 1:10, 1:100, 1:1,000 and 1:10,000. Ten microliters (µl) of the appropriate semen dilution sample were added to each buccal swab. The samples were extracted using Qiagen Protocol: Pretreatment for Epithelial Cells Mixed with Sperm Cells. Samples were then quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Results and discussion:

Blood dilution series

Full typing results were obtained from all 10-µL and 1-µL samples. No typing results were obtained from the 0.01-µL and 0.001-µL samples. At 0.1-µL, the results varied from obtaining a full DNA profile to dropout at 8 loci. Consideration must be given to the fact that the samples were not concentrated after extraction.

The average total amount of DNA recovered in this blood dilution series is reported in the following table:

Average Total DNA

Sample amount	DNA (without cRNA)	DNA (with cRNA)
10-µL	25.9-ng	71.2-ng
1-µL	11.04-ng	13.35-ng
0.1-µL	2.35-ng	2.1-ng
0.01-µL	0.17-ng	0.39-ng
0.001-µL	0.00-ng	0.05-ng

Carrier RNA (cRNA) is reported to increase the amount of DNA recovered. In this limited sample set, that appears to hold true for only the samples prepared with larger amounts of DNA, but not for the smaller amounts. Without concentration, interpretable DNA typing can be expected from samples with as little as 1-µL of blood. Concentrating the sample should allow for interpretable typing with samples containing as little as 0.1-µL of blood. As input DNA decreased below the minimum recommended level of 1-ng for the Identifiler kit, allelic dropout was observed.

Mock vaginal swabs

Full typing results were observed in all of the epithelial cell samples. This was expected as each sample was an undiluted buccal swab. Full typing results were obtained from the neat and 1:10 diluted semen samples. At a 1:100 dilution, dropout was observed at six loci in one sample while nearly complete typing results were obtained from the second. Dilutions of 1:1,000 and 1:10,000 provided little to no typeable results from the sperm cell fraction. The average total amount of male DNA recovered in this series is reported in the following table:

Average Total Male DNA (Sperm Fraction)

Sample	DNA
Neat semen	546-ng
1:10 sp	39.4-ng
1:100 sp	4.2-ng
1:1,000 sp	1.1-ng
1:10,000 sp	0.41-ng

Without concentration, interpretable DNA typing can be expected from vaginal swabs containing the equivalent of 1-µl of neat semen. Concentrating the sample should allow for interpretable typing from vaginal swabs containing the equivalent of 0.1-µl of neat semen. As input DNA decreased below the minimum recommended level for the Identifiler kit, allelic dropout was observed.

Mixture studies

Objective:
To assess the ability of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit to separate mixed epithelial cell and semen samples and obtain interpretable profiles

Methods:
Two sets of mixed epithelial and semen samples were prepared. Mock vaginal swabs were prepared by collecting buccal swabs from a female. The semen dilution series consisted of the following: neat, 1:10, 1:100, 1:1,000 and 1:10,000. Ten microliters of the appropriate semen sample were added to each buccal swab. The samples were extracted using Qiagen Protocol: Pretreatment for Epithelial Cells Mixed with Sperm Cells. Samples were then quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Results and discussion:
Separation between the epithelial cell fraction and the sperm cell fraction was achieved in all samples where typing results were obtained, resulting in mixture profiles that could be interpreted. As input DNA decreased below the minimum recommended level for the Identifiler kit, allelic drop out was observed. This allelic dropout does not allow for a complete DNA mixture profile interpretation.

Contamination assessment

Objective:
To assess the ability of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit to function without DNA cross-contamination.

Methods:
Blood samples and blank samples were placed on the EZ1 Advanced XL rack in alternating positions (i.e. blood sample, blank sample, blood sample, blank sample, etc.) Blank samples were extracted simultaneously with blood samples in two replicate extraction procedures. The amount of blood added to the positive samples was as follows: 10-µL, 1-µL, 0.1-µL, 0.01-µL, and 0.001-µL. The blood samples and blank samples were extracted using Qiagen Protocol: Pretreatment for Dried Blood. Samples were then quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Results and discussion:
One blank sample quantified above the level of "undetected" at 0.0007-ng/µL. However, none of the blank samples produced any detectable typing results. This demonstrates the ability of the instrument and kit to function without producing DNA cross-contamination.

Mock evidence samples

Objective:
To assess the ability of the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit to extract DNA from various substrates including hair, buccal swabs, bloodstained denim, cigarette butts and worn clothing items.

Methods:

Hair
Four hair samples (pulled hairs with roots) were extracted using Qiagen Protocol: Pretreatment for Hair.

Buccal swabs
Four buccal swabs were collected, dried and extracted using Qiagen Protocol: Pretreatment for Saliva (including the incubation at 95 °C for 5 minutes).

Blood on denim
Two bloodstains were prepared by spotting 10-µL of blood on denim swatches. The samples were extracted using Qiagen Protocol: Pretreatment for Stains on Fabric.

Cigarette butts
Four cigarette butts from two donors were extracted using Qiagen Protocol: Pretreatment for Cigarette Butts.

Worn items
Two baseball caps were swabbed with one swab each. The two swabs were dried and extracted using Qiagen Protocol: Pretreatment for Forensic Surface and Contact Swabs.

All mock evidence samples were quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Results and discussion:

Hair
Full typing results were obtained from each hair. The following table lists the amount of DNA recovered from each hair sample:

Sample	Total DNA
Hair 1	350-ng
Hair 2	18-ng
Hair 3	27.1-ng
Hair 4	543-ng

Buccal Swabs
Full typing results were obtained from each buccal swab. The following table lists the amount of DNA recovered from each buccal swab sample:

Sample	Total DNA
Buccal swab 1	368-ng
Buccal swab 2	447-ng
Buccal swab 3	472-ng
Buccal swab 4	787-ng

Bloodstains on denim
Full typing results were obtained from each sample. The following table lists the amount of DNA recovered from each bloodstained denim sample:

Sample	Total DNA
Denim/blood 1	37.3-ng
Denim/blood 2	20.3-ng

Cigarette butts
Typing results from the cigarette butt samples varied from obtaining full profiles to results in three loci. Samples were not concentrated after extraction. The following table lists the amount of DNA recovered from each cigarette butt:

Sample	Total DNA
Cigarette butt 1a	50.6-ng
Cigarette butt 1b	52.5-ng
Cigarette butt 2a	5.32-ng
Cigarette butt 2b	0.8-ng

Cigarette butts 1a and 1b were collected by donor #1 immediately after smoking. Both cigarette butts yielded a large amount of DNA and produced full typing results.

Cigarette butts 2a and 2b were collected from an ash tray after being discarded by donor #2. Results were obtained at eight loci and amelogenin for cigarette butt 2a without concentration. With pre-amplification concentration, a full DNA profile would be expected from cigarette butt 2a. Cigarette butt 2b produced a partial DNA profile at only three loci. One allele from this partial profile is foreign to donor #2. Even with concentration, a full DNA profile is not expected from cigarette butt 2b.

Worn items
The following table lists the amount of DNA recovered from each baseball cap swab:

Sample	Total DNA
Baseball cap swab 1	0.054-ng
Baseball cap swab 2	1.24-ng

Typing results were not obtained from swab 1 and even with concentration, a full DNA profile is not to be expected. Results were obtained at seven loci and amelogenin for swab 2 without concentration. With pre-amplification concentration, a full DNA profile would be expected from swab 2.

"Touch" DNA samples and comparison to Phenol/Chloroform/Iso-amyl alcohol (PCI) and Qiagen BioRobot M48 DNA extraction methods

Objective:
To compare the EZ1 Advanced XL robotic workstation and the DNA Investigator Kit with the PCI and M48 DNA extraction methods

Methods:

Steering wheel swabs
Each steering wheel was divided into three sections and each section was swabbed with one swab. Three steering wheels were sampled for a total of nine swabs. For each steering wheel, one swab was extracted using the EZ1 Advanced XL/Investigator Kit using Qiagen Protocol: Pretreatment for Forensic Surface and Contact Swabs, one swab was extracted using the PCI protocol and one swab was extracted using the Qiagen BioRobot M48.

Aluminum drinking can swabs
Three aluminum cans were collected from two different individuals for a total of six cans. One swab was used to sample the mouth area of each aluminum can. For each of the three swabs from one individual, one swab was extracted using the EZ1 Advanced XL/Investigator Kit using Qiagen Protocol: Pretreatment for Forensic Surface and Contact Swabs, one swab was extracted using the PCI protocol and one swab was extracted using the Qiagen BioRobot M48.

Drinking bottle swabs
Three bottles were collected from one individual and the mouth area of each bottle was sampled with one swab. Each swab was extracted using the EZ1 Advanced XL/Investigator Kit using Qiagen Protocol: Pretreatment for Forensic Surface and Contact Swabs, the PCI protocol or the Qiagen BioRobot M48.

Three different computer mice, each swabbed in triplicate, were sampled for a total of nine swabs. For each computer mouse, one swab was extracted using the EZ1 Advanced XL/Investigator Kit using Qiagen Protocol: Pretreatment for Forensic Surface and Contact Swabs, one swab was extracted using the PCI protocol and one swab was extracted using the Qiagen BioRobot M48.

Samples were then quantified using the Laboratory's current procedure for real-time PCR. Amplification was performed on the AB 9700 thermal cycler using the AB AmpFℓSTR Identifiler kit. Where possible, a target of 1-ng input DNA was used for amplification. Samples with less than 1-ng input DNA were amplified using 10-µL of extract without concentration. Samples were analyzed on the AB 3130 Genetic Analyzer and interpreted using AB GMID software version 3.2.1.

Results and Discussion:

Steering wheel swabs
A complete DNA profile was obtained from one swab extracted by the M48. No DNA profile was obtained from one swab extracted by PCI (most likely due to inhibition) and partial DNA profiles were obtained from the remaining seven. Two swabs extracted by the EZ1 Advanced XL were re-injected for 10 seconds with mixed results. The number of loci detected increased for one partial DNA profile and decreased slightly for the other. The same was also true for two swabs extracted using the M48 protocol.

	EZ1 XL/Inv. Kit	PCI	M48
Steering wheel swab	**Total DNA in 50-µL**	**Total DNA in 50-µL**	**Total DNA in 50-µL**
A	0.88-ng	6.85-ng	18.6-ng
B	1.59-ng	4.97-ng	1.34-ng
C	0.57-ng	4.84-ng	0.95-ng

Aluminum drinking can swabs
Five of the six swabs exhibited complete DNA profiles. One swab extracted by PCI exhibited no DNA profile (most likely due to inhibition).

	EZ1 XL/Inv. Kit	PCI	M48
Aluminum can swabs	**Total DNA in 50-µL**	**Total DNA in 50-µL**	**Total DNA in 50-µL**
D	57.5-ng	63.5-ng	125-ng
E	368-ng	Undet.	367-ng

Bottle swabs

Two of the three swabs extracted resulted in complete DNA profiles. One swab extracted by the M48 resulted in a partial DNA profile. The M48 swab was re-injected for 10 seconds which resulted in an increase of loci detected for that sample.

	EZ1 XL/Inv. Kit	PCI	M48
Bottle swabs	Total DNA in 50-µL	Total DNA in 50-µL	Total DNA in 50-µL
F	2.84-ng	8.35-ng	1.13-ng

Computer mouse swabs

Four of the nine swabs exhibited complete DNA profiles. One swab extracted by the EZ1 Advanced XL exhibited a DNA profile that was missing two alleles. Two swabs extracted by PCI exhibited DNA profiles missing either one or two alleles. Two swabs extracted by the M48 exhibited partial DNA profiles. These samples were re-injected for 10 seconds which resulted in an increase in the number of loci detected for both.

	EZ1 XL/Inv. Kit	PCI	M48
Comp mouse swabs	Total DNA in 50-µL	Total DNA in 50-µL	Total DNA in 50-µL
G	2.96-ng	7.75-ng	2.83-ng
H	5.1-ng	14.8-ng	3.12-ng
I	29.7-ng	104-ng	19.1-ng

General conclusions

Qiagen has produced the EZ1 Advanced XL robotic workstation and the EZ1 DNA Investigator Kit for the extraction and purification of DNA from a wide range of forensic samples. This validation examined the ability of the instrument and kit to function in the routine extraction of DNA samples from a variety of forensic sources.

The instrument and kit was able to generate reproducible results in the study using a blood dilution series.

The sensitivity of the extraction procedure was examined with both blood and epithelial cell/semen mixtures. Full DNA profiles can be expected from blood samples as small as 0.1-µL. Interpretable typing of sperm fractions can be expected from vaginal swabs containing as little as 0.1-µl of neat semen. The key factor is the amount of input DNA for the amplification process. Adherence to the recommended amount of input DNA for the Identifiler kit will consistently provide typing results.
The extraction procedure allowed for successful separation and interpretation of epithelial cell and semen mixtures.

The instrument and kit demonstrated the ability to extract samples without cross-contamination.

Hair samples, buccal swabs and bloodstains on denim were all successfully extracted and typed using the appropriate Investigator kit procedures. Cigarette butts yielded variable results. This may be due to a small amount of sample or degradation of the DNA. Based on this study, the condition of cigarette butts must be evaluated prior to extraction with the EZ1 Advanced XL and

DNA Investigator Kit. Samples for "wearer's" DNA must also be evaluated prior to Extraction as this study showed variable results with a limited sample of "wearer's" DNA substrates.

After comparing the quantitative and typing results obtained from the extraction of touch DNA samples using the EZ1 Advanced XL/DNA Investigator Kit, PCI and M48, it is apparent that the EZ1 Advanced XL/DNA Investigator Kit has the ability to perform as well as the M48 and in some cases better than PCI, especially when inhibition may be an issue. While quantitative values for samples extracted with PCI were often greater than those extracted with the EZ1 Advanced XL/DNA Investigator Kit and M48, it did not always result in complete DNA profiles. Certainly, additional typing information could be obtained with the use of centrifugal filter devices for those samples yielding lower than expected quantitative values, especially when in conjunction with increased Capillary Electrophoresis (CE) injection times. Centrifugal filter devices and modified CE injection times are routinely used in our Laboratory.

The EZ1 Advanced XL robotic workstation and the DNA Investigator Kit provide a useful platform for the extraction of DNA from a wide range of forensic samples, including both evidence and reference samples. Ultimately, the DNA analyst will need to evaluate the evidence being extracted and make the decision about which extraction method to utilize.

EZ1 Advanced XL Robotic Workstation and EZ1 DNA Investigator Kit Validation Study, pages 1-10
Reviewed by H. Klann, DNA Technical Leader: _____

Appendix E

Validation of the Quantifiler Duo® DNA Quantification Kit on the ABI 7500 Real-Time PCR System

The Applied Biosystems (AB) Quantifiler Duo® DNA Quantification Kit (Quant Duo) was validated using the AB 7500 Real-time PCR Instrument. The Quant Duo assay allows for the quantification of amplifiable male DNA and total human DNA in a single sample. In addition, the assay aids in the detection of PCR inhibitors. Studies were conducted to evaluate and compare the sensitivity, accuracy and reproducibility of the Quant Duo assay to the current CSF-HUMTH01 quantification system. This validation also evaluated the use of the Quant Duo kit on a variety of casework-type samples. Finally, a contamination study was conducted to further evaluate Quant Duo's performance.

I. Sensitivity and Assay Comparison Study

Method: The Quant Duo pooled male DNA standard was quantitated using the UV/VIS spectrometer in triplicate. The amount was averaged and serially diluted to contain 100, 25, 6.25, 1.56, 0.391, 0.097, 0.024, 0.020, 0.015, 0.010, 0.006, and 0.005-ng/uL DNA. This set of dilutions was quantitated over three reaction plates, each containing at least three replicates of the series, over the course of three different days. The same set was also quantitated using the current CFS-HUMTH01 assay over two reaction plates, each sample run in duplicate.

Summary of Results:

a) The measured quantities obtained from Quant Duo varied 24%, on average, than expected quantities of the serially diluted standard.
b) Quant Duo was able to detect human DNA, reproducibly, at quantities down to 10-pg/uL and male DNA down to 15-pg/uL as compared to the CSF-HUMTH01 assay which detected human DNA as low as 5-pg/uL.
c) With the exception of the 100-ng/uL dilution, all other dilutions quantitated less in the Quant Duo assay than the CSF-HUMTH01 assay.
d) At low inputs of single source male DNA (<25-pg/uL), differences between male and human DNA amounts were observed indicating a mixture.

Discussion:

The measured quantities obtained from the serially diluted series were averaged and compared to expected quantities. For the Quant Duo runs, observed quantities were similar for expected values in general. The average percent difference from the actual amount of DNA was approximately 24%. For the CSF-HUMTH01 assay, the average percent difference from the actual amount of DNA was approximately 38%. For both assays, percent differences, for the most part, increased with lower amounts of input DNA (Table 1A-1B). This greater variation at lower concentrations of DNA is due to stochastic effects and is consistent with Quant Duo's published developmental validation[1].

In terms of lower limits of detection, Quant Duo was able to detect human DNA reproducibly, as low as 10-pg/uL and male DNA down to 15-pg/uL. Of the 52 replicates of input amounts between 5-15-pg/uL, positive human and male results were obtained 40 times. However, 12 of these low-level samples were undetected. As a result, quantitation of samples less than 10-pg/uL using the Quant Duo assay may lead to false negatives (Table 1C). However, no false positives were detected. Therefore, quantitation results which detect male DNA do establish the presence of male DNA. Since the human target has been shown to be more sensitive than the male target, the human DNA quantitation values should be used for dilution purposes for amplification set-up. In addition, analysts may concentrate then re-quantify samples that have undetected amounts of DNA to ensure detection of low-level samples. Similarly, the DNA analyst may recommend Y-STR typing depending upon the sample type(s) and the case circumstances.

The CSF-HUMTH01 assay had a lower limit of detection for human DNA, which was down to 5-pg/uL. Of all the replicates run using the CSF-HUMTH01 assay, no low-level samples gave false negative results. When the averaged measured quantities of the serial dilutions were compared between both assays, all dilutions quantitated lower in the Quant Duo assay than the CSF-HUMTH01 assay (with the exception of the 100-ng/uL standard). The CSF-HUMTH01 assay indicated 9-61% more DNA with the same serial dilutions run on the Quant Duo assay. The differences between the Quant Duo and CSF-HUMTH01 may be attributed to the different primers (multi-copy nature of the CSF-HUMTH01 probe versus the haploid/diploid nature of the Quant Duo primers). Samples were then amplified with Quant Duo concentration amounts and examined for STR peak height evaluation in section II.

Since the single source male DNA (pooled male) standard was used to make the serial dilutions, it was expected that the total human and male DNA values would be equal using the Quant Duo assay. However, minor differences were detected in every sample. The Male: Female ratios obtained are calculated in Table 1A. At 6.25-ng/uL and above, more male DNA was detected than human. At low concentrations of input DNA (less than approximately 25-pg/uL), Quant Duo results could indicate a mixture of DNA when the sample is in fact single-source.

References:
TABLE 1A: Sensitivity titration: averaged measured and expected quantities for Quant Duo.
TABLE 1B: Sensitivity titration: averaged measured and expected quantities for CSF-HUMTH01.
TABLE 1C: Sensitivity titration: Quant Duo vs. CSF-HUMTH01.

II. Quant Duo Sensitivity for Peak Height STR Evaluation

Method: One female oral sample (SPR1-3) and one male blood sample (SPR1-2) were extracted using the BioRobot EZ1 and quantitated using both the Quant Duo assay (in duplicate) and the CSF-HUMTH01 assay. Using the averaged quantitation value from the Quant Duo assay, both samples were serially diluted to amplify 1.5, 1.0, 0.75, 0.50, 0.25, 0.10 and 0.05-ng of DNA.

Summary of Results:

a) Peak heights ranged from 200-2,000 Relative Fluorescent Units (RFU) for samples amplified with 1.0-ng of input DNA.

b) Full profiles were obtained down to 500-pg (sample set 1) and 250-pg (sample set 2) of input DNA.
c) Both samples measured lower in the Quant Duo assay than in the CSF-HUMTH01 assay.

Discussion:

When comparing quantitation values between Quant Duo and the CSF-HUMTH01 assay, both samples quantitated less using Quant Duo, roughly 46% and 15% less for the 2 samples used (Table 2A). As a result, target input DNA amounts for Identifiler amplification were evaluated. Using the quantitation data from Quant Duo, both samples were amplified with 1.5, 1.0, 0.75, 0.50, 0.25, 0.10 and 0.05-ng of DNA and run on CE8 (our most sensitive ABI 3130 Capillary Electrophoresis instrument). The peak heights were obtained and evaluated.

As expected, in general, lower amounts of input DNA resulted in lower peak heights (Table 2C). Our current assay recommends an input amount of 0.7-1.0-ng of DNA for Identifiler amplification. For the first sample set amplified (SPR1-2) with 1.0-ng of input DNA, peak heights averaged 861 RFU (±227 RFU). The peak height range for samples amplified with 0.75-1.0-ng of input DNA was 357-2,042 RFU. For the second sample set (SPR1-3), peak heights averaged 313 RFU (±95 RFU) for 1.0-ng of input DNA. These peak heights are lower than the 0.75-ng input sample suggesting an error in the dilution. The peak heights for the second sample set ranged from approximately 168-1,880 RFU for samples with 0.75-1.0-ng of input DNA. Based on the Identifiler internal validation study[ii], which used the CSF-HUMTH01 assay, peak heights averaged 1,837 RFU (std dev = 688 RFU) for samples amplified with 1.03-ng of DNA. This indicates that peak heights for samples quantitated with the Quant Duo assay are lower than the CSF-HUMTH01 assay. However, as these different samples were run on different days, a direct comparison is problematic.

Full profiles were obtained for samples amplified with 500-pg of input DNA for sample set 1 and 250-pg for sample set 2 (Table 2B). Few alleles were detected with samples amplified at 100-pg or less of DNA. These results are consistent with samples quantitated with the CSF-HUMTH01 assay in the Identifiler validation. As a result, it is recommended that the target amount for Identifiler amplification not deviate from the current 0.7-1.0-ng input amount of DNA.

References:

TABLE 2A: STR evaluation: Quant Duo vs. CSF-HUMTH01 assay sample quantitations.
TABLE 2B: STR evaluation: Percent of alleles correctly called vs. input DNA for Quant Duo.
TABLE 2C: STR evaluation: Average peak height vs. input DNA for Quant Duo.
GRAPH 1: Peak height vs. Input Amounts of DNA for Quant Duo.

III. Reproducibility/Precision Study

Method: The data from the dilution sets used for the sensitivity study was analyzed for Quant Duo's reproducibility. The C_t values were used to evaluate differences across replicates in a single plate and over multiple days. To evaluate the reproducibly of the standard curves, the C_t values of the standards were averaged and their standard deviations calculated. In addition, the reproducibility of both the slope and R^2 values of the standard curves for all runs were recorded.

Summary of Results:

a) Reproducible results for the sample dilutions were achieved using the Quant Duo assay as comparable standard deviations were seen across replicates in a single plate and over multiple days.
b) In general, higher standard deviations for both the human and male targets were observed with samples at lower concentrations.
c) Both the human and male targets produced reproducible linear-log standard curves between increasing C_t value and deceasing quantity of DNA template.

Discussion:

The reproducibility of the Quant Duo assay was examined by comparing the C_t values of the dilution sets used for the sensitivity study across a single plate, over multiple days. Standard deviations across each plate were averaged and compared. As expected, higher standard deviations were observed with lower concentration samples. Over multiple days, the standard deviations of the individual runs were comparable (TABLE 3A). For all replicates standard deviations ranged from 0.03 to 1.36 for the human target, 0.04 to 1.41 for the male target, and 0.01 to 0.19 for the Internal PCR Control (IPC) target. These results are consistent with Applied Biosystems' *Developmental Validation of the Quantifiler Duo DNA Quantification Kit* (Barbisin et al, 2009).

Seventeen standard curves were generated during the course of this validation. The standard curves for each Quant Duo assay were obtained from a series of eight dilutions from the Quant Duo pooled male DNA standard (50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068 and 0.023-ng/uL). Similar to other real-time assays, a linear-log relationship was obtained where the C_t value increased as the amount of template DNA decreased. In general, higher C_t values were observed for the male target over the human target. This was also observed in the developmental validation. Barbisin et al. explain this difference is a result of the haploid nature of the male target versus the diploid nature of the human target[1]. The human target, therefore, has a higher amplification efficiency and thus lower C_t value. For all runs, the human standard curves have slopes between -3.11 and -3.46 and Y-intercepts between 27.9 and 29.3. The male standard curves ranged from -3.16 and -3.48 in slope values and 28.1 and 29.3 in Y-intercept values. In addition, R^2 values (the measure of fit between the regression line and data points) were always greater than 0.98 (TABLE 3B).

References:

TABLE 3A: Reproducibility: Average and standard deviation (SD) of C_t values calculated for each sample dilution across three plates for Quant Duo.

TABLE 3B: Precision: Human and male standard curve slope, Y-intercept and R^2 ranges for Quant Duo.

IV. Mixture Study

I. Mixture Study I

Method: A sample set containing 0.05-ng/uL of male DNA with varying amounts of female DNA was prepared. The male to female mixtures were: 1: 0, 1: 1, 1: 5, 1: 20, 1: 100, and 1: 500. In addition, samples containing 0.05-ng/uL of female DNA with varying amounts of male DNA were prepared. The male to female mixtures were 0: 1, 5: 1, and 20: 1. The samples were quantitated one time each over three days. The samples were amplified using the average human (Ribonuclease P RNA component H1 - RPPH1 target) quantification result and a 1.0-ng input target amount.

II. Mixture Study II

Method: A sample set containing 0.0125-ng/uL of male DNA (approximately the assay's detection limit found in the sensitivity study) with varying amounts of female DNA was prepared. The ratio of male to female DNA in these samples was 1: 0, 1: 1, 1: 5, 1: 20, 1: 100, 1: 500 and 1: 1000. Several samples were also run containing 0.0125-ng/uL of female DNA and varying amounts of male DNA. The male to female mixtures were 0: 1, 5: 1, and 20: 1. The samples were quantitated in duplicate over three days.

Summary of Results:

a) Quant Duo successfully detected 0.05-ng/uL of male DNA in mixture samples up to a ratio of 1: 500, male to female parts (Mixture Study I). Using a lower male DNA concentration (0.0125-ng/uL), male DNA was detected in 85% of the mixed samples up to a ratio of 1: 1000, male to female (Mixture Study II).
b) The difference between the measured and expected mixture ratios from Mixture Studies I and II was, on average, 35% and 43%, respectively.
c) In Mixture Study I, mixture profiles were detected from samples containing DNA up to a ratio of 1: 20, and likewise for samples containing up to a ratio of 20: 1 (M: F). The mixture profiles from these samples (1: 20 and 20: 1 M: F samples) were partial. Full mixture profiles were generated from samples containing DNA up to a ratio of 1: 5 and 5: 1 (M: F).

Discussion:

Quantification results for mixture samples from Mixture Studies I and II are summarized in tables 4A and 4B, respectively. The Quant Duo kit successfully detected male DNA in samples up to a 1: 500 ratio (M: F) when using 0.05-ng/uL of male DNA (Mixture Study I). When 0.0125-ng/uL of male DNA was prepared in excess of female DNA (Mixture Study II), the Quant Duo successfully detected male DNA in 36 out of 42 (85%) mixture samples. Male DNA was detected in samples containing as high as 1000-fold excess of female DNA. However, six of the samples gave false negatives where no male DNA was detected. These false negatives were in no relation to the excess of female DNA present but more a reflection of the sensitivity of the Quant Duo assay (i.e. male DNA reproducibly detected down to 0.015-ng/uL). In addition, mixture ratios were calculated to examine the measured and expected ratios detected. These mixture ratios were calculated as follows:

Male DNA: Female DNA Ratio =

Male DNA / Male DNA: (Human DNA - Male DNA) / Male DNA

The difference between the measured and expected mixture ratios from Mixture Studies I and II was, on average, 47% and 42%, respectively. As shown in Tables 4A and 4B, measured mixtures were comparable for the most part to their expected values. For the 1: 0 male to female DNA mixture in Mixture Study I, however, Quant Duo detected more human DNA than male indicating female DNA was present. This is similar to the sensitivity study in which small differences between the human and male quanitations indicated mixtures when in fact the samples were single source. This variation and the variation of the expected measured ratios are most apparent at low concentrations. According to the Kit's manufacturer, (as stated in their developmental validation) this variation can be attributed to stochastic effects during the quantitation of male DNA at low concentrations.

Samples from Mixture Study I were amplified and typed to examine STR profiles and peak heights. Mixtures were detected in samples containing DNA up to a ratio of 1:20 (M: F) and likewise for samples with a ratio of 20: 1 (M: F). The profiles obtained indicated the presence of a mixture. However, the minor component produced only a partial profile. Full profiles from both the major and minor contributor were obtained from the 1: 1 and 1: 5 (M: F) and 5: 1 (M: F) samples. The two highest ratios, the 1: 100 and 1: 500 (M: F) mixed samples produced single source profiles only. The STR profiles generated from Mixture Study I are summarized in Table 4C.

Peak height data from the STR profiles and calculated mixture ratios (from peak heights) are shown in Table 4D. Mixture ratios were calculated from loci containing four alleles; these loci were TPOX, D18S51, and FGA. Mixture ratios were calculated for samples containing excess female DNA only. The calculated ratios from these loci are shown in Table 4E, as well as the quantitation mixture ratios from Mixture Study I (Table 4A). The calculated mixture ratios from the peak height data and quantification data are comparable to the expected mixture ratios.

References:

Table 4A: Mixture Study I- Averaged Human and Male Quantitation Values/ Expected and Measured Mixture Ratios.
Table 4B: Mixture Study II- Averaged Human and Male Quantitation Values/ Expected and Measured Mixture Ratios.
Table 4C: STR Profiles (Mixture Study I).
Table 4D: Peak height data/ Major: Minor Ratio (Mixture Study I).
Table 4E: Calculated mixture ratios from the peak height data and quantification data.

V. Degradation Study

Method: Six artificially degraded DNA samples, exposed to DNase I for 1, 5, 10, 15, 30, and 45 minutes, were used in this study. These samples were prepared by W. R. Hardy for use in the validation of the BioRobot M48 (M48). (See BioRobot M48 Validation for details of the sample

preparation). The six samples were quantitated once using the current CFS-HUMTH01 assay and the Quant Duo Assay. Samples were amplified using the Quant Duo human DNA concentration amounts, with an input of 1.0-ng.

Summary of Results:

a) CSF-HUMTH01 quantitation values for degraded samples consistently measured higher (57-84% higher) than the Quant Duo quantitation values.
b) For both assays, DNA concentration decreased with an increasing DNase I exposure of over 5 minutes.
c) Complete profiles were obtained for 5 out of 6 degraded samples. Only one allele was missing in the remaining sample.

Discussion:

Quant Duo and CFS-HUMTH01 concentration values for the degraded samples were compared. The degraded samples consistently measured higher with the CSF-HUMTH01 assay, between 57-84%, than the Quant Duo kit (Table 5A). This difference is attributed to the smaller amplicon size, 61 bps used in the CSF HUMTH01 assay, as opposed to the 140 and 130 bps used for the human and male targets, respectively for Quant Duo. Larger amplicon size provides a more accurate representation of amplifiable DNA for our current Identifiler STR markers. As shown in Table 5A, the higher degraded samples (exposed to DNase for longer time) showed the most difference in quantitation between the two assays. At samples exposed to DNase I for over 5 minutes, a decline in DNA concentration was observed in both assays (Graph 2). As expected, less DNA would be recovered in samples with more degradation.

The degraded samples were amplified using the Quant Duo human values and a 1.0-ng input target amount. Complete profiles were obtained for 5 of the 6 samples. The sample exposed to DNase I for 30 minutes exhibited dropout of one allele at CSF1PO. This sample (Deg30) may have been switched with the sample exposed to DNase I for 45 minutes. This suspicion is based on the dropout incident and lower peak heights, in general, observed with the Deg30 sample (Table 5C). As expected, the peak heights (RFU values) were lower for the higher molecular weight STR loci and decreased, in general, with increased exposure to DNase I (Table 5B-5C and Graphs 2-3)

Overall, Quant Duo provided a more accurate measure of amplifiable DNA present in the degraded samples over the current assay. If the CSF- HUMTH01 values were used for amplification, the samples would most likely have exhibited increased dropout, since the samples would have been diluted even further to reach the 1.0-ng target input.

References:

Table 5A: Quantitation Results for Degraded Samples.
Table 5B: Profiling Summary for Degraded Samples.
Table 5C: Peak Heights for Degraded Samples.
Graph 2: DNA Concentration vs. Samples with DNase I.
Graph 3: Peaks Heights vs. Samples with DNase I.

VI. Inhibition Study

Method: Eight potentially inhibited samples were quantitated using the Quant Duo and CSF-HUMTH01 assays. These samples were prepared by W. R. Hardy for use in the M48 and Identifiler validation studies. These samples included glove swabs and blood on various substrates like soil and sand. A list of the inhibited samples is described in Table 7 of the Known and Non-probative Evidence Study (Items I-1 to I-5B). To determine if potential PCR inhibitors were present, the cycle threshold (C_t) for the Internal PCR Control (IPC) was evaluated. In addition, the quantitation values of the samples between both assays were examined.

Summary of Results:

a) The Quant Duo assay successfully detected samples with PCR inhibitors using the IPC.
b) Using Quant Duo, quantitation results for severely inhibited samples cannot be used; dilution of the sample and inhibitors is necessary to generate DNA typing results.
c) Samples shown to be unaffected by inhibitors using the Quant Duo Assay were successfully typed when adequate DNA was present.

Discussion:

The IPC is used to detect the presence of inhibitors in the Quant Duo assay. When the C_t value of the IPC for a sample is greater than the C_t value of the standards, a PCR inhibitor may be present. If an inhibitor is indicated, sample dilution may be necessary prior to STR amplification. In addition, the IPC can distinguish between true negative samples or samples with PCR inhibitors. If the IPC fails to amplify completely (undetermined value), the sample may contain such a high level of inhibition that the IPC itself cannot amplify. As it acts as the internal positive control, an undetermined IPC C_t value could also indicate an inconclusive result, or a failed assay.

In this study, two of the eight samples exhibited evidence of inhibition. The samples were I-3A, blood on soil, and I-4A, blood on sand. For both samples, no C_t value was given for the three targets (human, male and IPC). Likewise, no amplifiable products were detected from these samples using the CSF-HUMTH01 assay (Table 6A). As a result, each sample was diluted prior to Identifiler amplification; a 1: 2 and 1: 10 dilution was chosen. No data was detected from the neat and 1: 2 dilution amplifications of items I-3A and I-4A. However, the 1: 10 dilutions generated a partial profile (dropout at D18S51 only) for item I-3A and a full profile for item I-4A (Table 6B). This demonstrates that both samples contained PCR inhibitors which affect both the real-time PCR amplification and Identifiler amplification. It is recommended that casework samples with complete inhibition be amplified neat (undiluted - for true low-level samples) and at a 1: 10 (or other appropriate) dilution prior to STR amplification.

DNA was detected in the remaining samples I-1, I-2, I-3B, I-4B, I-5A and I-5B. The IPC C_t values for these samples were within the normal range (as defined by the ABI Users Manual, C_t values approximately 29) and thus, did not indicate inhibition. The STR profiles obtained from these samples were reflective of their Quant Duo concentration amounts: No alleles were detected for item I-1 (0.003-ng/uL) and a partial profile was detected from item I-2 (0.029-ng/uL). The remaining samples, I-3B, I-4B, I-5A, and I-5B generated full STR profiles with the given Quant Duo concentrations.

This study demonstrates the importance of inhibitor detection and the potential impact of inhibitors on DNA detection and profiling. The Quant Duo assay correctly showed inhibition of

the IPC reaction for inhibited samples, I-3A and I-4A, which was further supported by the DNA profiling results. Also, samples shown to be unaffected by inhibitors using the Quant Duo Assay were successfully typed when adequate DNA was present.

References:
Table 6A - Inhibition Study: Quant Duo vs. CSF-HUMTH01 assay quantitation data.
Table 6B - Profiling Summary for Inhibited Samples.

VII. Known and Non-Probative Samples

Method: A variety of casework type samples were obtained from W. R. Hardy to evaluate Quant Duo. The samples were previously used in the validation of the BioRiobot M48 [iii]. The samples received were extracted both organically and using the M48 and previously typed. The samples chosen were a combination of degraded, inhibited and sexual assault evidence samples. Glove swabs, oral swabs mixed with hand cream, blood on sand/soil, and mock vaginal samples that had been differentially extracted were used. All samples were quantitated using the Quant Duo and the CSF-HUMTH01 assays. Samples were then amplified using the Quant Duo human quant results with an input target concentration of 1.0-ng/uL for Identifiler.

Summary of Results:
a) Quantitation results resulted in 43% less DNA, on average, in the Quant Duo assay than the CSF-HUMTH01 assay.
b) Using the Quant Duo concentration values, casework type samples amplified with 1.0-ng of input DNA had average peak heights of 1,285 RFU.
c) Mixture profiles were detected from known samples with mixture ratios up to 1: 22.5 and down to 1: 0.08 male to female DNA.

Discussion:

Quantitation values were obtained from all known samples using the Quant Duo and CSF-HUMTH01 assays. Both human and male values were obtained using Quant Duo. The average percent difference in quantitation results between the two assays was 43% when comparing human target results. With the exception of one sample, the CSF-HUMTH01 values were always higher than Quant Duo values. This mimics the general trend found throughout this validation study: CSF-HUMTH01 quantitation values were often higher than the Quant Duo values (Table 7A). A possible explanation for this trend is the different primers used in each assay which results in differences in amplicon length and assay efficiency.

All samples were amplified and typed using 1.0-ng (when available) of input DNA based on the Quant Duo human values. The peak heights ranged from 586-2,182 RFU with an average of 1,285 RFU. This is comparable to results reported in the Peak Height STR evaluation study of this validation. Samples with less than 1.0-ng of input DNA (0.194-0.403-ng/uL) showed allelic dropout as expected with average peak heights ranging from 102-568 RFU.

Allelic results for the inhibited samples were previously discussed (D. Inhibition Study). For the differentially extracted vaginal swabs, the epithelial cell fractions and sperm fractions were previously typed during the M48 validation. The samples were typed for this validation and the results compared (Table 7B). The 12 casework type samples varied from single source

to mixtures. Seven of the samples were previously typed as single source. The mixture ratios for these seven samples were calculated from Quant Duo results and correlated to the STR profiles obtained. Two of the single source samples (S1-EC1 and S1-EC2) gave mixture ratios of 1: 135 and 1: 157 (male to female DNA). Their STR profiles depicted single female samples. This correlates to the sensitivity of the mixture study where male alleles were undetected in all samples with over 1: 20 male to female DNA ratios. Four of the single male samples (S2-SP1, S2-SP2, S3-SP1, and SP3-SP2) gave low mixture ratios, both positive and negative, and averaged a ratio of 1: 0.05 (M: F). As seen in the sensitivity study, small differences between the primer efficiencies of the human and male targets suggest a mixture when the sample is in fact single source. Negative mixture ratios, or when the male quantitation values are greater than the human value also indicate a single donor. These results were confirmed with the STR profiles obtained. Analysts should therefore be aware of small differences between human and male values with single source samples and expect it to occur routinely. Sample S3-EC2 was previously typed as a single female profile with one carryover allele. When using the Quant Duo assay, this sample had a 1: 7.5 male to female ratio. When typed at 1.0-ng, a female profile was detected with 5 carryover alleles from the sperm fraction.

For the four casework type samples that previously typed as mixtures, Quant Duo detected differences between the human and male values. The ratios ranged from 1: 11 to 1: 22 male to female DNA. These samples all typed major female with minor carryover alleles that belonged to the sperm fraction. The two other samples (S1-SP1 and SP1-SP2) which were major male profiles with carryover from the epithelial cell fraction had mixture ratios of 1: 0.08 and 1: 0.423 male to female, respectively.

The calculated mixture ratios were then correlated to the peak heights of the detected alleles (Table 7B). For the most part, the expected peak height ratios were comparable to the measured Quant Duo mixture ratios.

The results of the known and non-probative study demonstrate that Quant Duo can indicate the presence of a mixture in a sample. These ratios can be used to predict whether minor profiles can be detected at the STR typing level. Analysts should note however, slight differences between the human and male target amplification efficiencies at the low level can mimic mixtures when the sample is in-fact single source. Therefore, analysts must use caution typing these low level samples as their quantitations might not accurately predict minor components of STR profiles.

References:
Table 7A - Knowns: Quant Duo vs. CSF-HUMTH01 assay sample quantitations.
Table 7B - Knowns: Calculated mixture ratios from Quant Duo and peak height data.

VIII. Contamination

Method: A contamination study was conducted which involved setting up runs with alternating sample and blank wells. The plates were prepared for mixture study II. The samples and blanks were run in duplicate over three reaction plates. The blank wells were filled with master mix and 2-uL of sterile water. Quantitation and C_t values were examined.

Summary of Results:
Two out of 60 blanks gave a positive result for the human target using the Quant Duo Kit.

Discussion:

Quantitation results from the three plates were evaluated. The first run gave a positive result in one blank well for the human target (0.00107-ng/uL and C_t = 37.52). Another plate gave one blank well with a positive result for the human target (0.000971-ng/uL and C_t = 37.72). All blank wells in the last plate were negative. Therefore, over the three runs two out of 60 blanks gave a positive result for the human target. With the exception of the two positive blanks, the C_t values for the remaining blanks were "Undet" for the human and male targets. The Quant Duo User's Manual states that laboratories have reported low-level signals (C_t values < 40) detected in the negative control samples using real-time PCR quantitation assays. The manual also reports that "such levels may be considered background and may not produce detectable product when the AmpFlSTR kits are used." The current LAPD DNA protocol requires STR amplification and typing of all samples to confirm the presence or absence of DNA in a sample.

IX. Conclusion

The results of this internal validation study demonstrate that the Quant Duo kit using the ABI 7500 Real-Time PCR System can provide quantitative information for a variety of forensic samples. The kit has the ability to simultaneously quantify the total amount of amplifiable human DNA and male DNA as well as the presence of inhibitors in a sample.

The sensitivity of the Quant Duo assay was examined and compared to the current CSF-HUMTH01 assay. Although less sensitive than the current quantitation assay, the Quant Duo kit detected total human and male DNA down to 10-pg/uL and 15-pg/uL, respectively.

Degraded samples were successfully detected using the Quant Duo kit. The findings in this study conclude Quant Duo more accurately measures amplifiable DNA in degraded samples than the current CSF HUMTH01 assay. This can be attributed to the larger amplicon produced with Quant Duo's primers. The 140, 130 and 130-bp fragments produced by the human (RPPH1), male (SRY) and IPC targets better correlate to the STR amplicons produced during Identifiler amplification.

Inhibited samples were detected using the Quant Duo kit. In this study, our inhibited samples had an undetermined IPC Ct value. Since the samples used for this study were so badly inhibited, the IPC was unable to amplify. Neat and diluted amplifications of these samples further confirmed the presence of inhibitors. These inhibited samples would have previously gone unnoticed using the CSF HUMTH01 assay. The detection of PCR inhibitors is an important addition to our quantitation assay as it will provide analysts with the ability to distinguish between true negatives and samples requiring further purification or dilution prior to DNA typing.

The ability to measure male DNA and total DNA in a sample was evaluated in this study. The Quant Duo Kit successfully detected male DNA samples up to a 1:500 ratio (Male: Female) when using 0.05-ng/uL. When a very low amount of male DNA, approximately 0.0125-ng/uL, was prepared in excess of female DNA, the Quant Duo successfully detected male DNA in 85% of the mixture samples. DNA typing of these samples demonstrated that full mixture profiles can be detected from samples containing up to a ratio of 1:5 (Male: Female) and partial mixture profiles can be detected up a ratio of 1:20 (Male: Female).

Use of the Quant Duo kit will aid the laboratory in obtaining successful DNA typing results with less time and resources than our current assay. The time required to process difficult samples (i.e., degraded and inhibited samples) will decrease, as they will tend to be more easily detected. Additionally, the ability to measure human and male DNA will provide useful information to the analyst and the potential capacity to select samples likely to produce DNA typing results.

Los Angeles Police Department – Scientific Investigation Division
Serology/DNA Unit – S. Rosner and A. Calderaro – Rev. 090710

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Reviewed by H. Klann, DNA Technical Leader: _

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