Rapid DNA technologies at the crime scene
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Chapter 5

Objective Data on DNA Success Rates can Aid the Selection Process of Crime Samples for Analysis by Rapid Mobile DNA Technologies

Abstract
Mobile Rapid-DNA devices have recently become available on the market. These devices can perform DNA analyses within 90 min with an easy ‘sample in–answer out’ system, with the option of performing comparisons with a DNA database or reference profile. However, these fast mobile systems cannot yet compete with the sensitivity of the standard laboratory analysis. For the future this implies that Scene of Crime Officers (SoCOs) need to decide on whether to analyse a crime sample with a Rapid-DNA device and to get results within 2 h or to secure and analyse the sample at the laboratory with a much longer throughput time but with higher sensitivity. This study provides SoCOs with evidence-based information on DNA success rates, which can improve their decisions at the crime scene on whether or not to use a Rapid-DNA device. Crime samples with a high success rate in the laboratory will also have the highest potential for Rapid-DNA analysis. These include samples from e.g. headwear, cigarette ends, articles of clothing, bloodstains, and drinking items.
5.1 Introduction

Nowadays, DNA analysis and comparing DNA profiles is a key element of forensic science. While in reality the DNA analysis process from crime scene to result can take days, weeks or even months, the TV-series CSI performs fictional DNA analysis within minutes. This so-called ‘CSI-effect’ affects the public expectations (1). Science is starting to catch up with fiction and the actual forensic community is urging to speed up the DNA analysis process. Especially in high profile cases such as terrorism cases or serial murder and rape cases every minute can count. In this light the Netherlands Forensic Institute (NFI) has recently developed a fast DNA analysis track, called DNA 6-hours (2). Another technological trend is the introduction of mobile Rapid DNA devices that can perform DNA analysis within 90 minutes with an easy ‘sample in – answer out’ system: the so-called Rapid DNA analysis. The unique selling point of the mobile DNA-technology is that it can be taken to the crime scene and operated by the Scene of Crime Officers (SoCOs). This enables to start up the DNA analysis process immediately. The introduction of mobile DNA devices in the criminal justice system is therefore inevitable.

Rapid DNA devices are designed for several purposes. In the field of medicine, for instance, Rapid DNA technologies are being developed to rapidly detect viruses, bacteria or infectious diseases such as malaria or HIV from samples (3-5). These technologies also hold out promise for forensics; for instance, to determine the presence of the male and female variants of the amelogenin gene to indicate the presence of DNA as a screening test (6).

In addition, several companies in the field of forensics are working on rapid human identification systems to indicate the presence of DNA or to perform complete DNA profiling outside the laboratory with easy-to-use handheld systems. These systems operate as fully integrated and automated DNA analysis systems that produce high-quality STR-profiles suitable to perform comparisons with a DNA database or reference profile. Such technologies include the RapidHIT200 by IntegenX, ParaDNA by LGC forensics and DNAscan by NetBio. Other promising technologies are IntrepID by Lockheed-Martin and ZyGEM, Portable DNA Analyzer by NEC and MIDAS by the former Forensic Science Service.

Several studies on these mobile technologies have shown that robust buccal swab DNA profiles can be used for identification purposes (7-9). This progress has even led to the establishment of a quality assurance standard for DNA data-basing laboratories that perform Rapid DNA analysis using a Rapid DNA instrument for the analysis of reference samples (i.e. offender, arrestee, detainee or casework reference sample) for direct comparison with or inclusion in the Combined DNA Index System (CODIS) (10). This further ensures the quality and integrity of typing data from mobile devices.
Mobile Rapid DNA systems are currently used for the analysis of buccal samples when comparison with a DNA database is the objective. This is due to the fact that these samples contain sufficient DNA for mobile analysis. It is unclear at present what quantity and quality of DNA is needed to obtain profiles with Rapid technologies. The quantity of DNA that is needed for these Rapid DNA systems is high compared to standard laboratory DNA analysis. Sensitivity studies report different DNA quantity requirements (7-9, 11-14). For instance, partial DNA profiles were obtained from samples containing 5 ng DNA on a cotton swab (12). This study also showed that when the DNA was directly added to the sample cartridge, complete profiles could be obtained from 500 pg of DNA, and partial profiles with 65% of the alleles present could be detected down to 25 pg of DNA. NetBio even demonstrated that a complete STR profile could be obtained from 1 ng of purified DNA (7). However, the potential consequences of mobile DNA technologies for the analysis of crime samples are not discussed in these studies.

With this limitation, some studies on Rapid DNA do show successful typing results from crime scene DNA samples (7, 14-16), indicating that mobile technologies can provide investigative leads (15). The threshold for analysing DNA samples – i.e. the actual sensitivity of the mobile devices – is unclear, as there are limited studies on this matter. However, the sensitivities of the mobile devices will be lower than standard analysis at the laboratory and the sample is also (partly) consumed when using a mobile analysis technology. On the one hand, using these devices may offer an early investigative lead; on the other hand, it entails the risk of losing a sample that could have led to a profile in the lab. It is important to recognise both the opportunities and the risks associated with analysing crime samples with mobile technologies.

When mobile Rapid DNA devices are integrated at the police forensic department, SoCOs have to decide whether to use a Rapid DNA device to analyse a crime sample or to forward the crime sample to the laboratory, in a situation of uncertainty. This is because the results and effects of using a Rapid DNA device are unknown. To reduce the uncertainty, information should be made available about the possibilities and risks of rapidly analysing crime samples. Although information on the success rates of crime samples analysed with Rapid DNA devices is still insufficient, relevant information about these outcomes can in first instance be obtained from laboratory data. All forensic DNA laboratories have DNA typing data available from different trace exhibits and with different DNA contents. Based on these data, the outcomes of Rapid DNA devices can be estimated.

At the NFI we have a dataset containing 2260 DNA crime samples (obtained in a previous study (17)). This dataset was used to estimate the potential results obtained with the less sensitive mobile Rapid DNA analysis systems. Estimations based on these data might support the SoCOs in their decisions to either use a mobile Rapid DNA device to analyse the crime sample or to send the sample to the Crime Lab.
5.2 Materials & Methods

For this study we used a set of 2260 analysed crime samples from 28 different trace exhibit categories obtained in a previously published study (17) (Table 1). From these samples we obtained the case files with details on crime samples. Based on the testimonies from the reporting scientists we obtained information on the concentration of the DNA in the extracts of the samples and the characteristics of the DNA profile. The DNA profiles were classified as 1) single DNA profiles; 2) mixed DNA profiles that meet the criteria for storage in the Dutch national DNA database; 3) complex DNA profiles that do not meet the criteria for storage in the DNA database but contain typing data that can be used for exclusion; and 4) no typing result, when the profile data contains too little information for a meaningful comparison.

This dataset was used to analyse the potential impact of the lower sensitivity levels of the mobile DNA analysis systems. The literature suggests that mobile technologies are able to process DNA samples with DNA quantities of 100 pg/μL (6, 11) in the extract. Some results of these studies even show that extracts with DNA quantities of at least 25 pg/μL (11) were able to yield partial profiles. To illustrate the impact on obtaining profiles with less sensitive devices, we arbitrarily decided on two sensitivity levels for the mobile technology to estimate the potential results, a somewhat conservative level of 100 pg/μL and a more sensitive level of 25 pg/μL. Although currently unrealistic, this sensitivity level may well be achieved in the future.

Laboratory data on DNA typing results were used to estimate the potential of mobile DNA technologies with a lower sensitivity level. The success rates of numerous crime sample categories were evaluated at an analytical threshold value of 100 pg/μL and of 25 pg/μL.

Due to this reduced sensitivity of the mobile DNA technology compared to the lab, we needed to define an additional outcome of this Rapid DNA device, namely the false negative. A false negative indicates that the DNA in the extract generated a profile in the laboratory but the amount of DNA in the sample fell below the sensitivity level of the mobile device (arbitrarily set at either 100 pg/μL or 25 pg/μL). This means that the extract yielded a profiling result in the lab, but expected not to provide a profile when analysed with a Rapid DNA device.

For each crime sample category we analysed the actual DNA typing results. For a number of categories we analysed the DNA typing results at thresholds of 100 pg/μL and 25 pg/μL. The DNA profiling results above and below these sensitivity levels were evaluated. If the laboratory obtained a profiling result from samples that contained DNA below these sensitivity levels (set at 100 pg/μL and 25 pg/μL), they were marked as a false negative.

The potential impact of these lower sensitivity levels on the analysis results can be used to indicate the effect of any sensitivity level for DNA analysis in the future.
**Table 1. Actual Observed DNA Profiling Results**

The actual observed profiling results are ranked from highest to lowest success rates (single + mixed + complex DNA profiles). Traces above the dotted line indicate the most successful trace categories. *Based on Table 3 from Mapes. et al., 2016 (17)*

<table>
<thead>
<tr>
<th>Trace exhibit</th>
<th>N (Σ=2260)</th>
<th>Observed profile results (%)</th>
<th>Concentration (pg/μL)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single</td>
<td>Mixed</td>
</tr>
<tr>
<td>Ball cap</td>
<td>100</td>
<td>42</td>
<td>39</td>
</tr>
<tr>
<td>Balaclava</td>
<td>100</td>
<td>46</td>
<td>29</td>
</tr>
<tr>
<td>Headwear (other)</td>
<td>98</td>
<td>27</td>
<td>34</td>
</tr>
<tr>
<td>Cigarette end</td>
<td>100</td>
<td>84</td>
<td>3</td>
</tr>
<tr>
<td>Sock</td>
<td>64</td>
<td>38</td>
<td>25</td>
</tr>
<tr>
<td>Sleeve cuff</td>
<td>70</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>Blood</td>
<td>100</td>
<td>68</td>
<td>6</td>
</tr>
<tr>
<td>Collar</td>
<td>77</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>Fabric glove (inside)</td>
<td>100</td>
<td>33</td>
<td>24</td>
</tr>
<tr>
<td>Drinking items</td>
<td>100</td>
<td>57</td>
<td>6</td>
</tr>
<tr>
<td>Shoe</td>
<td>48</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Latex glove (inside)</td>
<td>87</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Torch</td>
<td>77</td>
<td>27</td>
<td>12</td>
</tr>
<tr>
<td>Handbag grip</td>
<td>100</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Lighter</td>
<td>23</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Handle motor/bike</td>
<td>67</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Screwdriver</td>
<td>100</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Knife grip</td>
<td>100</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Car items</td>
<td>100</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>(Fire) weapon grip</td>
<td>100</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Glasses</td>
<td>32</td>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>Hand-tools (other)</td>
<td>100</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Keys</td>
<td>43</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Zip tie</td>
<td>99</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Cartridge case</td>
<td>100</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Crowbar</td>
<td>78</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Tape</td>
<td>44</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Gas cylinder</td>
<td>53</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*On average 2 outliers per exhibit were removed (min=1, max= 6). For example, secured bloodstains (category “blood”) show a mean concentration of 1459.3 pg/μL; after removing 1 outlier with a concentration of 54.8 ng/μL, the mean concentration was 920.2 pg/μL.*

### 5.2.1 DNA Analysis Process of the Selected Samples

**DNA sample preparation**

The traces we used in this study were secured from the crime scenes by SoCOs and sampled for DNA analysis at the police laboratories. The sampling was performed by the police laboratories using NFI standards and protocols. Most samples were secured by swabbing the exhibit using a dry cotton swab. At the police laboratories the swabs were transferred to special containers and sent to the NFI. Cigarette ends were cut from...
the cigarette and placed in the container. Exhibits such as balaclavas and fabric gloves were sampled by the stubbing procedure (18).

**DNA extraction and quantification**
DNA extraction from the secured samples and the profiling were performed at the NFI. In the course of this study the DNA quantification method used for DNA extracts was the Quantifiler® Duo DNA quantification kit with the 7500 Real-Time PCR System (Applied Biosystems™ - AB). The manufacturer of the quantification kit reports a DNA concentration range of 50 ng/μL to 23 pg/μL (19).

**DNA profiling**
All Short Tandem Repeat (STR) DNA profiles included in this study were obtained with the Next Generation Multiplex (NGM) DNA analysis system (AB). The NGM DNA analysis system determines the genetic information on 15 polymorphic DNA-loci and the sex specific locus Amelogenin (20). DNA amplification (29 PCR cycles) and fragment analysis were performed according to the manufacturer’s recommendations except for validated in-house adaptations (i.e. enhanced detection of PCR fragments (21)). The reporting scientist performed the interpretation and statistical evaluation of the DNA profile comparisons. DNA profiles for entry, comparison and storage in the DNA database must meet minimal criteria (22). In some instances, intelligence-based database searches on complex profiles were performed as indicated by the reporting scientist.

### 5.3 Results & Discussion

The purpose of this study was to understand the potential impact of analysing crime samples with a mobile Rapid DNA analysis system, which can produce profiling results within 2 hours but is less sensitive than laboratory techniques. A dataset of 2260 DNA samples, containing 28 different categories of trace items, was analysed for this purpose. Table 1 shows the actual observed profiling results of the 28 trace exhibit categories ranked from highest to lowest success rates. DNA samples from ball caps, for instance, show a total of 94% obtained profiles of which 42% single, 39% mixed and 13% complex profiles. At the other end of the spectrum, no profiling results were obtained from DNA samples from gas cylinders.

DNA success rates are related to the concentration of the DNA in the extract obtained from the crime scene (17). Samples with low quantities of DNA are therefore less appropriate for analysis with less sensitive techniques, such as Rapid DNA devices. Crime samples that show a high success rate in the laboratory will therefore also have the highest potential for Rapid DNA analysis. The dotted line in Table 1 indicates the categories with the highest success rates. The DNA categories below this line show...
success rates ranging from 55% all the way down to 0%. The categories with the highest success rates consist of ball caps, balaclavas, other headwear, cigarette ends, socks, sleeve cuffs, bloodstains, collars, fabric gloves and drinking items. These categories show DNA success rates ranging from 71% up to 94%.

The objective of using a mobile Rapid DNA device is to quickly obtain informative knowledge on the donor of a crime DNA sample through a rapid profiling procedure, which can immediately be compared to the profiles in the DNA database or to known reference samples. To obtain this goal, it is recommended to limit the rapid DNA analysis of traces at the crime scene to DNA samples with high success rates.

To evaluate the potential impact of a technique with a lower sensitivity level we focused on the categories with the highest (>70%) success rates. It is noted that samples in these categories (above the dotted line in Table 1) mainly contain saliva-stained exhibits and/or have fabric-type surfaces, while samples from categories with lower success rates consist of items with smoother, plastic types of surfaces. This illustrates that the difference in surface material is an important factor that should be considered when choosing traces for (Rapid) DNA analysis.

DNA samples from these rather successful trace exhibit categories, of which 70% or more resulted in a profile, were selected and further analysed to understand the impact of applying techniques with different sensitivities for profiling DNA samples. For this purpose we took two hypothetical sensitivity values: 100 pg/μL and 25 pg/μL. Figure 1 clearly shows the impact if the samples from these “high success rate” categories were to be analysed with a less sensitive technology. A false negative indicates that the quantity of DNA in the extract is below the sensitivity threshold. In these cases it is expected that the less sensitive mobile analysis system will not yield a profiling result.

Many samples in the “high success rate” categories show DNA concentrations lower than 100 pg/μL. If these samples were analysed with a technology that allows for a sensitivity of 100 pg/μL DNA in the extract, this would result in loss of information. Samples from most categories would then produce no profiling results in more than half of the cases, with a high number of false negatives where the standard laboratory method would have obtained a valid typing result.

For instance, profiling results were obtained from balaclava samples in 92% of the cases at the laboratory (46% single profiles, 29% mixed profiles and 17% complex profiles). With a sensitivity level of 100 pg/μL, it is expected that the balaclava samples would show a profiling result in only 61% of the cases. In 31% of the cases the less sensitive mobile technology would produce a false negative result. In these cases the mobile analysis system would fail to produce potentially valuable DNA profiling information, and by using the system, investigators might run the risk of losing potentially valuable DNA profiling data. The 31% false negative results consisted of 13% single DNA profiles, 10% mixed profiles and 8% complex profiles.
The information in Figure 1 can be used to decide whether to analyse a sample with a fast but less sensitive mobile DNA-analysis system, or to analyse it in the laboratory. This decision will depend on the possibility to analyse mixed and/or complex DNA samples with mobile devices and on the extent to which false negatives are acceptable in a certain situation. If, for instance, the Rapid DNA devices are only designed to profile and compare single DNA profiles, probably only samples from cigarette ends and blood should be considered for fast mobile analysis.
However, if Rapid DNA devices enable the analyses of mixed DNA samples at a sensitivity level of 100 pg/µL (see Figure 1), then the picture changes. For instance, if a 70% success rate with a Rapid DNA device is acceptable, thus accepting 30% false negative results (complex profiles and false negatives), then one can safely decide to analyse samples from cigarette ends (10% false negative results) and bloodstains (30% false negative results).

The picture would change dramatically if future generations of mobile DNA devices achieve even more sensitive analyses. If a sensitivity level of 25 pg/µL could be reached and the technology also enables the analysis of mixed samples, then samples from ball caps (17% false negative results), balaclavas (22% false negative results), fabric gloves (21%) and headwear (29%), although complex profiles do not always lead to valuable information, they can sometimes exclude or include a person’s profile and can therefore provide relevant information. Consequently, if these complex profiling data cannot be processed, the number of false negative results will increase. Rapid DNA-devices that can also analyse complex profiles would therefore be most optimal.

With the current state of Rapid DNA devices, where DNA quantities of at least 100 pg/µL in the extract are needed, these systems cannot yet make a significant contribution to the crime scene for the analysis of biological traces. However, such a decision depends also on the percentage of false negatives that is considered acceptable, and this most probably varies depending on the type of case. For the efficient use of a Rapid DNA device, the decision to accept a certain number of false negatives is crucial. The Criminal Justice System needs to decide on an acceptable risk of losing evidence. For instance, accepting false negatives could be more justifiable in burglary cases than in a murder case. On the other hand, in terrorism cases or serial murders every second can count. In those cases a fast typing result can be crucial to identifying a suspect. Obviously, the number of traces available at the crime scene must also be taken into account.

An important issue that should be mentioned about these mobile Rapid DNA devices is that they do not allow for the quantification of a sample prior to analysis. As stated in a previous article, the limitation of a Rapid DNA system is the inability to quantify the amount of DNA added to the PCR step (23). The development of a mobile quantification module might be crucial for an optimal mobile analysis of casework samples. Information on the DNA quantity can help decide whether to progress the DNA sample with the Rapid DNA device or to forward the sample to the forensic laboratory. Such a technique is not available so far, so that SoCOs need to make decisions on analysing DNA samples with uncertainty. Knowledge of DNA success rates that take reduced
sensitivities into account, as shown in Figure 1, can then offer them some degree of support. The results of this study should not be used to rush implementation, but this study can help to develop new working methods for the Criminal Justice System for the use of these mobile Rapid DNA technologies at the crime scene.

5.4 Conclusion

Until actual data on the sensitivity of mobile Rapid DNA technologies for the analysis of crime scene samples become available, only laboratory data on DNA success rates, as shown in Table 1, can aid the crime scene sample selection process. Our study illustrates that it is crucial to understand the possible risks of losing profiling data (Figure 1) before an objective decision can be made to use a Rapid DNA device on a crime sample. This will reduce the number of false negatives (samples that contain enough DNA for laboratory analysis but produce no typing result with the mobile technology) and allow for a better use of fast mobile DNA analysis and the potential identification of perpetrators.

The results of this study can help SoCOs to make evidence-based decisions on the crucial decision: to analyse a DNA sample with a Rapid DNA analysis device and accept false negatives and to potentially identify the perpetrator quickly; or to forward the sample to the laboratory, where it takes longer before the results are obtained but where the probability of obtaining a profiling result is greater due to the higher sensitivities of the laboratory techniques.

It is claimed that with the use of mobile Rapid DNA technologies, perpetrators can be identified within hours (24). The mission of the SoCO is to assist the legal system in solving crimes through the investigation of the forensic evidence and to identify perpetrators quickly. However, it is unacceptable if the new mobile DNA technology entails a high risk of losing potentially incriminating evidence. The data from this study combined with comprehensive selection criteria for the crime scene workers can reduce that risk.

5.5 References

Chapter 5

DNA Success Rates for Rapid Mobile DNA Technologies