Comparison of the variables affecting the recovery of DNA from common drinking containers

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Abstract

As the boundaries of forensic DNA profiling continue to expand, less obvious sources of biological evidence are being collected at crime scenes for DNA profiling. One example is the recovery of biological evidence from common drink containers, such as bottles and cans, which have been found at crime scenes. There are many variables that may have an impact on recovering a DNA profile from such exhibits. In this research, the effects of person to person variation, time, type of drink (including alcoholic and non-alcoholic beverages), and type of drink container, were assessed for their impact on the major analytical outcomes of the DNA process. The results show that the α-amylase activity varies from individual to individual and is reduced in the presence of some alcoholic drinks. A reasonable DNA yield was obtained from all samples, however, the concentrations exhibited significant person to person variation. The type of drink container influenced the DNA yield with cans giving a higher yield than bottles of the same drink type. To a reduced extent the presence or absence of alcohol affected the overall DNA yield and when partial or failed DNA profiles were produced they were more likely to be associated with alcoholic drinks than non-alcoholic drinks.

Keywords: Forensic; DNA; STR; SGM Plus™; α-Amylase; Saliva; Multivariate analysis

1. Introduction

The advance of DNA technology has seen the application of forensic biology to criminal cases increase dramatically. DNA profiling has been applied to many forms of biological material such as blood, semen, bone, teeth, hair, and cigarette butts [1]. In New Zealand, blood and semen exhibits still tend to dominate with over 75% of criminal cases requiring the analysis of these types of sample [2], however crime scene examiners are now attuned to collecting a broader variety of exhibits for DNA analysis. Exhibits such as discarded clothing and food remnants [3,4], touched or handled surfaces [5], and drinking containers such as bottles and cans [6] are now yielding useful DNA information on a regular basis.

Over the past 2 years in New Zealand, the submission of common drinking containers for DNA analysis has increased dramatically (Fig. 1). This is due in part to the development of a DNA Databank and its applicability to the investigation of volume crime [7,8]. In accordance with the advancement of DNA profiling methodology, the success of DNA analysis from these exhibits has also increased (Fig. 1).

There are several variables involved in the analysis of this type of exhibit in a forensic context. Often these are beyond the control of the scientist or crime scene examiner. As with any forensic pursuit, however, it is important to attempt to quantify and understand the factors influencing the outcome of the scientific process. In this research, volunteers drank a variety of common beverages from common drink containers. The effects of person to person variation, the type of drink (including alcoholic and non-alcoholic beverages), the type of drink container and time were assessed for their impact on the major analytical outcomes of the DNA process. In particular, attention was focused on the detection...
of α-amylase, the yield of human DNA and the production of a STR profile suitable for evidential purposes. Observed trends were then tested to assess their statistical significance.

2. Materials and methods

2.1. Sample collection

Six subjects, four females and two males, were asked to consume a variety of alcoholic and non-alcoholic beverages from either a bottle or a can (Table 1). The subjects were expected to drink out of all containers as they normally would. Each subject consumed each type of beverage twice, to allow testing at different time periods.

All containers were left to dry at room temperature for 24 or 48 h after consumption. A double-swabbing technique was used to sample the cellular material from the mouth and neck area of the bottles, and from the mouth area of the cans [9]. The time period and swabbing method were chosen to simulate sample collection from scenes of volume crimes, such as burglaries, where a crime scene examiner would attend the scene within 24–48 h, retrieve an exhibit and collect the swab from the exhibit, such as a drink container. Prior to sampling for extraction, the swabs were left to dry at room temperature for at least 48 h. The wet and dry swabs for each container were combined at the extraction stage and processed as one sample.

Each of the six subjects was asked to submit a buccal swab that was used as a reference during the experiment.

2.2. DNA extraction and quantitation

A modified Chelex® 100 extraction procedure was used to extract DNA from all samples [10]. Prior to incubation with Chelex® 100 at 56 °C, 20 μl of proteinase K (10 mg/ml) was added to 200 μl of extracted DNA solution.

In order to estimate whether or not the salivary material was retrieved from the drink containers, a test for α-amylase activity was carried out on the extraction supernatants of all samples [11]. Approximately 1/4 of a ground 45 mg Phadebas® tablet was combined with 500 μl of extraction supernatant and incubated at 37 °C for 15 min. The reaction was stopped with 1 ml 0.5 M NaOH and samples centrifuged at 13,000 rpm in a microcentrifuge for 3 min to pellet the tablet remnants. The intensity of the colour in the supernatant represents the intensity of the enzymatic reaction and level of α-amylase activity. This was assessed visually with reference to prepared controls. For consistency purposes, the

![Fig. 1. The number of cases submitted to the New Zealand DNA Databank between 1998 and 2000 in which the primary exhibit was a common drinking container. The success rate indicates the proportion of these cases (as a percentage value) that yielded a DNA profile suitable for inclusion on the DNA Database. For a crime sample, a minimum of 10 alleles is required for a sample to be included on the DNA Database.](image-url)
results were evaluated by the same analysts and scored on an ascending scale of 1–4. Intensity data was collated for \( \alpha \)-amylase test results and categorised by the individual subject, the type of drink and the type of drink container.

The extracted DNA was quantitated using the ACES\textsuperscript{TM} 2.0\textsuperscript{+} Human DNA Quantitation System in accordance with the manufacturer’s instructions [12,13]. The lowest detectable standard contained 0.04 ng DNA. The concentration of DNA detected during quantitation was categorised by the individual subject, the type of drink and the type of drink container.

The extracted samples were amplified according to manufacturer’s instructions using the AmpFISTR\textsuperscript{®} SGM Plus\textsuperscript{TM} multiplex (Applied Biosystems, Foster City, CA) in a 50 \( \mu \)l volume reaction [14,15]. A 1.5 \( \mu \)l aliquot of amplified product was analysed using the ABI Prism 377 Gene Sequencer (Applied Biosystems, Foster City, CA). DNA fragment analysis was performed for the 10 AmpFISTR\textsuperscript{®} SGM Plus\textsuperscript{TM} STR loci and the sex determination locus Amelogenin using Genescan Analysis\textsuperscript{®} (version 3.1) and Genotyper\textsuperscript{®} (version 2.5) software. Internal validation studies have shown that the AmpFISTR\textsuperscript{®} SGM Plus\textsuperscript{TM} multiplex (SGM Plus\textsuperscript{TM}) will amplify efficiently using between 0.2 and 1 ng of template DNA with the optimal amount of DNA concentration being between 0.4 and 0.5 ng (Cullen et al., personal communication). In our study, all samples with a visible level of DNA at quantitation (\( \geq 0.04 \) ng) were amplified. As the maximum volume of DNA able to be added to the SGM Plus\textsuperscript{TM} PCR is 20 \( \mu \)l, this meant the range of DNA template amplified was between 0.08 and 0.8 ng, with an optimal template amount of 0.4–0.5 ng achieved where possible. Samples for which no DNA was detected in the quantitation process were not amplified and no further action was taken.

The average peak height for all 10 STR loci and the Amelogenin locus was calculated to assess the DNA profile. This data was categorised by the individual subject, the type of drink and the type of drink container. Peaks below 50 relative fluorescent units (RFU) were not analysed in this study. For all peaks smaller than 50 RFU, a value of zero was assigned and the locus failed. Profiles were considered to be “full” when the genotypes for all 10 STR loci and Amelogenin could be determined. Profiles were considered to be “partial” or “no result” respectively if any or all of the STR loci could not be designated according to the guidelines described.

2.3. DNA profiling method

In these experiments four response variables were considered. These were:

- \( \alpha \)-amylase—the relative amount of \( \alpha \)-amylase activity;
- DNA—the DNA yield;
- profile—the failure or success of obtaining a usable DNA profile;
- intensity—relative peak height combined for all STR loci.

The profile intensity is a linear combination of the peak height measurements for each of the STR loci. The peak heights of D3S1358, HUMvWF31A, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, HUMTH01 and HUMFIBRA in RFU quantify, to some extent, the amount of DNA present. There is a good reason to think that these values will measure a similar quantity—i.e. they are highly correlated. In order to investigate this, a principal components analysis (PCA) was undertaken [16].

PCA analysis on the variables D3S1358, HUMvWF31A, D16S539, D2S1338, Amelogenin, D8S1179, D21S11, D18S51, D19S433, HUMTH01 and HUMFIBRA showed that the first principal component explains 96% of the variation in the data, hence the first principal component (D3S1358) is sufficient to explain the variation present in the 11 SGM Plus\textsuperscript{TM} loci. The first principal component will be referred to as PCA1, which is equivalent to “intensity” in the observed results.

The response variables in this experiment are thought to be affected by four factors. These factors are:

- person—person to person variation;
- beverage—the type of drink;
- time—24 or 48 h between drinking and swabbing;
- container—the type of container (glass bottle or aluminium can).

This experiment has multiple responses as well as multiple factors. A standard multivariate analysis of variance (MANOVA) model was proposed for three of the response variables (\( \alpha \)-amylase, DNA yield, and intensity) [17]. In using the MANOVA model there are some assumptions made that may not be justified, so univariate ANOVA models for each response were considered as well [18].

The additional hypothesis that there is a difference in outcome between beverages served in glass bottles and beverages served in aluminum cans was formally tested using a linear contrast [18].

The response as to whether a successful profile has been obtained, is binary. For this reason, modeling this response with a normal distribution is not appropriate, therefore, a traditional treatment for binary response data, a generalized linear model (GLM), has been used [19].

3. Results and discussion

3.1. Person to person variation

The \( \alpha \)-amylase activity is found in numerous human bodily fluids and in extremely high levels in adult human saliva [20]. In forensic laboratories, this biological feature is utilised to assess the presence or absence of saliva on an
Table 2
Output from ANOVA procedure for reduced model

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>Pr(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Response: α-amylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person</td>
<td>5</td>
<td>42.30</td>
<td>8.46</td>
<td>28.23</td>
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</tr>
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<td>Beverage</td>
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<td>31.91</td>
<td>4.56</td>
<td>15.21</td>
<td>&lt;1E−7</td>
</tr>
<tr>
<td>Time</td>
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<td>0.17</td>
<td>0.17</td>
<td>0.56</td>
<td>0.46</td>
</tr>
<tr>
<td>Person:beverage</td>
<td>35</td>
<td>18.53</td>
<td>0.53</td>
<td>1.77</td>
<td>0.03</td>
</tr>
<tr>
<td>Residual</td>
<td>47</td>
<td>14.08</td>
<td>0.30</td>
<td>0.02</td>
<td>0.89</td>
</tr>
<tr>
<td>(B) Response: DNA yield</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person</td>
<td>5</td>
<td>0.27</td>
<td>0.05</td>
<td>3.24</td>
<td>0.01</td>
</tr>
<tr>
<td>Beverage</td>
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<td>1.33</td>
<td>0.19</td>
<td>11.41</td>
<td>&lt;1E−7</td>
</tr>
<tr>
<td>Time</td>
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<td>0.11</td>
<td>0.11</td>
<td>6.42</td>
<td>0.01</td>
</tr>
<tr>
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<td>0.72</td>
<td>0.02</td>
<td>1.23</td>
<td>0.25</td>
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<tr>
<td>Residual</td>
<td>47</td>
<td>0.78</td>
<td>0.02</td>
<td>0.02</td>
<td>0.90</td>
</tr>
<tr>
<td>(C) Response: PCA1 (intensity)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person</td>
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<td>228.93</td>
<td>45.79</td>
<td>10.14</td>
<td>&lt;1E−5</td>
</tr>
<tr>
<td>Beverage</td>
<td>7</td>
<td>195.90</td>
<td>27.99</td>
<td>6.20</td>
<td>&lt;1E−4</td>
</tr>
<tr>
<td>Time</td>
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<td>20.16</td>
<td>20.16</td>
<td>4.46</td>
<td>0.04</td>
</tr>
<tr>
<td>Person:beverage</td>
<td>35</td>
<td>321.01</td>
<td>9.17</td>
<td>2.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Residual</td>
<td>47</td>
<td>212.25</td>
<td>4.53</td>
<td>0.02</td>
<td>0.93</td>
</tr>
</tbody>
</table>

exhibit. A high level of α-amylase activity indicates the presence of saliva, however, as individuals exhibit a large amount of variation in the levels of α-amylase in saliva, a low level of α-amylase activity does not indicate the absence of saliva. The examination of the α-amylase test results for all six subjects revealed that the levels of α-amylase recovered from all sample types varied from person to person. Some subjects showed consistently low levels of α-amylase activity (average α-amylase reading subject C = 0.3), one showed consistently high levels (subject D = 2.4) and others tended to cluster around an overall mean intensity value of 1.5 (subject A = 1.6, subject B = 2.0 and subject E = 1.7). Results from the univariate analysis of variance confirm what has been observed here (Pr(F) < 1E−8, Table 2A). Earlier studies examining α-amylase activity have shown that its levels can vary not only from person to person but with the time of day that sampling and testing is undertaken [20,21].

Significant variation was observed from person to person in the amount of DNA recovered from the extraction process whereas the intensity of profiles obtained from the six subjects showed a degree of similarity (data not shown). Results from the data analysis indicate the person to person variable has a significant effect on both DNA yield (Pr(F) = 0.013, Table 2B) and DNA profile intensity (Pr(F) < 1E−5, Table 2C). Variation between individuals exists as well as variation between beverage types and not all people respond in the same way to the same beverage.

3.2. Type of drink

The average α-amylase activity was significantly lower for the mixed-spirit alcoholic drinks and Stolichnaya, in contrast to the other alcoholic and non-alcoholic drinks (Fig. 2A). However, even though these drinks contain alcohol as a component, samples from containers of beer did not show lower α-amylase activity when compared to the non-alcoholic drinks. Substrate media, such as types of fabrics containing saliva stains, has been suggested as a factor that may have effect on detection of α-amylase activity [22]. It is possible that a similar effect is induced from constituents of the various drink types. The potential for false positives was removed as testing each drink type confirmed they had no residual α-amylase activity.

The type of drink was seen to have the most profound effect on the resultant DNA concentration. Similar to the results for α-amylase activity, the DNA concentration was also observed to be lower for the mixed-spirit alcoholic drinks and the Stolichnaya. Overall, the average DNA yield for all alcoholic drinks (0.04 ng/μl) was slightly lower than for all non-alcoholic drinks (0.06 ng/μl). Low values were also recorded for the milk bottles and 24 h water bottle samples (Fig. 2B). The general trends of these results suggest that the overall chemistry of some drinks, particularly in the presence of alcohol, can affect the yield of DNA following extraction.

The type of drink also appeared to have an effect on the quality of the STR profile produced. Once again, the results show a variation between the alcoholic and non-alcoholic drinks. The average peak height for DNA profiles obtained from non-alcoholic drinks was greater when compared to profiles from alcoholic drinks (Fig. 2C). This observation may represent some inhibitory effects from the components of the alcoholic drinks on the PCR analysis, or alternatively, it may be related to the effects of drink type on the extracted DNA yield which have then carried on to affect the outcomes of the profiling process. As there was a slightly reduced DNA yield for the alcoholic drinks, the average volume of DNA template added was subsequently higher. This further complicates the determination of cause for this observed effect on the intensity of the DNA profiles. The amount of template DNA added to a PCR is fundamental to its success. Thus, the accuracy of the quantitation system is a crucial aspect to assessing the outcomes of the DNA profiling process. There is some suggestion that the current methods are variable, therefore, any conclusions drawn from the DNA profile intensity must be weighed against the subjectivity and accuracy of the ACES® 2.0+ Human DNA Quantitation System.

Of the 96 samples analysed in this project, there were 73 full profiles, 10 partial profiles and 13 samples where no DNA profiles were obtained. Of the 23 samples which did not yield a full profile, only 4 (3 partial and 1 no result) have originated from non-alcoholic drinks. This observation suggests that where a partial profile or no result was observed, there was a greatly increased likelihood that it had originated from an alcoholic drink type. In some cases this was observed regardless of the DNA concentration amplified. As the generation of the DNA profile is the ultimate step of
Fig. 2. (A) α-Amylase intensity, as a function of the drink type, shows that for some alcoholic drinks the α-amylase activity is reduced. (B) DNA concentration as a function of the drink type shows that for alcoholic drinks (BB, XB and SB) the DNA concentration is reduced. Milk (MB) and water bottles (WB24) also showed reduced DNA concentrations. The cans were observed to return a higher DNA yield than bottles containing the same drink type. (C) DNA profile intensity as a function of the drink type shows that for the alcoholic drinks (BB, BC, XB, SB) the DNA profile intensity is reduced. The overall average for alcoholic drinks (≥800 RFU) is significantly less than the overall average for non-alcoholic drinks (≥2000 RFU). Low intensity peak heights increase the likelihood of partial or no result profiles. These results relate to the sum of peak heights at the D3S1358 locus.
this forensic process, any impact at this stage is seen as integral to the overall success of analysis.

Data analysis shows the type of drink (beverage) is a significant factor for each of the three responses tested (Table 2A–C). The results of the GLM procedure (Table 3) show that once again the significant factors are person and beverage. This is evidence that the probability of a profile “failing” is dependent on the person who provided the DNA and the beverage type. Comparison of the fitted probabilities for individual beverage types show quite dramatically that the alcoholic beverages have a much greater probability of failing than the non-alcoholic drinks (Fig. 3).

### 3.3. Type of container

The comparison of the α-amylase activity recovered from samples taken from the beer and Coca-Cola cans, with those recovered from the bottles for the same drinks, revealed that α-amylase intensity levels were higher for samples recovered from cans (Fig. 2A). A similar observation was noted for the DNA yield obtained from beer and Coca-Cola cans, compared to the bottles of the same drink type (Fig. 2B). These results may be related to the person to person variation already observed, however, there are a number of differences in the physical design and composition of the two types of drinking vessels which may affect the way the drink is consumed and subsequently the amount of biological material transferred.

As with the α-amylase activity and the DNA yield, the DNA profile intensity of the Coca-Cola can was greater than for the bottle, however, a less noticeable reversal of this trend was seen for the beer can when compared to the beer bottle (Fig. 2C). The results for the beer cans were slightly incongruous as the DNA yield for this drink type was one of the

### Table 3
Analysis of deviance model for profile failure

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Deviance</th>
<th>Residual d.f.</th>
<th>Residual deviance</th>
<th>F value</th>
<th>Pr(F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response: profile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Null</td>
<td>95</td>
<td>76.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person</td>
<td>5</td>
<td>12.65</td>
<td>90</td>
<td>63.49</td>
<td>6.60</td>
<td>&lt;1E-4</td>
</tr>
<tr>
<td>Beverage</td>
<td>7</td>
<td>17.61</td>
<td>83</td>
<td>45.89</td>
<td>6.57</td>
<td>&lt;1E-4</td>
</tr>
<tr>
<td>Time</td>
<td>1</td>
<td>1.16</td>
<td>82</td>
<td>44.73</td>
<td>3.03</td>
<td>0.09</td>
</tr>
<tr>
<td>Person:beverage</td>
<td>35</td>
<td>21.81</td>
<td>47</td>
<td>22.92</td>
<td>1.63</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Fig. 3. Estimated probabilities of “failure” by beverage type. This shows that profile failure is more likely for alcoholic beverages. The figure has been adjusted such that the real distribution of the points is obscured, each of the bars actually contains 12 data points.
highest for all drinks tested, yet the resultant DNA profile intensities were the lowest. This may reflect the limitations of the quantitation method to accurately determine higher concentrations of DNA. The actual concentration may have been slightly over-estimated and the amount of template added to the PCR is subsequently below the optimal value.

The linear contrast to assess container type effect is a weighted sum of the relevant means, with the restriction that the sum of weights is zero. The only response variable for which something sensible can be said about the contrast is the DNA yield response. The other two variables (α-amylase and DNA profile intensity) have significant interactions between the person and the beverage, hence, it does not make sense to talk about the effect of the beverage/container type alone as it is confounded with the individual drinker. An estimate of the contrast and its standard deviation gives a t-statistic of approximately 5.47. The 5% critical value is approximately 1.96, hence this is strong evidence of a non-zero difference between bottles and cans.

3.4. Time

The time variables examined in this experiment, 24 and 48 h, had no significant effect on the α-amylase intensity levels obtained. Although significant, the effect of time on the DNA concentration (over the two time periods examined) was less significant than the effects of these other variables. There was little difference between the DNA profile intensity over the two time periods examined. In instances where a difference was noted, the profiles obtained from the samples recovered after 24 h had higher average peak heights than those from the 48 h period. This is consistent with the expected effects of time on the integrity of the DNA, however, the net effect was minimal and not considered to be of great consequence.

The output from the ANOVA procedure shows that time can affect DNA yield but doesn’t appear to affect mean α-amylase activity (Table 2A–C).

The output from the MANOVA procedure (Table 4) shows that there appears to be an interaction between person and beverage, and that the other main effects are important. However, there is no person/time interaction and no beverage/time interaction.

4. Conclusion

The requirement for analysis of common drink containers as forensic exhibits has increased recently, particularly through the application of forensic DNA databases to the investigation of volume crime. By scrutinising selected variables in the DNA profiling analysis, some distinct trends have been observed. Time was the least significant variable over the 24 and 48 h periods examined. There was significant person to person variation with respect to α-amylase activity and DNA yields. The type of drink, in particular alcoholic versus non-alcoholic, significantly affected the DNA yield and the resultant DNA profile. In both cases a reduced return was associated with samples from alcoholic drinks. The α-amylase activity was also reduced in samples taken from alcoholic drinks. When partial profiles or no results were obtained they were more likely to be associated with samples from alcoholic drinks than from non-alcoholic drinks. Overall, beverage type and person to person variation significantly affected the likelihood of obtaining a profile. The type of drink container also significantly affected DNA yield as in all cases cans gave a higher yield than bottles of the corresponding drink type.

The findings of this research do not prescribe a need for alteration to the analysis of common drinking containers as exhibits in forensic investigations. They may assist in the prioritisation of items for analysis, for example when faced with a choice, an analyst may opt to analyse samples from a non-alcoholic drink container ahead of samples from an alcoholic drink container. However, due to the person to person variability seen in this research, a cautionary approach to selective analysis is advised. This research offers supportive data to assist in post-analysis interpretation of findings in relation to this, quantifying to some degree the major factors influencing the outcome of this particular scientific process.

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[12] ACES™ 2.0+ Human DNA Quantitation System, Life Technologies Inc.


