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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Introduction

The Columbus Police Crime Laboratory (CPCL) has recently been validating newer methods to more effectively process our current casework backlog. A significant number of cases we receive are sexual assaults. Typically, the processing of sexual assault cases involves numerous differential extractions that are time-consuming and require hands-on sample manipulation and multiple tube transfers. These cases are also assigned a priority number upon submission and given a turnaround date for completion. In an attempt to decrease the amount of time required for sexual assault sample processing, CPCL validated Promega's Casework Direct (CWD) extraction kit as a way to reduce lengthy incubation and hands-on laboratory work for sexual assault samples. At the time, CPCL was also validating QIAGEN's QIAcube robot to help streamline processing of differential casework samples. The CWD kit is used for the rapid processing of swabs from casework samples prior to quantification[1]. There is a short 30 minute incubation at 70°C with no subsequent purification of the lysate required for STR amplification. The lysate can be quantified and amplified using existing instrumentation and protocols. The kit contains the Casework Direct Reagent and 1-Thioglycerol, which is used as a reducing agent. The CWD lysate also requires the addition of 5X AmpSolution Reagent to be added to Plexor® HY quantifications. This method was originally meant to be evaluated strictly as a Y-screening tool. However, it was noticed upon review of the first data sets that CWD consistently yielded higher DNA quantification values and was much faster and easier to complete. The validation of CWD with high and low level DNA samples was added to possibly use this as our main extraction method (excluding hairs).

Methods

Differential/Blood

Differential, blood, and mock touch-sexual assault type samples were extracted using a combination of our current method (manual separation), QIAGEN's QIAcube robot, and the CWD kit. CPCL used the provided manufacturer protocols with some slight variations: our validated CWD reagent volume was 300µl (recommended 100-400µl), and QIAGEN Buffer G2 was used in place of diluted ATL buffer for the QIAcube, along with purification on the EZ1 using the Large Volume protocol with the addition of MTL buffer and carrier RNA.

Differential samples were prepared by adding approximately one drop of semen to female buccal samples. Two swabs per set were prepared and one swab used for each extraction method. Additional differential samples were obtained by using cuttings from previously extracted proficiency samples. Blood samples were prepared by using cuttings from previously extracted proficiency samples. Semen dilution samples were prepared by adding one drop of semen to the appropriate amount of sterile water. One drop of each dilution was added to two sterile swabs per dilution.

Samples were quantified using Promega's Plexor® HY quantification kit (4µl of 5X AmpSolution added for CWD samples) and amplified with Promega's Fusion 6C® amplification kit. Separation was performed on the Applied Biosystems 3500 CE instrument and analyzed with GeneMapper™ ID-X v1.4 software.

Touch

Various "touch" or low-level mock DNA samples were collected from items typically encountered in casework; this included firearms, tools, clothing, bottles/cans, and cigarette butts. Two swabs were

collected for each sample to use as a comparison. Samples were extracted using our current extraction method and the CWD kit. A volume of 300µl CWD reagent was used for most samples; a small subset of firearm and tool swabs had 250µl added to determine if this improved the DNA concentration. Cigarette butts were extracted in 200µl of CWD reagent.

The incubation temperature was modified from 70°C to 80°C and the incubation time from 30 minutes to 60 minutes in separate studies to see if these changes had an effect on the amount of DNA recovered.

Results

Differential/Blood

Twelve differential samples (11 + reagent blank) were extracted using the QIAcube, CWD kit, and our current manual differential extraction procedure. All samples were quantified and the [Auto] yields are demonstrated in **Figure 1**. These results demonstrated that all methods produced comparable yields; however, the CWD samples yielded much higher DNA concentrations when looking at the total yields for each method. These samples were also amplified and analyzed using the Fusion 6C® STR-DNA kit. A target amount of approximately 1ng was used to normalize samples. All samples were analyzed according to our current analysis parameters and the electropherograms compared across all three methods. The results demonstrated that all three methods produced equivalent results.

Twelve touch-type samples (11 + reagent blank) were extracted using the QIAcube, CWD kit, and our current manual differential extraction procedure. The differential extraction procedure was used on these samples to mimic actual casework sample processing. All samples were quantified and the [Auto] yields are demonstrated in **Figure 2**. The QIAcube and CWD samples were amplified and analyzed using the Fusion 6C® STR-DNA kit. A target amount of approximately 1ng was used to normalize samples. All samples were analyzed according to our current analysis parameters and the electropherograms compared between methods. The QIAcube and CWD methods produced comparable (if not better) yields when compared to our current manual differential method. Additionally, all samples generated the expected profiles based on quantification results.

Eleven blood samples (10 + reagent blank) were extracted using the CWD kit and our current EZ1 extraction procedure. All samples were quantified and the [Auto] yields demonstrated in **Figure 3**. These results showed the CWD kit outperformed our current EZ1 procedure across all samples. The CWD samples were also amplified and analyzed using the Fusion 6C® STR-DNA kit. A target amount of approximately 1ng was used to normalize samples. All samples were analyzed according to our current analysis parameters and the electropherograms compared between methods. Based on this data, it was shown the CWD samples can be reliably and accurately analyzed using our current analysis methods.

A sensitivity study was performed using 12 semen dilutions and extracted with the QIAcube and the CWD kit. All samples were quantified and the [Auto] yields are demonstrated in **Figure 4**. The DNA yields decreased with increasing dilutions ratios as expected. The samples were amplified and analyzed using the Fusion 6C® STR-DNA kit. A target amount of approximately 1ng was used to normalize samples. All samples were analyzed according to our current analysis parameters and the electropherograms compared between methods. The peak RFUs for both extraction methods decreased as expected the decreasing amounts of input DNA. No increased stochastic effects were observed.

Touch

Ten “touch” DNA samples were extracted using the CWD kit and our current EZ1 extraction method. The incubation time was increased to 60 minutes for one set, while the second set was kept to 30 minutes. All samples were quantified with no significant change in [Auto] values observed between incubation times.

Ten additional “touch” DNA samples were extracted using the CWD kit and our current EZ1 extraction method. The incubation temperature was increased to 80°C for one set, while the second set was kept at

70°C. All samples were quantified with no significant change in [Auto] values observed between incubation temperatures.

Five additional cigarette butts were extracted using the CWD kit and our current EZ1 extraction method. A volume of 200µl of CWD reagent was used. All samples were quantified and with no significant difference in [Auto] values observed. The samples were also amplified and analyzed using the Fusion 6C® STR-DNA kit. A target amount of approximately 1ng was used to normalize samples. All samples were analyzed according to our current analysis parameters and the electropherograms compared between methods. CWD samples demonstrated more drop-out of alleles compared to the EZ1 samples, likely due to increased inhibition in the CWD samples.

Conclusions

The results of the validation demonstrated that the CWD extraction kit is a robust extraction method that can be used in place of our current differential extraction method as a Y-screening tool. This has enabled 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 by not having to run as many differential samples. For lower level samples it may be necessary to perform an additional extraction to maximize DNA yields, but the benefits of quickly obtaining Y-screening results from sexual assault samples outweighs the slight disadvantage of having to re-extract some samples. The previous sexual assault case workflow has been altered to incorporate CWD as the initial extraction method for Y-screening. As necessary based on the quantification data, additional cuttings are taken from those samples with too high of an [Auto]/[Y] ratio (~>40). CPCL has also demonstrated the CWD kit to be a worthwhile alternative to our current high-level DNA samples and is currently our preferred method of extraction. The shortened extraction time enables us to more effectively process batches with high-level DNA samples. At this time, results from low-level DNA samples do not support the use of CWD as a final extraction method for these types of samples.

Figure 1

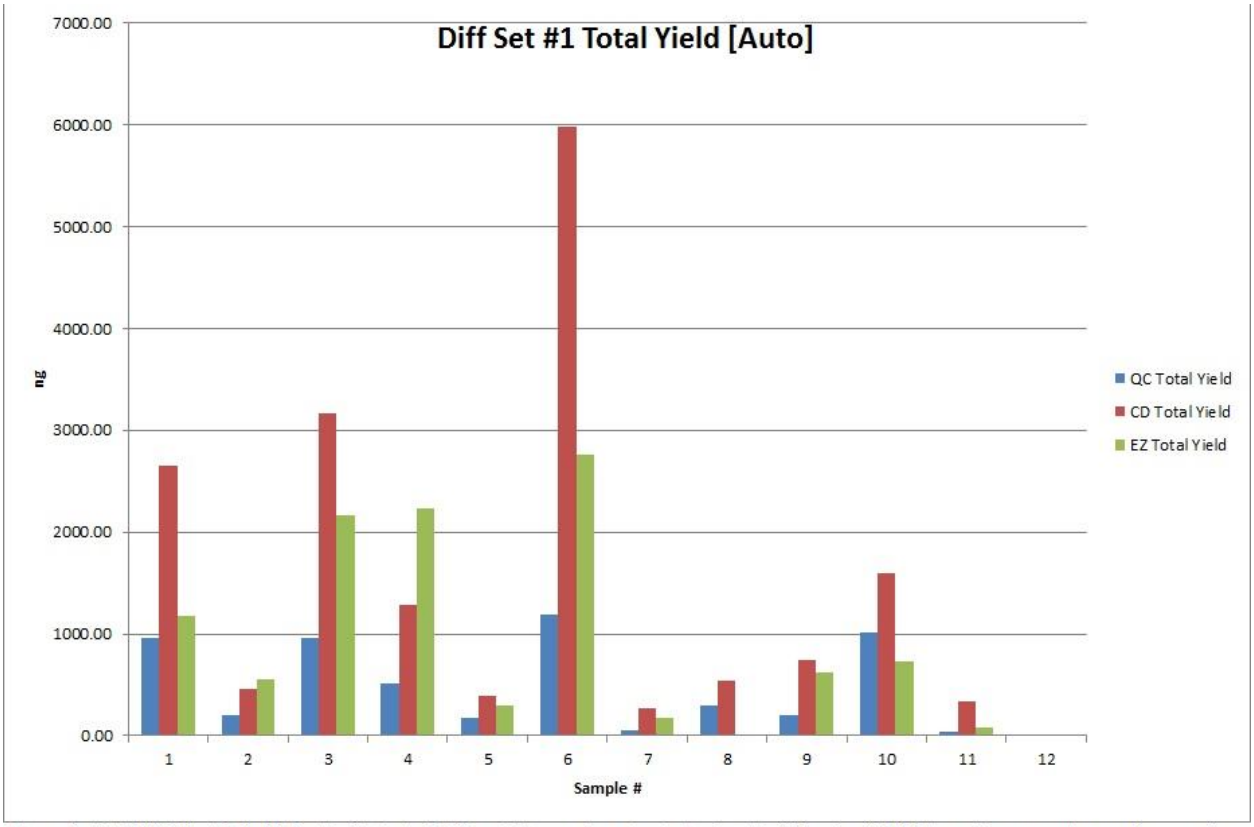

Figure 1. Total DNA [Auto] yields for the first set of differential samples extracted using the QIAcube, CWD kit, and the current manual separation +EZ1 method.

Figure 2

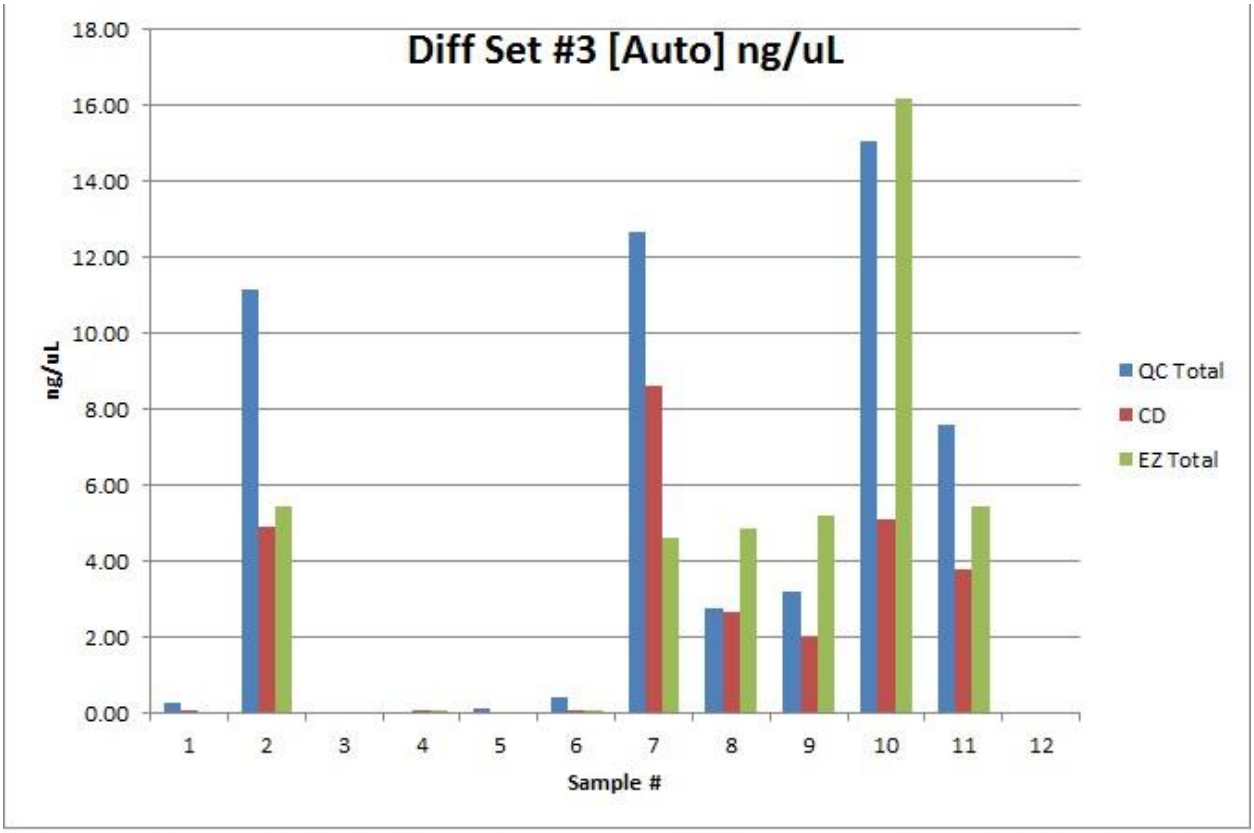

Figure 2. Total DNA [Auto] yields for "touch"-type sexual assault samples extracted using the QIAcube, CWD kit, and the current manual separation + EZ1 method.

Figure 3

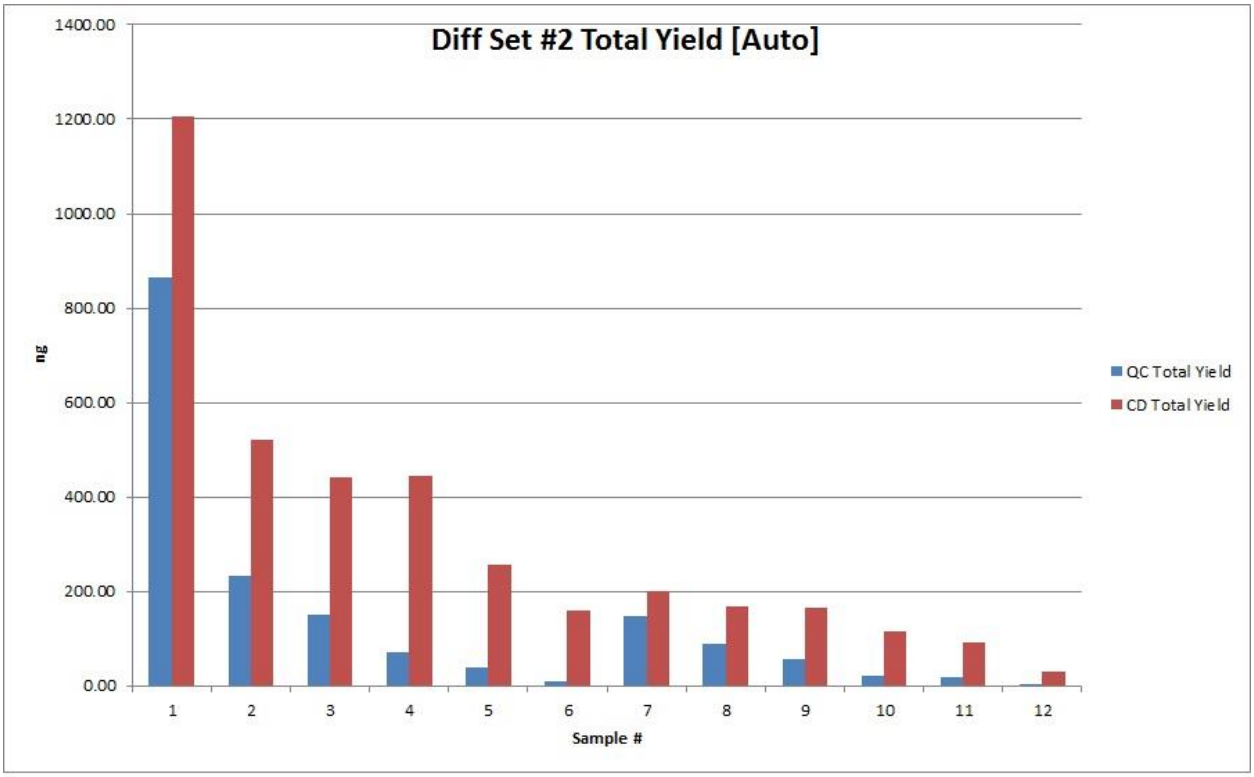

Figure 3. Total DNA [Auto] yields for sensitivity study with semen dilution series extracted using the QIAcube and CWD kit.

Figure 4

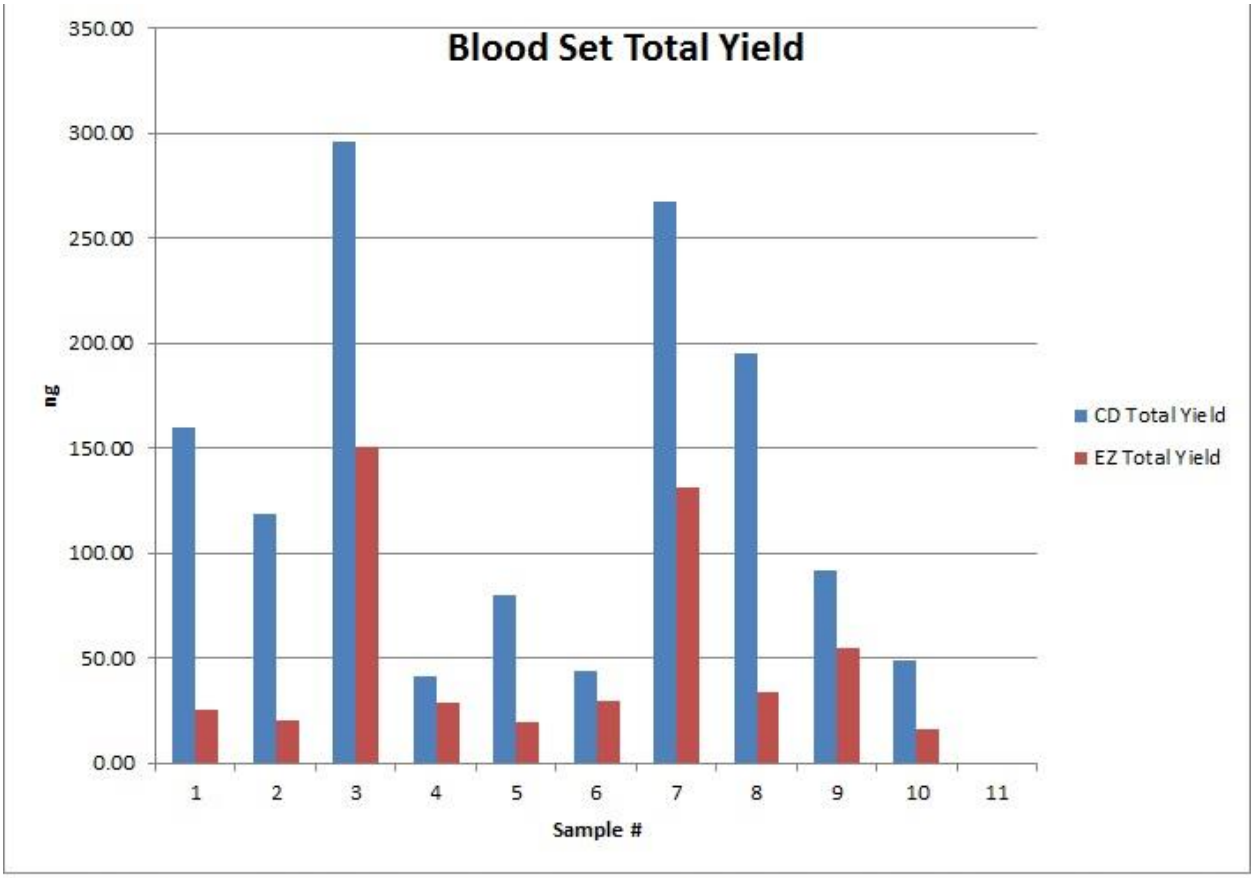

Figure 4. Total DNA [Auto] yields for blood samples extracted using the CWD kit and the current EZ1 method.

References

1. Promega Corporation. Rapid Processing of Swabs from Casework Samples Using Casework Direct Kit, Custom. Application Note #AN300: 1-6, 2016.