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The Maxwell® 16 Low Elution Volume System for Forensic Casework: Implementation and Routine Use in a Forensic Laboratory

By Heike Felske-Zech, Kai Steffen Edler, Igor Martytschan, Christin Gruber and Frank Heidorn
Division of Forensic DNA Services, Department of Legal Medicine, Justus-Liebig University, Giessen, Germany

INTRODUCTION

The increasing number of forensic casework samples in our department forced us to increase our workflow efficiency. One way to raise sample throughput and unburden laboratory staff is automation. Various automated systems for DNA extraction are already on the market; some of which have been tested in our laboratory. We expected a high sample throughput of about 100 samples per day, easy handling and high DNA concentration and yield, especially for samples with very low cell adherence (e.g., forensic touch samples). These expectations were based on results generated with our present DNA isolation procedure: cell disruption by proteinase K treatment, Chelex® extraction and ethanol precipitation.

After various tests we chose the Promega DNA IQ™ Casework Sample Kit for Maxwell® 16 (Cat.# AS1210). For about 6 months nearly all forensic casework samples were extracted using this system. With a single Maxwell® 16 Instrument we realized a throughput of up to 500 samples per week. Here we describe our observations during the testing phase of the system and our modifications to establish its use in routine laboratory work.

MAXWELL® 16 LEV SYSTEM

The DNA IQ™ Casework Sample Kit for Maxwell® 16 was described recently (1). In our laboratory, cell lysis of nearly all forensic casework samples was performed using the Tissue and Hair Extraction Kit (for use with DNA IQ™) following the standard protocol. Only for lysis of hair samples was an optimized standard protocol used.

For DNA purification after sample lysis, the Maxwell® 16 Instrument was used with the DNA IQ™ Casework Sample Kit. Buffers and DNA IQ™ Resin are provided in a plastic cartridge. The cartridges are opened in a special cartridge rack, and the sample is transferred to the first well of the cartridge. A tube with elution buffer (50 µl) is added to the rack, as well as a specialized plunger, which encircles the metal magnetic bars in the Maxwell® 16 Instrument to protect the sample against cross-contamination. The extraction procedure yields a highly purified DNA solution. Although the Maxwell® 16 Low Elution Volume (LEV) configuration allows a range of 25–50 µl of elution buffer, we have validated our protocols with 50 µl elution volumes for all samples. This ensures sufficient DNA concentration, even with stain samples having low DNA content.

The total extraction time is about 2 hours, including cell lysis, cartridge preparation and run time. Sixteen samples can be processed simultaneously.

The Maxwell® 16 System removed PCR inhibitors more effectively than routine methods like Chelex® extraction. There was no need for ethanol precipitation or sample repurification.

Table 1. DNA Yield From Different Sample Types Extracted with the DNA IQ™ Casework Sample Kit for Maxwell® 16 (Approximate Final Elution Volume of 35 µl).

Sample	DNA Concentration (ng/µl)
Blood (20 µl)	0.44
Buccal cell sample	12.3
Fingernail	8.5
Semen stain	32.9
Chewing gum	0.16
Cigarette butt	1.4
Cup	0.10
Bottle	0.24
Hair (with root)	7.4
Faeces	0.55
Envelope sealing flap	1.7
Touch samples	
Key	0.012
Neck chain	0.26
Keyboard	0.19
Mouse	0.14
Refrigerator door	0.011
Railing	0.22
Glove	0.028
Stone	0.12
Watch	0.033

CASEWORK SAMPLES: YIELD AND AMPLIFICATION SUCCESS

To evaluate amplification success, various routine samples were extracted and amplified with the STR system SE33 (ACTBP2). Analysis of PCR products was performed using an ABI PRISM® 310 Genetic Analyzer. Prior to amplification, DNA was quantified by real-time PCR. DNA concentrations ranged from 0.012 to 32.9 ng/µl (Table 1). All samples were successfully amplified at the SE33 locus. After several months of experience with the Maxwell® 16, we can say that yields are similar to those using Chelex® extraction. Normally, sufficient DNA quantities were obtained from samples like cigarette butts, buccal swabs, blood, saliva or semen stains. Even with problematic materials such as faeces

or stones, we extracted sufficient DNA to generate a complete DNA profile for the German DNA Analysis Database. However, more than 75% of our samples are forensic touch samples, frequently with a very small amount of cell adherence. Therefore, there is still a great number of negative samples after extraction with the Maxwell® LEV System; even the most efficient DNA extraction procedure would not yield sufficient DNA concentration from such samples.

CASEWORK SAMPLES: DNA PURITY

During early testing, we noticed that DNA samples extracted with the Maxwell® LEV System were pure and PCR inhibitor-free. After processing thousands of samples with this system, we believe that this is one of the most positive features of this system compared to other methods.

About 65% of samples processed using Chelex® extraction were re-purified because the samples were cloudy or discolored and/or had an increased IPC C_T value, which is an indication of PCR inhibition. For re-purification we used spin columns based on silica-gel technique. Due to DNA loss after re-purification it was often impossible to get sufficient DNA for amplification from low-DNA samples such as stones with Chelex® extraction. When using the Maxwell® 16 Instrument, we did not see any cloudiness or discoloration of the samples. The number of samples with increased IPC C_T values decreased to less than 1%, allowing us to examine problematic sources of biological materials such as a stone that was used in a home invasion. Figure 1 shows the complete DNA profile obtained from such a stone. From this stone we extracted 0.025 ng/µl DNA and successfully amplified DNA using

the Promega PowerPlex® ES System. We received a complete DNA profile for the German DNA Analysis Database.

As a result of the high DNA purity, we can add a higher DNA volume per reaction without the risk of PCR inhibition. Thus it is possible to increase the number of positive samples with very low DNA concentrations (below 0.05 ng/µl).

An additional positive effect of the Maxwell® LEV System is less hands-on time for laboratory staff. Furthermore, the elimination of multiple quantifications before and after re-purification reduces costs significantly and counteracts the higher cost of the Maxwell® 16 System compared to manual Chelex® extraction.

HANDLING

Both the Maxwell® 16 Instrument and DNA IQ™ Casework Sample Kit were rapidly incorporated into our laboratory routine. However, preparing the plasticware is a bit laborious. You must ensure the foil is completely removed after opening the cartridges and verify that the plungers are intact. We ask the supplier of the plasticware to improve his quality standards. To that end, Promega is working with the plasticware supplier to address these issues and improve ease of handling.

The cartridge rack with elution tubes is heated during the extraction process, so evaporation may result in a loss in volume of the extracted DNA. Of the 50 µl elution volume that we add, approximately 35 µl remains after the process. The user should optimize the volume of elution buffer on a case-by-case basis. To avoid volume losses, the user should minimize the time the samples remain in the instrument after purification,

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and so the timing of other steps of the analysis might need to be adapted to accommodate the process cycle of the instrument.

Use of the Maxwell® 16 System reduces the number of steps involved in DNA extraction. After sample lysis you only have to prepare the cartridges to obtain an optimized elution volume of highly purified DNA. Steps like ethanol precipitation or sample repurification are not necessary. This reduces manpower and efforts to avoid sample mixups and contamination. After examining thousands of samples we documented no cross-contamination.

SAMPLE THROUGHPUT

Our department requires analysis of 100 samples per day. This amount can be managed with a single Maxwell® 16 Instrument, which can extract 16 samples simultaneously in one run of 30 minutes. We purchased two additional cartridge racks to prepare new cartridges during the extraction run to ensure continuous operation. We think that the throughput can be maximized to more than 200 samples per day.

SUMMARY

With the DNA IQ™ Casework Sample Kit for Maxwell® 16 and the Tissue and Hair Extraction Kit (for use with DNA IQ™) we were able to extract DNA from various forensic casework samples. Due to the low elution volume of 50 µl we obtained sufficient DNA even from samples with very low DNA adherence. Compared to routine methods like Chelex® extraction, the Maxwell® 16 System removed PCR inhibitors more effectively. There was no need for additional steps like ethanol precipitation or sample repurification. This reduced manpower and efforts to avoid sample mixups and contamination. After purchasing two additional cartridge racks, we can process about 1,000 samples per month with the Maxwell® 16 LEV System as our standard extraction method. We think that the throughput can be maximized further.

The increased efficiency in the analysis of forensic casework samples due to the high DNA purity is evident in all parts of our laboratory.

REFERENCES

1. Mandrekar, P. *et al.* The Maxwell® 16 low elution volume system for forensic casework. *Profiles in DNA* **10(2)**, 10–12.

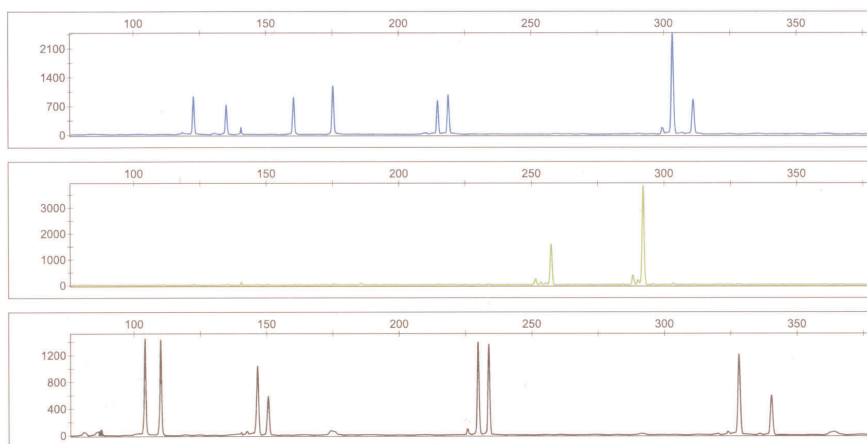


Figure 1. STR analysis of DNA extracted from a stone (a forensic casework sample from a home invasion). DNA was extracted using the Tissue and Hair Extraction Kit and DNA IQ™ Casework Sample Kit for Maxwell® 16. DNA (0.1 ng) was amplified with the PowerPlex® ES System (reaction volume of 12.5 µl), and amplified products were analyzed using an ABI PRISM® 310 Genetic Analyzer.