Use of Promega's Casework Direct Extraction Kit as a Y-Screening Tool and for the Rapid Processing of Touch DNA Samples

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Columbus Forensic Services Center

- Service the City of Columbus, Ohio
  - population approx. 1,000,000
- Forensic Biology, Firearms, Drug Chemistry, Questioned Documents, Latent Print Processing and Latent Print Comparisons
- (9) DNA analysts + DNA Technical Leader
- 2017 received 2,280 lab requests and released 2,269 reports

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Methods: Y-screening with manual differentials

- Samples
  - Use a "Direct to DNA" approach (SAEK swabs)
  - Triage samples for 1st or 2nd round processing
- Extraction
  - Differential extraction on the Qiagen EZ1® robot
  - Sperm fraction (SF) spotted on slide (5µl) following last wash step
- Quantification
  - All samples using Plexor® HY System
- Amplification
  - Fusion 6C® and PowerPlex® Y23
- Loading
  - 3500 (STR and Y-STR) Genetic Analyzers

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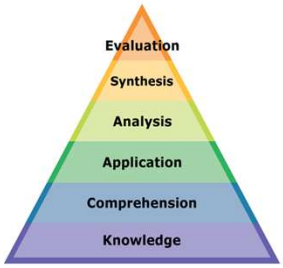
Concerns

- Time (screening/lab processing)
- Identification of body fluids (saliva and semen)
- How to triage SAEKs
  - Every case is different
  - Multiple suspect / consensual partner cases
  - Male victim cases
  - Female suspect cases
- What to take forward
  - STR testing
  - Y-STR testing
  - Both



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"Direct to DNA" Approach

- Initial processing includes SAE kit orifice swabs:
  - Vaginal and anal
  - Oral (depending on history)
  - Also most relevant swabs (i.e. thighs, neck, breasts, etc.)
- Differential extractions performed on vaginal/anal, thighs
- No initial semen screening performed on SAE kit items
- Subsequent re-processing of additional swabs and/or clothing may be needed
- Y-screening [Auto]/[Y]
  - <10 STR
  - 10-40 STR and/or Y-STR
  - >40 Y-STR
  - ≤0.001ng/µl total male DNA – stop at quant

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Considerations Over Time

- Analysts given more discretion over sample processing:
  - Oral swabs only on a case-by-case basis
  - Epithelial fractions not purified initially, only when needed
  - Only stain/search slides for samples that are amplified
  - Y-STR analysis may be more beneficial regardless of [Auto]/[Y] ratio (low quant value)
- Traditional semen screening on clothing & bedding
  - Touch on a case by case basis
- Amplification decision based on:
  - [Auto]/[Y] ratio ranges
  - Total human and male quant values

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Where do we go from here?

- Processing a lot of differential samples up front
  - No initial screening, so must treat anything with potential semen as a differential
  - Very time-consuming, waste of reagents/consumables
- Multiple rounds of processing
  - "Triage" samples based on medical report/case synopsis
  - Not everything from SAEK processed initially
- Many sperm slides very "dirty"
  - Hard to view sperm
  - Tried different slides and dyes: didn't really help

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Introduction

- Promega Casework Direct Kit, Custom
  - Used for rapid processing of swabs from casework samples prior to quantification
  - Used as a male screening tool for sexual assault swabs/cuttings
  - No subsequent purification of the Prototype Casework Direct lysate is required prior to STR amplification
  - Kit contents:
    - Casework Direct Reagent
    - 1-Thioglycerol (as a reducing agent)
    - 5X AmpSolution™ Reagent- must be added to Plexor® HY quantifications
  - Casework (CW) tubes and spin baskets recommended for this procedure to eliminate the need for tube transfers*
    - * Issue with tubes not sealing very well without the spin basket; decided against using them for Casework Direct processing; currently use QIAGEN Lyse + Spin 2mL tubes

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Introduction

- Casework Direct
  - Simple
    - 2-step procedure
  - Fast
    - 30 min incubation
    - 5 minute centrifuge spin
  - No wash steps
  - Minimal potential for DNA loss
  - Use lysate for both quantification and amplification
  - ~$6.40 per sample
- EZ1
  - Preprocessing steps
  - 1-2 hour incubation times
  - Cartridge-based purification
  - Additional machine run time
  - Multiple wash steps
  - Multiple transfer steps
  - Potential for DNA loss
    - Binding efficiency of DNA to resin
    - Washes
    - Elution efficiency
  - ~$9.90 per sample

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Methods – Differential & Blood

- Differential and blood samples were extracted using the QIAcube, Casework Direct, and current extraction (EZ1) methods
- Extractions were performed following the provided manufacturer protocols, with a few minor variations
  - Recommended 100-400µl – validated 300µl for Casework Direct*
    *300µl determined to be best volume for amount of swab extracted
- One swab per sample was used for each differential sample and approximately 3mm^2 cuttings per blood sample were used
- All extracted samples were quantified with Plexor® HY
  - Recommended addition of 5X AmpSolution™ Reagent to Reaction Mix
- PowerPlex® Fusion 6C amplification
- 3500 capillary electrophoresis
- GeneMapper™ ID-X v1.4

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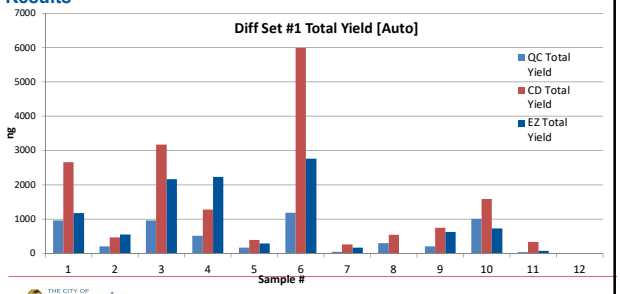
Results



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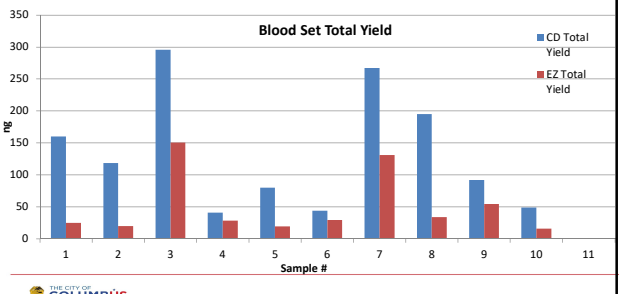
Results



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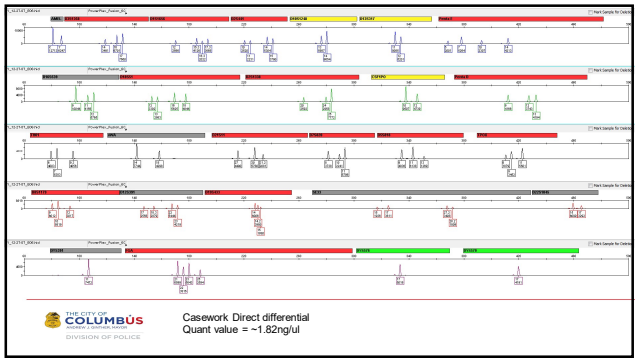
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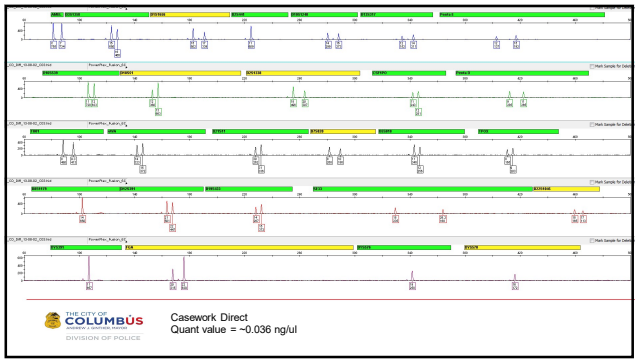
Casework Direct differential
Quant value = ~1.82ng/ul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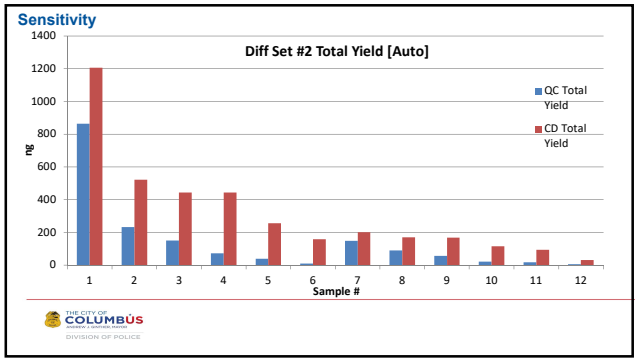
Casework Direct
Quant value = ~0.036 ng/ul


Sensitivity
Diff Set #2 Total Yield [Auto]
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Sample #
QC Total Yield
CD Total Yield
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Methods: Trace Samples

- 30 minute incubation vs. 60 minute incubation
- 70ºC vs 80ºC incubation temperature
- Addition of 250µl and 200µl vs 300µl of CWD reagent
- No significant difference in DNA recovery
  - CWD extract too dilute and microconning not recommended
  - EZ1 still the preferable extraction method for low-level samples at this time

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Methods: Cigarette Butts

	EZ1	CWD
Samples	[Auto] ng/uL	[Auto] ng/uL
Cigbutt_1	0.2748	0.8771
Cigbutt_2	0.0211	0.0186
Cigbutt_3	0.4323	0.1948
Cigbutt_4	0.0002	N/A
Cigbutt_5	0.1366	0.0343

- No significant difference in quant values for cigarette butts extracted using CWD vs. EZ1
- More dropout observed in F6C CWD profiles when amplified versus EZ1
- Overall, CWD extracts too dilute and currently no method to concentrate samples

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Considerations, Casework Direct

- High-level DNA samples will continue to extract over time, may need to re-quant if leaving stored in fridge for an extended period of time before amplification
- Consumption samples will be extracted normally, not with Casework Direct
- For lower level samples it may be necessary to perform an additional extraction to maximize the DNA yields, but the benefits of being able to quickly obtain Y-screening results from sexual assault samples will outweigh the disadvantage of not always being able to proceed directly to amplification with some samples
  - Based on more recent casework data, CWD typically yields higher quantification values
  - Usually not beneficial to re-extract on EZ1 unless as a differential

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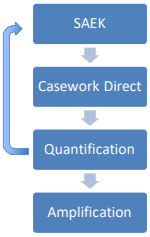
SA Case Workflow

- May be necessary to re-extract samples from SAE kit or clothing items
- Re-extraction may be performed as a low-level DNA sample or a differential (on QIAcube)
- DNA profile may determine whether a sample needs re-extraction



- Will allow us to more efficiently process differential samples (less hands-on lab work by the analyst)
- Obtain quantification data for both fractions up front; saving both time and money
- Maintain consistency by addition of automated separation and wash with use of the QIAcube

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CWD Y-Screen Case Example

Sample	[Auto]	[Y]	[Auto]/[Y]
CWD vaginal	178.0050	0.0732	2433.1418
EF Diff vaginal	28.8832	0.0022	12877.5738
SF Diff vaginal	0.6540	0.1351	4.8423
CWD anal	0.0787	0.0111	7.1203
CWD oral	5.1713	N/A	N/A
CWD external genital	11.2217	0.0182	615.4170
EF Diff external genital	18.1827	0.0085	2126.6474
SF Diff external genital	0.1768	0.0660	2.6770
CWD inside of jeans	0.0087	0.0010	8.8902
CWD lips/face	0.0085	N/A	N/A
CWD neck	0.0485	0.0133	3.6539

Impacts on Casework

- No "limit" on sample number for initial Casework Direct extraction
  - Process samples faster and reduce TAT
- Very few samples require a differential extraction
  - Validation of STRMIX® has helped for interpretation and reporting of mixture samples
  - Use of differential extractions for samples with high [Auto]/[Y] ratio (~>40)
- No real improvement of "touch" SA samples upon re-extraction on the EZ1
- Better resolution of minor F6C alleles with higher [Auto]/[Y] ratios (range of 30-40)
- Lower [Y] yielding more interpretable STR profiles
  - F6C validation showed loss of minor male @ ~0.008 ng/ul [Y]
  - Able to obtain interpretable male profiles from samples @ ~0.004 ng/ul [Y]

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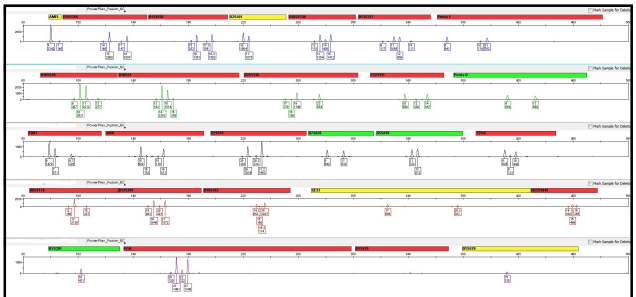
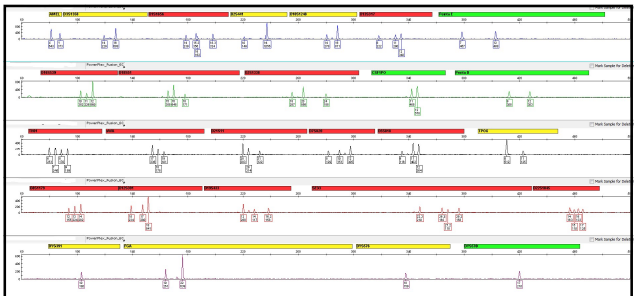


[Auto] = ~0.037 ng/ul
[Y] = ~0.004 ng/ul

face and neck swabs



[Auto] = ~0.010 ng/ul
[Y] = ~0.004 ng/ul

neck swabs

Conclusions, Casework Direct

- The Casework Direct extraction method is a robust extraction method that can be used in place of our current differential extraction method as a Y-screening tool
- It enables us to obtain quantification data from both the epithelial and sperm cell fractions in one short extraction process
- Furthermore, it is often possible to proceed with amplification and analysis which provides additional time and cost savings
- The Casework Direct extraction method is also a worthwhile alternative to our current high-level DNA samples - the shortened extraction time enables us to more effectively analyze batches of these sample types
- Results from low-level DNA samples do not support use of Casework Direct as a final extraction method – looking at other alternative methods

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Looking into the future…

- Alternative "touch" extraction method currently being evaluated
- Preliminary data with Promega's Maxwell® Extraction Robot shows better recovery of DNA versus current EZ1 method
- Can accommodate a larger number of samples per robot and requires less processing time

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Looking into the future…

- Post-PCR clean up method is currently being validated using Qiagen's QIAquick® PCR purification kit
- Used to remove impurities introduced during amplification that can affect subsequent downstream applications
- Looking to also try this "clean-up" method with Casework Direct extracts both pre- and post-PCR

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Thank You!

- **Promega**
  - Kim Huston
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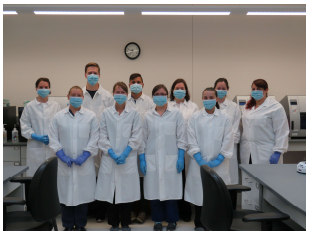

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QUESTIONS?

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