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Final Summary Overview

Evaluating the Efficiency of the Use of the Qiagen® QIAsymphony® with High Throughput Y-screening as an Alternative to Conventional Serology

Purpose of the Project

Sexual Assault Kit (SAK) backlogs have been a prevalent topic in news stories in recent years throughout the United States. According to the Rape, Abuse and Incest National Network (RAINN), there are over 300,000 victims of rape/sexual assault per year (1). The goal of this project is to develop and evaluate an accurate high throughput screeniug method for the presence of male DNA (otherwise known as Y-screen) as an option when addressing forensic DNA laboratory SAK backlogs. The unique aspect to this method is taking a larger initial cutting from the evidence as compared to traditional serology or other Y-screen methods and extract the cells from the sample with Phosphate Buffered Saline (PBS). This extraction lysate is referred to as the LCS or liquid cellular slurry. A set amount of the LCS is further extracted utilizing DTT based extraction buffer on the Qiagen® QIAsymphony® that will lyse all cells present in the LCS. Quantitative PCR with simultaneous male and human DNA quantitation setup is then performed on the extracted LCS. Analysis or screening of samples based on the presence or absence of male DNA can then occur. If necessary, a differential separation and extraction can be performed on the LCS. The LCS should be a homogenous sampling prior to screening which should create an accurate representation of the DNA on the evidence. This project will compare and evaluate laboratory efficiency of the LCS method, the newly released SWGDAM recommended method and conventional serology. Analyst labor time and reagent and supply costs will be logged for the cost analysis.

(1) https://www.rainn.org/statistics/scope-problem

Project Subjects

In order to best emulate samples that are collected during a sexual assault examination, volunteers were given specific instructions on how to collect the requested samples. Institutional review board (IRB) approval was obtained and documented for the volunteer sample collection. Three sample kits (Female, Male and Post-Coital) were prepared and distributed at a presentation to potential volunteers. The volunteers provided two types of samples for the studies. The first types of volunteer samples were "neat" saliva, semen, oral swab, vaginal swab and anal swab samples. It was requested that the "neat" semen samples be collected directly into a conical tube to reduce contamination possibilities. The "neat" semen samples were added to swabs creating samples resembling those commonly found in a sexual assault kit including: vaginal, anal and oral swabs. When possible, "neat" semen was added to actual oral and anal swabs from female volunteers. After the actual oral and anal swabs were exhausted, "neat" semen was added to swabs that were created with liquid saliva from female volunteers to "mimic" oral and anal swabs. The second type of volunteer sample was post-coital swabs. These samples included post-coital orifice swabs (vaginal, oral and anal) and body surface swabs where oral contact was made to best simulate samples collected during a sexual assault examination. The time since intercourse and other variables such as showering, brushing teeth, etc. were documented accordingly. When possible, samples were collected in triplicate at 8 to 12 hour intervals for up to 72 hours after intercourse. These samples were utilized for the studies performed throughout the length of this project in the hopes of creating situations similar to real-life.

Project Design and Methods

It should be noted the studies that the design and studies changed during the course of this grant as outlined on the semi-annual report to adjust to staffing changes and more importantly current

processes utilized during sexual assault kit screening. One key factor was the release of the SWGDAM's *Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits* released in December of 2016. This recommendation was adopted by MUFSC and in turn the focus shifted from comparing the LCS method to conventional screening to comparing the LCS method to both conventional screening and the SWGDAM method.

Optimization of the LCS: A study was performed to optimize the creation of the LCS (i.e. determining how efficient the PBS-soak was at obtaining the DNA-containing cellular content from the swab.) This study also investigated whether a nutator or thermomixer (with no heat) would produce a higher DNA yield. To further optimize the methodology, additional variables such as incubation time and temperature were explored. The volume of PBS added to the substrate to maximize the space in the spin tubes utilized by the MUFSC was evaluated by increasing the volume of PBS until the substrate no longer dried out in the spin basket as the spin basket was immersed in the PBS. During this study, the sample input into the quantitation reaction was increased from 2uL to 6uL to identify a possible correlation between an increase in the sample input and an increase in sensitivity.

Caps versus foil study: Based on evaporation that was noted when utilizing the recommended elution trays for the QIAsymphony, a brief study was performed to determine the best way to seal the tray after elution for long term storage. Rubber septa strip caps and foil plate covers were compared. Half of an elution tray was covered with foil and half was covered with rubber strip caps and the elution tray was stored for 9 days.

Conventional serology versus 10% LCS method: Serial dilution of semen was performed and divided into three sets. Conventional serology was performed on one of the serial dilutions sets.

The LCS method was performed on two sets of the serial dilution and performed in quadruplicate.

SWGDAM versus 50% LCS method: Twenty mock SAKs that included a total of 44 samples were created from the volunteer sample submissions. All samples were cut with sterilized scissors that were washed in a Decon™ ELIMINase™ and ethanol wash. The following table illustrates how the samples were cut for Y-screen and differential extraction.

Sample Size	Extraction Method
1/2 of each swab	LCS Y-Screen method
1/8 of each swab or ~0.5cm^2 of fabric	SWGDAM Y-Screen method
Remaining sample (~1/2 of each swab or fabric)	Differential extraction

Table 1: Processing Procedure for Cutting Samples for Y-Screen and Differential Extraction.

Once samples were cut for Y-screen, 300µL of PBS was added to the samples that were being utilized for the LCS method. These samples were then placed on a ThermoMixer® set at 900 rpm overnight at room temperature. The liquid and substrate were transferred into a spin basket tube and spun down for five minutes at 13,200 rpm. The substrate was transferred into a compatible micro-centrifuge tube. 200µL of supernatant was immediately transferred into a new micro-centrifuge tube for any potential future testing (e.g. p30 testing). The remaining 100uL of PBS and cell pellet was vortexed to create the LCS. 50µL of the LCS was transferred into a micro-centrifuge tube to be used for the 50% LCS screening method. The remaining 50uL of the LCS was stored in refrigerator (2-4°C) for potential DNA extraction.

The 50µL of the LCS and SWGDAM sample cuttings were extracted on the Qiagen QIAsymphony® SP instrument with the QIAsymphony® DNA Investigator Kit following the MUFSC QIAsymphony Male DNA screening procedure. Extractions were quantified using

Applied Biosystems® Quantifiler™ Trio DNA Quantification Kit and Applied Biosystems® 7500 Real-Time PCR System using the HID Real-time PCR Analysis Software v1.2 following the Quantifiler Trio MUFSC procedure.

Y-Screen samples were analyzed after quantification. Any extract with a male percentage (calculated by dividing human male ng/uL by small autosomal ng/uL and multiplying by 100) greater than 85% were taken forward to amplification directly from the Y-Screen tray (if necessary appropriate dilutions were made.) Based on the Y-Screen quantitation data, samples can then be chosen to be extracted. For this grant, all samples were differentially separated regardless of the quantitation results. Samples following the SWGDAM method were re-cut taking a larger amount forward to differential (Table 1) and STR typing. For samples following the LCS method, the remaining 50uL LCS were taken out of the refrigerator storage to be differentially extracted. All samples that were differentially separated followed the MUFSC procedure for differential extraction using the QIAcube® and EZ1® Advanced XL instruments from QIAGEN.

Differential extracts were quantified using the same procedure as the Y-screen extracts previously. Extracts were analyzed after quantification to determine samples that had a concentration requiring dilution or concentration. For this grant study, all samples were taken forward to STR amplification regardless of the quantitation results.

Where applicable, extracts were diluted or concentrated following the MUFSC guidelines for normalizing extracts. Extracts were amplified at a full (25uL) reaction volume following the MUFSC procedure on amplification using Applied Biosystems® GlobalFiler® PCR Amplification Kit on the Applied Biosystems® Veriti® 96-Well Thermal Cyclers.

The amplified product was then prepared for Capillary Electrophoresis on the Applied Biosystems® 3500xL Genetic Analyzer utilizing the MUFSC procedure for GlobalFiler® utilizing a 15 second injection time.

Analysis of the samples was performed using the data interpretation guidelines of MUFSC with an analytical threshold of 140 RFU (relative fluorescence units) and a stochastic threshold of 500 RFU. Analysis was performed using AB® GeneMapper® ID-X Software v1.5.

Cost analysis: Cost tracking spreadsheets comparing conventional serology to the LCS method were developed utilizing the MUFSC's laboratory supply costs (reagents, consumables, PPE and cleaning supplies) at the time of analysis and actual and estimated labor time where appropriate. Equipment costs were tracked but not included as the equipment is shared with other laboratory projects/procedures. An attempt was made to obtain an accurate cost per sample considering that certain supplies are used per sample and others are used per sample/case/batch. Other factors also include whether a supply is used daily, weekly or monthly. It should be noted that this cost analysis is based on the procedures and experience of the MUFSC.

Data Analysis

Optimization of LCS: Quantitation results were recorded in tabular format comparing the agitation methods and time and temperature variable. Specifically, for each sample the quantitation in ng/uL was determined. From that value, the percent recovery and potential loss were calculated. Results were compared between the quantitation reactions when adding 2uL of the sample extract to the reaction versus 6uL of the sample extract.

Caps versus foil study: The amount of volume loss was measured and a heat map was developed to demonstrate the loss of extract in each sample well.

Conventional serology vs 10% LCS method: Quantitation values and conventional serology results were recorded in tabular format for comparison. During the grant period the MUFSC operational DNA laboratory validated the SWGDAM method. for male DNA screening demonstrating the effectiveness of this method versus conventional serology method.

SWGDAM versus 50% LCS method: A mock case file was created for each case which included an inventory, the Y-screening results for the SWGDAM method and the 50% LCS method, the electropherograms with analysis comments, case allele chart with appropriate quantitation results, amplification targets and conventional serology results. A summary was compiled to compare the screening results from the SWGDAM method, the 50% LCS method and conventional serology to the STR results to determine which screening method best predicted the STR result obtained. Four categories were assessed with the twenty mock cases comparing the Y-screen result with the STR result with the goal of obtaining a male DNA profile. These four categories were: best sample, vasectomized/low sperm, best sample for amplification direct from y- screen and best actual post coital sample. The quantitation grading system was also evaluated to determine if it accurately predicted the STR results.

Cost analysis: Cost analysis was performed utilizing spreadsheets that tracked all of the relevant supplies. The spreadsheet tracked labor separate from laboratory supplies. The following table summarizes the cost analysis comparison of conventional serology and the LCS method on the QIAsymphony.

Costs per sample	Conventional Serology	LCS on QIAsymphony
Labor cost	$34.65	$25.99
Supply cost	$9.37	$13.27
Total cost	**$44.02**	**$39.26**

Table 2: Estimated cost per sample comparing conventional serology and LCS method.

Project Findings

Optimization of LCS: As expected, we determined the thermomixer was more efficient at removing cells from the swab. In addition, it was determined that adding heat had no measurable effect on the efficiency of removing the cells. However, due to possible sample variation this step could be re-evaluated for future research. It was initially concluded that 500uL of PBS will be used for incubation. However, after processing several samples with500uL a bubble was created when removing the spin basket. This created a possible contamination concern. The volume was reduced to 300uL to eliminate this from occurring. Initial attempts indicated that increasing the amount of DNA added to the quantitation reaction from 2uL to 6uL would not increase the sensitivity in relation to the volume. This was not explored further for this study.

Caps versus foil study: Foil is the preferred sealing method. However, either sealing method could be utilized as the results were not significantly different. The decision for choosing caps versus foil may most likely be a quality decision and based on the comfort level a lab may have with the different techniques.

Conventional vs 10% LCS methods The 10% LCS method was not as sensitive as conventional serology. The inconsistent results at the lower end of the serial dilution (0.063ng/uL) prompted an investigation into considering how to increase the sensitivity. Two options were explored. The first, increasing the volume of extract added to the quantitation reaction from 2uL to 6uL – see optimization of the LCS. The second option was to look at extracting 50% of the LCS versus the previously studied 10% - see SWGDAM method versus 50% LCS method.

SWGDAM method versus 50% LCS method: The following table and notes below summarize the findings from the 20 mock cases that were processed with the SWGDAM and LCS methods for a total of 88 samples for comparison in the four categories.

Category	LCS Method	SWGDAM Method	Both Methods Equivalent
Best Sample	4	5	11
Vasectomized/Low Sperm	0	3	2
Direct Amp from Y-Screen	3	3	4
Actual Post-Coital	0	1	4

Table 3: Case tally per category.

It was noted that the SWGDAM method routinely yielded more DNA than the LCS method. This could be because the substrate was in the extraction buffers for the SWGDAM method versus the LCS method. This may have given the SWGDAM method a slight advantage. A trend was observed showing that a higher percentage of male to female DNA was obtained from the LCS method. One concern is that the SWGDAM method outperformed the LCS method for the low sperm/vasectomized cases. This may be due to the lack of recovery of the cellular material from the swab and may be a topic for further research. Half of the cases (10 of the 20 cases) could be amplified directly from the y-screen tray to effectively circumvent a differential separation/extraction and additional quantitation step. This is an advantage that both the SWGDAM and LCS methods have over conventional serology which requires differential separation. All 88 samples were graded based on the y-screen quantitation results. 7 of the 88 grades were inconsistent with the STR results. This could be expected because the differential separation may alter the ratios of male and female DNA in the sample. Further work to optimize the grading system may prove beneficial to allow for reliable prediction of STR results from the screening method results. It is our opinion that a key element in the grading system prediction is

utilizing a robust extraction procedure (e.g. QIAsymphony) with a reliable quantitation kit (e.g. Quantifiler Trio) allowing for direct amplification from the Y-screen extraction tray.

Cost Analysis: As expected, conventional serology is labor intensive with low supply costs while the reverse is true with the LCS method. Total cost per sample was not significantly different between the two methods. Although we attempted to factor in the effects of batching cases in the cost analysis, we do not feel that this is fully reflective of the cost savings utilizing batch analysis with the LCS method as compared to performing conventional serology on one case at a time. For example, using the LCS method, direct amplification from the y-screen tray is a benefit that was not factored into the cost analysis at this time. Taking advantage of this option could eliminate the possibility of a differential extraction and additional quantitation reaction. Factors such as this could significantly tip the balance of cost between conventional serology and Y-screen (SWGDAM or LCS methods).

Implications to Criminal Justice Policy and Practice

Backlogs are a prominent issue in forensic DNA laboratories throughout the country. Performing a Y-screen utilizing the Qiagen® QIAsymphony® provides more accurate sampling, increased correlation to DNA STR results and a streamlined workflow all of which will increase the laboratory's efficiency. Being able to directly amplify from the Y-screen tray saves valuable time and resources. Employing a sampling plan based on a grading system of the Y-screen results to predict STR results would be valuable to any forensic DNA laboratory. This would allow for informed decisions regarding sample selection, sample preservation and appropriate DNA analysis technology.