Improved Performance for Forensic Casework: Extraction and Isolation Updates for the Maxwell® 16 Instrument.

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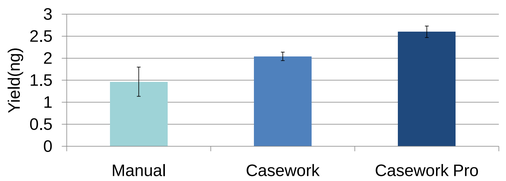
1. Abstract

The DNA IQ™ System is an established chemistry for the recovery of DNA from casework samples. Successful recovery of DNA from most casework samples depends upon the efficiency of two separate processes. Extraction efficiency refers to the recovery of sample from a solid support such as a swab or cutting of fabric. Isolation efficiency refers to the recovery of DNA from the extracted sample that is achieved during purification.

We have recently improved the performance of the DNA IQ™ System on the Maxwell® 16 instrument. We accomplished this enhancement through independent improvements in extraction and isolation chemistries. First, we designed a new LEV plunger using a proprietary material that increases the isolation efficiency of the DNA IQ™ System chemistry as performed on the Maxwell® 16 instrument. We can demonstrate the effect of our LEV plunger redesign upon isolation efficiency using liquid samples. Second, we have achieved improved extraction efficiency by introducing an optimized extraction buffer chemistry that precedes the DNA isolation process.

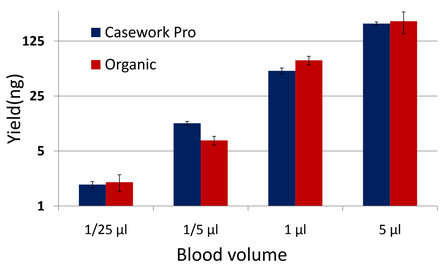
These changes resulted in increased DNA yield across a variety of samples, using the DNA IQ™ System compared with organic extraction.

2. LEV Plunger Redesign Improves Isolation Efficiency of the DNA IQ™ Casework Pro for Maxwell® 16



Pooled contact swabs were isolated with DNA IQ™ System performed manually, with the original Casework kit (old plunger) and the Casework Pro kit (new plunger) on the Maxwell® 16 instrument. The Casework Pro kit showed improved isolation efficiency over the other DNA IQ™ system formats.

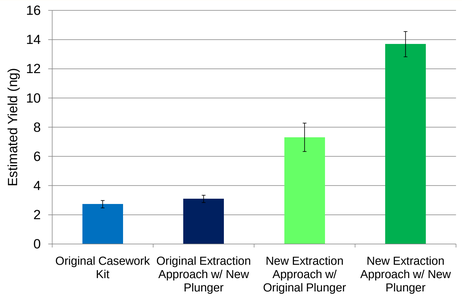
3. DNA IQ™ Casework Pro Provides Comparable Isolation Efficiency to Traditional Organic Isolation



Liquid blood volumes were isolated with DNA IQ™ Casework Pro and a traditional Organic extraction method using the Microcon YM-100 concentrator unit (n=3).

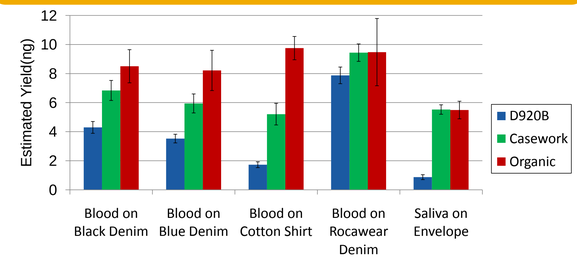
Isolation efficiency with DNA IQ™ Casework Pro was comparable to that isolated with the organic method across the input volumes tested.

4. Improvements to Isolation and Extraction Result in Significantly Improved Yield.



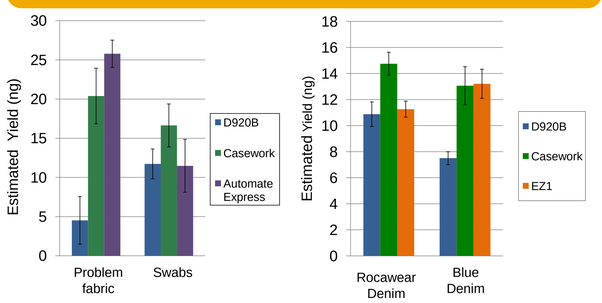
Liquid blood dried onto 5x5 mm squares of pantyhose (each bar represents n=8 trials). Yield from the Original Maxwell® 16 Casework kit (Left) is improved through application of the New LEV plunger and New Extraction Approach (at Right). All samples were quantified with the Plexor® HY System.

5. Yield Comparisons to Organic Extraction.



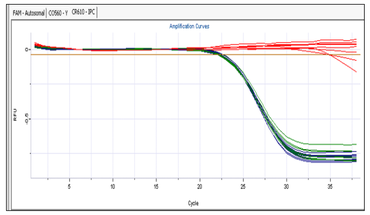
Volumes of whole blood or dilute saliva were dried onto the series of substrates noted. Samples were then extracted with the Incubation Buffer from the Tissue and Hair Extraction Kit (D920B) or Casework Extraction Buffer (Casework) and isolated using the DNA IQ™ Casework Pro Kit for Maxwell® 16. Replicates were processed through a traditional Organic Extraction process (Organic) for comparison. N=8 replicate extractions were performed for each approach. All samples were quantified with Plexor® HY System to estimate yield.

6. Automated Isolation System Comparisons Using Difficult Samples



Equal volumes of a dilute blood suspension were dried on a series of substrates. These samples were isolated with the Maxwell® 16 instrument , Qiagen BioRobot EZ1 instrument or the Automate Express™ Forensic Extraction Instrument. Samples were extracted prior to isolation in the Maxwell® 16 Casework Pro kit using either the original (D920B) buffer or the new buffer (Casework) . All samples were quantified with Plexor® HY System as indicated. N=6 Replicate extractions were performed for this trial.

7. DNA IQ™ chemistry results in eluates free of inhibitors to amplification- Blood on Leather.



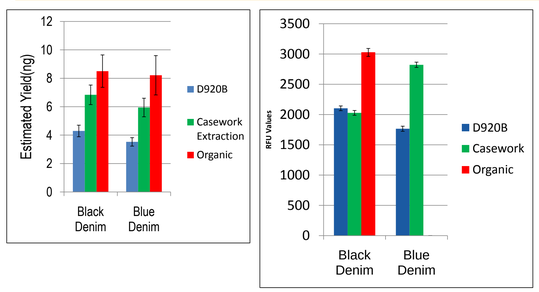
Organic

DNA IQ™ eluates

Plexor® HY System provides information about inhibition of PCR in a separate IPC channel. A shallowing of the Plexor® HY Curve in the IPC channel denotes inhibition of amplification.

Blood dried on leather was extracted with an Organic extraction process (Red) using the Vivacon 2 concentrator unit, or with the Original DNA IQ™ Incubation Buffer (Blue) and isolated with the Casework Pro Kit for the Maxwell® 16 instrument, or with the New DNA IQ™ Casework Extraction Buffer(Green) and isolated with the Casework Pro Kit for the Maxwell® 16 instrument. N=8 replicate isolates were obtained with each method.
Note: No inhibition was apparent in any of the Maxwell® 16 isolates.

8. Quantitation Results for DNA IQ™ Casework Pro Eluates are Predictive of STR Amplification Success.



Volumes of whole blood or dilute saliva were dried onto a series of substrates. These samples were extracted and isolated with the DNA IQ™ Casework Pro Kit for Maxwell® 16 or with a traditional organic process. Isolates were quantified with Plexor® HY System (at left) and amplified with PowerPlex® 16HS System. RFU Values from PowerPlex®16HS System are reported for each sample set. Plexor® HY and PowerPlex®16HS values represent n=8 replicate extractions +/– 1 SEM.

9. The DNA IQ™ Casework Pro Kit Produces Reliable Yield and STR Performance from Difficult Substrates

Sample types Tested
Blood on Black Denim
Blood on Blue Denim
Blood on Leather
Blood on Cotton Sheet
Blood on 60:40 polycotton blend
Blood on Rocawear T shirt
Blood on Rocawear Denim
Blood on Carpet
Blood on Cotton Shirt and soil suspension
Blood on Blue Denim with Soil suspension
Blood on Black Denim with Soil Suspension
Saliva on Marlboro Red
Saliva on Camel Blue
Saliva on Envelope
Blood on Gore-Tex® Fabric
Blood on Fabric with Lubriderm® Lotion
Blood on Fabric with Vaseline® Lotion
Blood on Fabric with B&B Shea Cashmere® Lotion
Blood on Red Corduroy Fabric

Nineteen combinations of sample and substrate type were extracted with Incubation Buffer or Casework Extraction Buffer, followed by DNA IQ™ Casework Pro isolation on Maxwell® 16. A traditional organic process utilizing a Vivacon device was performed for comparison . Samples were processed through Plexor® HY quantitation and PowerPlex® 16HS amplification and scored for two metrics:

- **Yield of at least 50% of that of the highest performing treatment set**
- **Generation of a full PowerPlex® 16HS STR Profile**

Treatment Set	Yield	% Full Profiles
Incubation Buffer	31.6%	94.7%
Casework Buffer	78.9%	94.7%
Organic Extraction	68.4%	78.9%

10. DNA IQ™ Casework Pro Kit for Maxwell® 16 can be Used to Successfully Process a Variety of Sample Types.

Sample description	Estimated Yield (ng)
Liquid Blood	6.29+/–0.46
Blood on Black Denim	6.84+/–0.69
Blood on Blue Denim	5.94+/–0.65
Blood on Rocawear® Black Denim	9.44+/–0.60
Blood on Cotton Fabric	5.21+/–0.74
Blood on 60:40 PolyCotton Blend	7.38+/–0.55
Blood on RocaWear T-shirt	2.40+/–0.42
Blood on Brown Leather	6.77+/–0.76
Blood on Treated Carpet	0.68+/–0.07
Blood on Cotton Fabric with Soil	0.41+/–0.27
Blood on Blue Denim with Soil	7.75+/–0.43
Blood on Black Denim with Soil	2.92+/–1.25
Saliva Suspension	3.76+/–0.60
Saliva Cigarette Butt paper-Camel Blue	4.30+/–0.31
Saliva Cigarette Butt paper- Marlboro Red	2.24+/–0.27
Saliva on Adhesive panels from Envelope	5.53+/–0.33
Blood on Gore-Tex® Pieces	7.44+/–0.59
Blood soaked onto Fabric with Lubriderm® Lotion	6.38+/–0.39
Blood soaked onto Fabric with Vaseline® Lotion	8.13+/–0.55
Blood volumes on Red Corduroy Fabric	5.34+/–0.48

The Casework Extraction Buffer was used to extract various simulated forensic sample types, followed by isolation in the DNA IQ™ Casework Pro Kit. In each case, Plexor® HY estimates were used to estimate yield from 8 replicate extractions. Each sample type is represented with a mean and +/–1 SEM.

11. Conclusions

1) Improvements to the extraction and isolation efficiency of the DNA IQ™ chemistry, as utilized on the Maxwell® 16 instrument, result in a significant improvement in the yield obtained from various forensic sample types.

2) The Casework Extraction buffer on the Maxwell®16 instrument is competitive with a broader set of sample types than the Incubation buffer.

3) DNA isolated with the DNA IQ™ chemistry is free of inhibitors, and shows robust performance in downstream processes, such as quantitative PCR, and STR Multiplex amplification.

12. Acknowledgements

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