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

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Introduction

In Germany some spectacular criminal cases focused not only the attention of the police but as well of the public on the great possibilities of DNA-Technologies in criminal investigations. So the murdering of a famous designer from Munich, Rudolf Moshammer in 2005 could be resolved after a few days by a match in the German database. This leads to a change in the legal regularities at the end of 2005 which made it much easier for the police to give an order for DNA-Analysis of forensic casework samples. So today the power of DNA-Technology is not only used for resolving serious crimes like homicide, raping or sexual abuse but also for the investigation in home invasion, burglary or robbery. This led to a doubling of the number of crime scene samples also in our DNA-Lab from 2005 until now. In the year 2007 we examined nearly 13,000 samples in more than 5,000 criminal investigations in nearly all kinds of crimes. The constant growing of the number of samples forced us to increase our work flow efficiency. One way to unburden the laboratory stuff is automation e. g. of DNA-Extraction.

Our routine DNA-Isolation procedure through 2007 was cell disruption with Proteinase K digestion, Chelex® extraction and ethanol precipitation.
Reference stains from suspects or victims were extracted in a King Fischer device using the DNA-IQ-System. For special samples, e. g. evidences from rape cases with need of differential lysis, we use organic extraction.

Based on the results from Chelex® extraction we expect for an automated extraction procedure comparable DNA yields, also for samples with low amounts like forensic touch samples. Moreover an automated DNA extraction procedure should be easy to handle and a throughput of 100 samples per day fulfils our needs.

After looking for the optimal solution for our lab, we established the Maxwell® 16 low Elution Volume System for forensic case work at the beginning of 2008. Until now we extracted more than 9,000 forensic casework samples with the Maxwell® System.
So we collected a lot of data about the efficiency of DNA extraction with the Maxwell® System from establishing phase, quality insurance and, what could be most interesting for you, the routine work in our forensic lab.
First of all I will make you familiar with the Maxwell® System.

The Maxwell® Low Elution Volume System: Features , Handling and Sample Throughput

The system consists out of three elements: the Hair and Tissue Kit for cell disruption by Proteinase K and DTT treatment, the Maxwell® 16 DNA-purification Kit and the Maxwell® 16 Instrument for performing the purification process.
The first step, Proteinase treatment with the Hair and Tissue kit, follows the standard protocols provided by Promega.
After cell disruption samples are mixed with the lysis buffer from the DNA-IQ Kit. For the purification in the Maxwell® device the sample is placed in a special cartridge which is the essential part of the Casework kit. These cartridges contain in different wells lysis- and washing-buffer and the DNA IQ resin, paramagnetic particles with binding capacity to DNA. The cartridges are covered with plastic film which must be removed. Then you have to place a specific plunger in the first well, to protect the magnetic rod in the Maxwell® instrument. These rods have the function to transport the paramagnetic beads from one well to another in a special program.
For DNA-elution a tube must be placed and filled with elution buffer. The volume of the elution buffer must be optimized for your needs. We use 50µl and get back ~ 35µl due to evaporation during the purification process, because the cartridge rack is heated continuously.
The sample is put into one special well. The rack with the cartridges is placed in the Maxwell® device. After purification you will find the DNA in the elution tube.
The time need for the extraction process is for lysis 60 min., roundabout 15 min. for preparation of the plastic ware and 30 min. purification in the Maxwell® device, all together 1 h 45 min. The Maxwell® device can handle 16 samples in one purification process. The sample throughput is only limited by the working time of the device. We bought two additional cartridge racks to prepare samples during running-time. So we could enhance the sample throughput to more than 100 samples per day, which fits our needs. We think, up to 200 samples per day will be no problem.
We compared the positive and negative features of handling the Maxwell® and extracting DNA with Chelex®. With the Maxwell® system you have to handle plastic ware, which is a little bit time consuming and laborious. For Chelex® extraction and ethanol precipitation different tubes must be labelled and several centrifugation steps are required. So you have fewer steps in DNA extraction with Maxwell®. Fewer steps cause less chance for sample interchange or cross contamination. In our lab we haven't seen any cross contaminations with the Maxwell® System.

DNA-Yield and DNA-Concentration: Laboratory and Quality Insurance Samples

One of the most important features of a DNA-extraction method is the DNA Yield and the DNA concentration which you get from a sample.
During the testing phase of the Maxwell® System we extracted a number of various forensic samples which you get normally enough DNA for a great number of PCR-reactions. This slide shows the DNA-concentration and yield after extraction. The DNA-Quantification is done with Real Time PCR. We get sufficient DNA for every tested sample like semen stains, fingernails or cigarette butts. All swabs from saliva stains give a positive result. From a hair root we extracted the great amount of more the 250ng DNA.

May I put your attention to the samples “blood” and “chewing gum”. We gave them directly into the sample well of the cartridge without Proteinase K digestion or other steps for cell disruption and it works. All DNA-samples could be amplified successfully. The positive typing for such kind of samples meets our expectations.

During the establishing of the Maxwell® we examined a set of 30 cups. We got DNA-amounts from 0.5 to 500ng. All samples could be typed successfully. The big difference of the amount of DNA we got from the different cups focuses one common problem with real samples. The amount of cell adherence, here from a saliva stain, and also the way of collection have a great influence on the DNA amount you get from a casework sample.

For quality insurances we prepared a defined sample for both extraction methods – Chelex® and Maxwell®: 1µl liquid blood dried on filter paper. From 50 Maxwell® extracted samples we got DNA-concentrations between 0.01 and 0.49ng/µl. The mean concentration was 0.17 which is 6ng DNA absolutely. We compared this result with 50 samples from the Chelex® extraction There we got a mean of 6.7ng DNA absolutely. We think the difference of the DNA-yield between Chelex® and Maxwell® is probably a statistical fluctuation. When we compare the results of different extractions within one method we also find differences of more than 5 times less ore more.

DNA-Yield and DNA-concentration: Forensic Casework Samples

To have a look on real forensic casework samples we statistically analysed a part of our casework samples. This part consists of samples, where only one or two different DNA evidences from one crime scene are sent to our lab for DNA-typing. These samples get a so called G-Number and the reports are documented in a special database. Statistical work within this database is easy to do. We can look for a special kind of samples and for the corresponding results of the analysis. And it is easy to compare the results from DNA-Extraction with Chelex® and Maxwell®. From January up to the end of August 2008 we analysed 3773 G-numbers samples. They all undergo DNA extraction with Maxwell®. In the year 2006 and 2007 the routine DNA extraction method was Chelex®.

Let us look at first at the results of cigarette butts. As you can see on the slide the number of samples was between 202 in 2006 and 174 through August 2008. With the typing success rate, we mean that part of samples which gives a full profile for the German database. The typing success rate differs from 70% in 2006 to 78% in 2008. So the rates are comparable. The reason for a negative result for cigarette butts is most common a wet sample collected in the outside.

For blood swabs the number of samples differs from 88 to 164, the success rate for each year is a little bit more than 90%, for Maxwell® as well as Chelex®.

So in conclusions for real forensic samples normally with a sufficient DNA-content as well as for the laboratory samples and quality insurance samples: Chelex® and Maxwell® give comparable results.

DNA-Yield and DNA-Concentration: Forensic Touch Samples

May we have a look at DNA-samples which often have a very low cell adherence. From forensic touch samples you regularly get a very low amount of DNA, often beneath the limit for a successful typing.

During the Maxwell® testing phase we collected various touch samples from different sources. We got results from 0.01 to 0.2ng/µl for this kind of samples. They all could be amplified successfully. Some of our collected samples did not give enough DNA for successful typing. They are not shown on the slide. DNA-extraction with Chelex® gave the same result: many samples with a sufficient DNA-Yield, many which did not give enough DNA. To get more data about the efficiency of Maxwell® extraction we compared our results for real forensic DNA samples as shown for cigarette butts and blood swabs before.

Many of our forensic touch samples we get from tools which were used e. g. for a house breaking and which the offenders have left at the crime scene.

We examined 88 hammers and screwdrivers in 2006 and 106 in 2007. All of them were Chelex® extracted. The success rate (full profile for the DNA database) was 35% respectively 31%. Up to august 2008 we extracted DNA from 62 hammer shafts and screwdrivers with Maxwell® and the success rate was 34%. So the efficiency of DNA extraction with Chelex® and Maxwell® seems the same, also for this kind of touch samples.

The next slide shows the success rate of swabs of forensic touch samples taken by the police. In most cases the samples are collected by the normal police officers on the spot and not by special forensic units. These swabs are e. g. collected in a stolen car, from a door knob after home invasion as well as from tools, because the police often decides that it will be easier to send the swab to our lab instead of the tool itself.

These swabs are the most common samples in our lab. Their number grew from under 2000 in 2006 to more than 3300 in 2007. Coeval the success rate decreased from nearly 16 to 14%. Until end of August we analysed nearly 2300 swabs of touch samples with a success rate of only 9.2%. For us there was one overall question: by what is that decrease caused? When we take again a look at the success rate from swabs we took ourselves we find, as mentioned before, nearly the same results for Chelex® and Maxwell®. The following results are from various samples like touch samples from tools or gloves, saliva samples or clothes. Swabs taken by the police are excluded. The success rate for these samples decrease much less: from 44.4% in 2006 over 41.7 in 2007 to 38.9% up to the end of August 2008. The difference between 2006 and 2007 is nearly the same as from 2007 to 2008. So the change of the extraction method seems not to be responsible for the decrease. We still have to answer this question.

So in conclusion for the forensic touch samples we can’t compare the efficiency of the DNA-extraction methods: The way of sample collection has a very big influence on the typing result.

At last I present you some data of DNA-analysis for a special scientific project of our department. We have to extract DNA from paraffin embedded tissue from dead bodies for genetic analysis of human genes. We made a parallel extraction of the material with Maxwell® and organic extraction. The DNA

yield for organic extraction was at mean 37ng and 44ng for Maxwell® extraction. So for this kind of samples the efficiency of Maxwell® is also shown.

DNA-Purity

The last point to evaluate the Maxwell® extraction in comparison to Chelex® is looking at the efficiency in removing PCR-inhibitors.
Already in the testing phase we observed an excellent purity of Maxwell® extracted DNA-samples. After processing more than 9000 samples we found that the most positive feature of the Maxwell® System is the efficient removing of PCR inhibitors.
When using Chelex® extraction we find 2/3 of the DNA-samples with need of repurification. The DNA extracts were cloudy or discoloured or had an increased C_T value of the internal positive control in the Realtime PCR quantification. These are indications of PCR inhibition. So we used spin columns based on silica gel technique for repurification. After purification the samples had to be quantified again. Using the Maxwell® extraction we don't see any discoloured or cloudy samples. Nearly no sample needs repurification due to an increased IPC C_T value. This diagram shows impressively the advantage of Maxwell® extraction versus Chelex® concerning the DNA purity. The Maxwell® System removes PCR inhibitors much more effectively than Chelex® extraction (figure 1).
The Maxwell® System extracted successfully pure DNA from evidences, which needs often more than two repurification steps. Here you see the typing result from a stone used by a home invasion. DNA is extracted with the Maxwell® System and 0.1 ng was amplified using Promegas PowerPlex® ES System. We get a full profile for the German DNA Database. The second example is a glove from another home invasion. In a first extraction with Chelex® there is a need of two repurification steps, before PCR inhibitors were removed for a successful typing. After amplifying 0.8ng DNA we get a mixture stain of at least two persons. To derive a DNA profile the main component does not emphasize clear enough. Regularly we make an additional DNA extraction from a different part of such a sample. Often we get a clear-cut result after this. So it was for this glove after second extraction with the Maxwell® System (figure 2). We amplified 0.4ng DNA with the PowerPlex® ES System and the sample shows a clear one person profile. The Maxwell® extracted DNA sample needs no repurification before amplification.

Conclusions

In conclusion DNA extraction with Maxwell® removes PCR inhibitors much more efficiently than extraction with Chelex®. There is less hand on time for the laboratory staff, because no repurification and additional quantification must be done any more. This also reduces the costs significantly and counteracts the higher costs of the Maxwell® System compared to the manual Chelex® extraction.

Last I want to give you a short summary of our experience after three quarters of a year DNA extraction with the Maxwell® System:
The Maxwell® System extracts sufficient DNA from different sources of forensic samples for successful DNA typing

The purity of the DNA samples is excellent, PCR Inhibitors are removed more effectively than with Chelex® ® extraction. So there is no need for repurification and additional quantification.
Compared with Chelex® you have to do fewer steps in the laboratory. Centrifugation and ethanol precipitation isn't even necessary. This reduces manpower and efforts to avoid sample mix-ups and contamination.
Extraction with the Maxwell® 16 LEV System optimizes the workflow efficiency in our lab and helps to handle the increasing number of forensic samples.
I close with the best regards from our two great technicians, who do all DNA-extraction work in our lab, Kai and Igor. I say thank you to them for their excellent work. And for making everything a little bit better e. g. bringing light into the Maxwell® device.

Figure 1

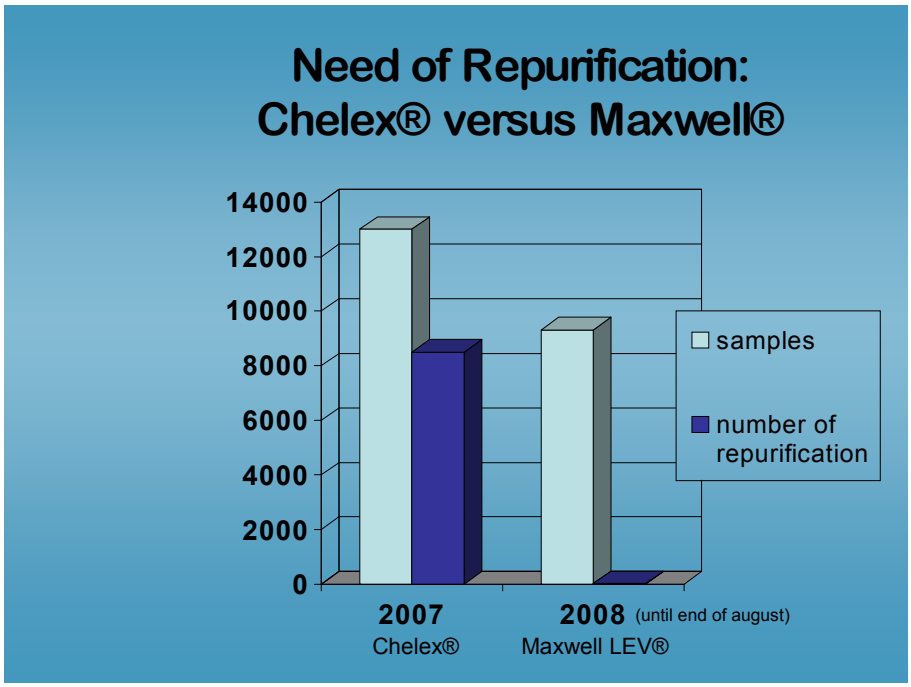
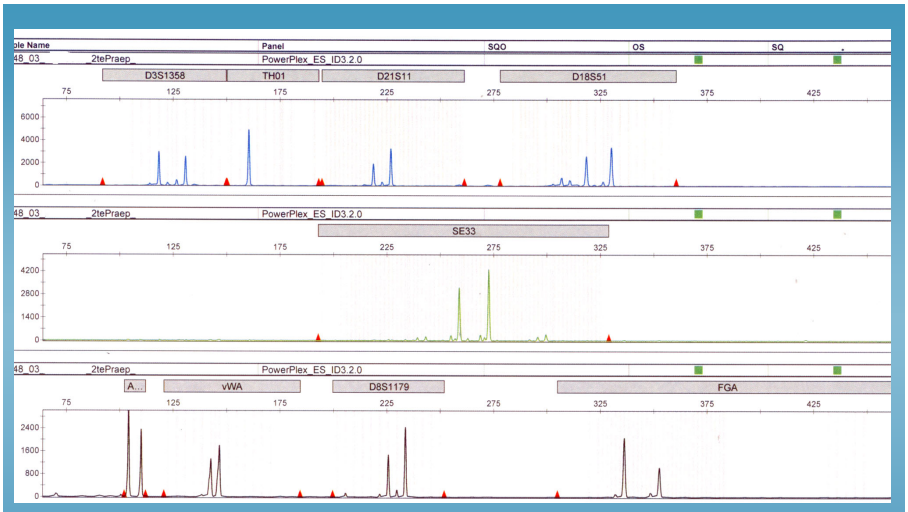


Figure 2



STR analysis of DNA after extraction with Maxwell® from a same glove.
The sample needs no repurification. 0.4 ng DNA was amplified with PowerPlex® ES System