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Reducing the Workload: Analysis of DNA Profiling Efficiency of Case Work Items

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ABSTRACT

Due to the increased sensitivity of genotyping kits developed in recent years, laboratories were able to recover more usable information from smaller amounts of DNA on items of evidence (e.g., touch evidence). This led to increased submission of touch or trace DNA items, which in turn increased the overall workload (van Oorschot, Ballantyne, and Mitchell 2010).

In this work, the data collected from approximately 650 cases and 2,000 evidence items was analyzed in order to study the work efficiency, to optimize evidence items processing and to give guidelines for reducing the workload. We examined three aspects: the DNA profiling efficiency for each item type; the number of samples required to obtain a DNA profile from an item; the number of items needed to be examined to obtain a database eligible profile for each case.

To examine DNA profiling efficiency of various items, the productivity Index (PI) grade was calculated on a scale of 0–10 which measures the success rate and the amount of work required for DNA profiling. To the best of our knowledge, this is the first study that measures the combination of DNA profiling success rate and the work required to obtain it.

Body fluids items (blood and saliva) were the most efficient biological sources for DNA profiling (PI 6) while clothing items were less efficient (PI 2.6). Touch items were the least efficient (PI 1.6).

The successful recovery rate of a single source or a major DNA profile increased when items were sampled twice rather than once. No significant increase was obtained when items were sampled more than twice.

Four to five items per case was found to be the optimal number of items to be tested in a case in order to reduce the workload and increase the number of DNA profiles included in the DNA database.

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Forensic science; DNA success rates; laboratory workload; forensic process

Introduction

In the past, the main sources of DNA on case items were body fluid stains such as blood, semen, and saliva (Walsh, Metzger, Higuchi 1991; Walsh et al. 1992). Such stains are easy to detect visually or by using a variety of presumptive tests, usually contain high DNA concentrations and rarely require mixture analysis. In recent years there were technological advances in collection, amplification and interpretation of trace DNA evidence from case-work items such as clothing and touched items (van Oorschot, Ballantyne, and Mitchell 2010). This type of biological material cannot be attributed to a specific body fluid or tissue and is deposited by direct or secondary transfer (Feine, Shpitzen, Roth, and Gafny 2016). Consequently, the workload in DNA casework laboratories increased for three different reasons. First, due to the increase in the recovery potential of items to provide sufficient DNA for amplification, more items are collected at a crime scene and sent to the lab. Second, the number of samples per item increased since the location of trace DNA on an item is unknown. Finally, interpretation of results became more complex due to an increase in the frequency of mixed DNA profiles. The increasing workload in our lab, as in other forensic labs (National Institute of Justice 2006; Peterson, Crim, and Hickman 2005; Nelson 2011; Maguire, Houck, Williams, and Speaker 2012) has caused a significant backlog in case processing.

Several studies examined the DNA yield from different case items, different sampling techniques, the percentage of DNA profile database inclusion and the percentage of cold hits in DNA databases (Castella

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and Mangin 2008; Bond and Hammond 2008; Mapes, Kloosterman, van Marion, and dePoot 2016). However, the data examined in these studies lack an analysis of the amount of work invested in DNA profile recovery. In addition, they did not address the overall success per case.

The aim of this work was to examine ways to manage the increasing workload. Three aspects were examined: (1) DNA profiling efficiency for each evidence item type; (2) number of samples required to obtain a DNA profile from an item; and (3) number of items needed to be examined in each case to obtain a database eligible profile.

To address these aspects, a method was created to calculate the efficiency of DNA profile recovery by estimating the work invested in processing the item in relation to the percentage of successful DNA profile recovery.

Materials and methods

DNA profiling

Case items were sampled by swabbing, cutting or tape lifting according to the lab S.O.Ps. Presumptive tests such as Kastle-Meyer, BlueStar® and Phadebas® (Tobe, Watson, and David 2007; Virkler and Lednev 2009) were performed prior to sampling, when required.

Samples were processed using the following kits and instruments: DNA extraction: AutoMate Express™ Forensic DNA Extraction System combined with PrepFiler Express™ or PrepFiler Express BTA™ chemistries (Applied Biosystems®, CA). Quantification: Quantifiler Duo DNA Quantification Kit (Applied Biosystems®, CA) and GeneAmp PCR System 9700 (Applied Biosystems®, CA). DNA amplification: Powerplex® ESI 16 kit (Promega, WI) in a GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems®, CA). Capillary electrophoresis was performed by the 3500xl Genetic Analyzer or 3130xl Genetic Analyzer (Applied Biosystems®, CA).

Data collection

The data consists of 644 cases and 2016 items processed in our laboratory during the first half of 2013.

Sexual assault, high volume crime, and murder cases have specific considerations in item sampling in our lab, thus can bias results of this work. The laboratory policy in high volume crime cases (burglary and motor vehicle theft) is to limit the item type to blood and saliva only (blood stains, cigarette butts, drinking and food items) and to perform only one attempt with each item to obtain a DNA profile. The processing policy of homicide and sexual assault cases is independent of workload considerations and thus not affected by the results of the present study. Therefore, they were excluded from this dataset. The dataset included crime types such as non-sexual assault, arson, robbery, terrorism, car accidents, drugs, and weapons. These cases mount up to approximately 50% of the total number of cases processed in our laboratory.

Data collected about the processing of case items was summarized and included the following:

- **Type of evidence items**:

The type and number of case items received per case and the number of samples taken from each item. Case items were grouped by type into three categories: body fluids, clothing and touch items (see Table 1).

- **Presumptive tests**: such as Kastle-Meyer (KM), BlueStar, Phadebas (if performed).
- **DNA profile interpretation**:

Table 1. Case-Items Categories

Item category	Items type	Number of items		Number of samples	
Body fluids	Total:	683	(34%)	837	(25%)
	• Cigarettes	152	(22%)	158	(19%)
	• Blood stains	374	(55%)	491	(59%)
	• Saliva items (chewing gum and other Phadebas positive items)	38	(6%)	60	(7%)
	• Drinking items (bottle necks, drinking cans. Not tested for saliva)	119	(17%)	128	(15%)
Clothing items	Total:	268	(13%)	607	(18%)
	• Clothing	85	(32%)	200	(33%)
	• Gloves	95	(35%)	192	(32%)
	• Head covering items (ski masks, helmets, scarfs, etc.)	88	(33%)	215	(35%)
Touch items	Total:	1065	(53%)	1845	(56%)
	• Cellphone	33	(3%)	37	(2%)
	• Cable tie	32	(13%)	35	(2%)
	• Weapons	141	(10%)	469	(25%)
	• Working tools	103	(9%)	150	(8%)
	• Packaging cloth items (bags, socks, and cloth)	93	(14%)	158	(9%)
	• Other (explosive device components, ropes, plastic bags, handles, lighters, etc.)	145	(40%)	301	(16%)
	• Adhesive tapes	422	(5%)	538	(29%)
	• Papers	57	(4%)	115	(6%)
	• Wires	39	(22%)	42	(2%)
Total		2016		3289	

Data obtained from each case item was designated into three categories. (1) A single source or major profile: The number of DNA profiles suitable for DNA database inclusion (a single source or a major profile that has at least 8 STR loci). (2) A mixture: the number of samples that contain a complex mixture profile, which can be used for suspect exclusion only. (3) A negative result: Samples that had insufficient DNA for amplification (less than 250pg) or failed to amplify.

In addition, the total number of profiles submitted to the DNA database for each item and case were counted.

A detailed workflow diagram is presented in Figure 1.

To study the marginal benefit of multiple sampling an effort was made to focus on items with numerous samples. Since the analyzed dataset did not contain an adequate number of items sampled 5 times or more, a second dataset was collected composed of 100 items

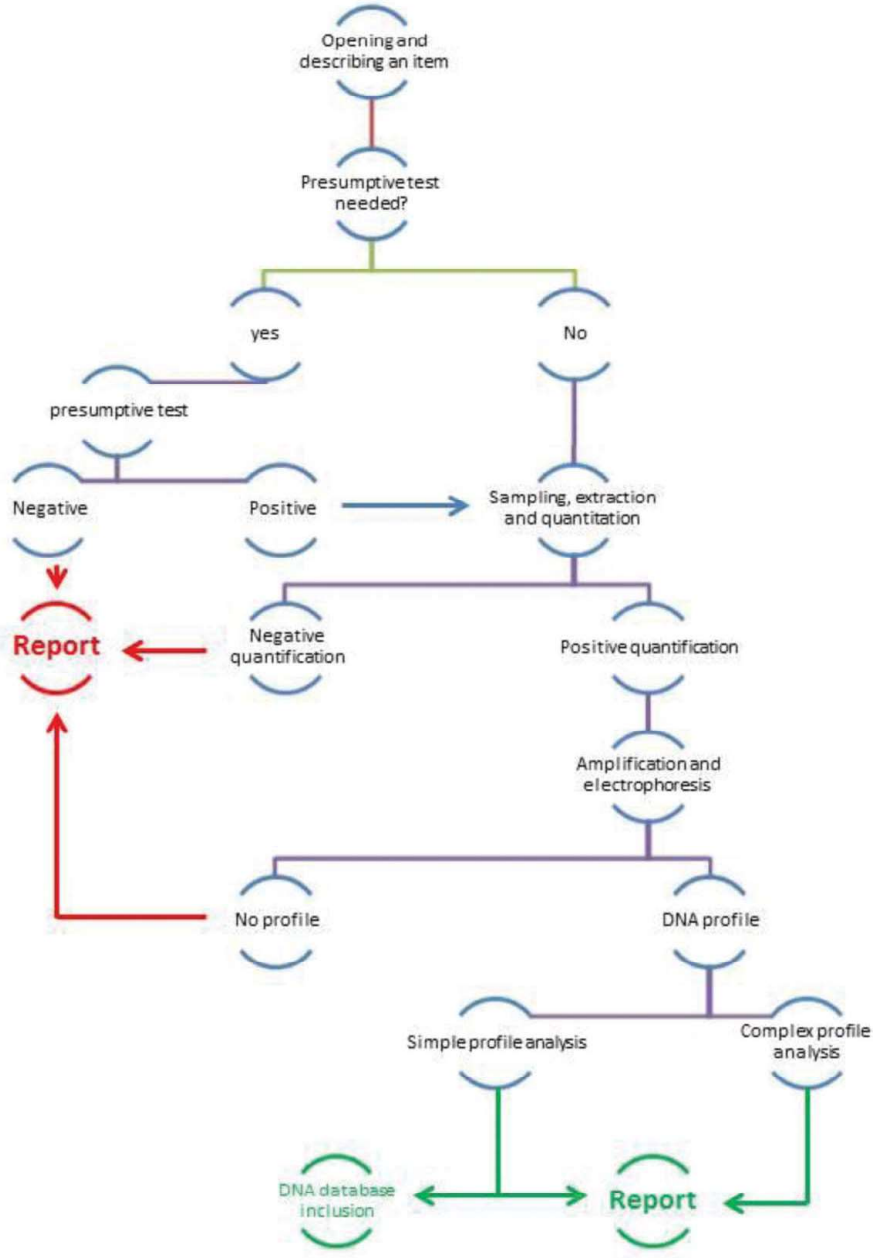

Figure 1. A detailed workflow diagram describing sample processing.

that have been sampled at least 5 times (upon first sampling session).

Productivity index (PI)

To evaluate the DNA profiling efficiency for each item type the DNA forensic testing was approached as a cost benefit system (Speaker 2009), where the benefit is the success rate of an item and the cost is the amount of work invested in testing the item (presumptive tests, sampling, and profile analysis). Success was defined as an item with at least one DNA profile suitable for DNA database inclusion, according to the Israel Police National DNA Database guidelines (a single or major DNA profile with at least 8 STR loci). All profiles included in the database were counted, regardless to whom they belonged. Success rate for each item type was calculated as:

$$\text{Success rate} = (\text{number of successful items}) / (\text{total number of case items}).$$

To calculate a normalized amount of work invested in each item, the actual amount of work on a specific item was calculated relative to the work that needed to be invested in an ideal item. An ideal item is considered an item which was sampled once with 100% success rate providing a DNA profile and without performing any presumptive tests.

The amount of work spent on a specific item was estimated by using an assigned work unit (AWU) scale. To this end, a survey was conducted among 10 analysts in our laboratory. They were asked to evaluate the amount of work required for each step of the DNA profiling process. The results of this survey served as a basis for AWU scale (shown in Table 2).

Table 2. The AWU (Assigned Work Unit) Index.

Work steps	AWU	Multiplication
Opening and describing an item	5	
Blood presumptive test	1	
Saliva presumptive test	5	
Sample extraction and quantitation	3	The number of samples taken
Amplification and electrophoresis	3	The number of samples with DNA
Simple profile analysis	5	The number of samples with single or major DNA profiles
Complex profile analysis	10	Samples with mixed profiles

Notes: for each step of DNA profiling process AWU was defined. In order to calculate the total AWU for each item the AWU for each step multiplied with the multiplication column as described. A cigarette butt for example has 5 AWU for opening plus 3 AWU for sampling extracting and quantitating plus 3 AWU for replicating and electrophoresis and additional 5 AWU for single DNA profile analysis resulting in 16 AWU.

The relative work unit (RWU) is defined as the total AWU calculated for each item divided by 16, the amount of AWU of the most efficient item, which is sampled once with no presumptive tests and provides a profile suitable for DNA database inclusion. For a calculation of a cigarette butt, see notes in Table 2).

As mentioned above, the efficiency for DNA profiling of an item type is the success rate divided by the work invested. Hence, the productivity Index (PI) of an item type was calculated as its success rate divided by the average RWU. To present the data in a scale of 0–10 we multiplied the fraction result by 10, providing the PI (success over work) grade:

$$PI = \frac{success\ rate}{average\ RWU} \times 10$$

In this study only the steps described in Table 2 were considered. These steps are commonly used in forensic labs worldwide. Other aspects influencing the amount of work invested can vary between labs and therefore can significantly influence the PI grade. In order to create a common basis for evaluating the items efficiency between labs, several aspects such as financial costs, report writing and Q.A were excluded.

Results and discussion

The results in this study cover three different aspects: first, the effectiveness of recovering DNA profiles from different case items by assigning a 0–10 PI grade. Second, the effect of number of samples per item on the probability of recovering a DNA profile and finally the optimal number of items sent to the lab per case for efficient DNA profiling.

Productivity index (PI)

Item type success rate

Among items examined in this study, 34% were from the body fluids category, 13% were clothing and 53% were touch items (Table 1).

Table 3 summarizes the success rate for each category; body fluids, clothing and touch item, and for each item type. The low success rate obtained from drinking items (35%) compared to other saliva items such as cigarettes (89%) can be explained by other

Table 3. Summary of DNA profiling results for different items.

Item category	Items type	Samples per item	Negative quantification	Distribution of Amplified Samples			Item Success rate	Relative Work Units	Productivity Index
				DNA profile	Mixture	No profile			
Body fluids	Total:	1.23	16%	93%	5%	2%	80%	1.28	6.2
	• Cigarettes	1.04	9%	97%	1%	1%	89%	1.03	8.6
	• Blood stains	1.31	8%	96%	2%	2%	90%	1.36	6.7
	• Saliva items	1.58	23%	85%	13%	2%	79%	1.83	4.3
	• Drinking items	1.08	53%	68%	21%	10%	35%	1.21	2.9
Clothing items	Total:	2.26	32%	50%	38%	13%	48%	1.79	2.7
	• Clothing	2.35	34%	60%	30%	10%	54%	1.76	3.1
	• Gloves	2.02	45%	45%	37%	18%	39%	1.45	2.7
	• Head covering items	2.44	20%	45%	44%	11%	51%	2.18	2.3
Touch items	Total:	1.73	64%	34%	36%	29%	17%	1.06	1.6
	• Cellphone	1.12	38%	62%	24%	14%	39%	1.00	3.9
	• Cable tie	1.09	57%	53%	33%	13%	25%	0.86	2.9
	• Weapons	3.33	71%	31%	30%	38%	18%	1.09	1.6
	• Working tools	1.46	79%	52%	31%	17%	14%	0.87	1.6
	• Packaging cloth items	1.70	54%	33%	35%	32%	18%	1.24	1.5
	• Other	2.08	52%	25%	46%	30%	19%	1.39	1.4
	• Adhesive tapes	1.27	64%	36%	39%	23%	12%	1.00	1.2
	• Papers	2.02	73%	28%	16%	56%	12%	1.10	1.1
	• Wire	1.08	83%	43%	29%	29%	8%	0.72	1.1

Notes: Sample per items: the average number of samples taken per item. Distribution of Amplified Samples – out of total amplified samples (beyond quantification threshold). Item success rate - the percentage of items from which at least one DNA profile suitable for DNA database inclusion was obtained.

means of use rather than direct contact with the mouth. Thus, some of these items may not actually contain biological material.

Success rate correlated to sample location

As shown in Table 3 the body fluids category has a considerably higher success rate (80%) compared to clothing (48%) and touch items (17%). Similar success rates were reported in a work published by Mapes et al. (2016). There are several explanations for this gap: first, when sampling an item containing body fluids, the location of the biological material is usually known owing to presumptive tests or visual identification of the stain. Second, body fluids are a good source for a single DNA profile compared to the properties of touch DNA (trace amounts and possible multiple donors) (van Oorschot, Ballantyne, and Mitchell 2010; Meakin and Jamieson 2013).

To examine whether prior knowledge of the biological material location on the item influences the success rate, the items were grouped into three categories: known location, assumed location, and unknown location of the biological material.

Figure 2 shows the correlation between the success rate of an item and the prior knowledge of the biological material location. The most successful items were those containing known location of body fluids (90%), i.e., cigarettes buts. With items where the location was assumed (bottle necks for example) the success rate was reduced by more than a half (from 90% to 40%).

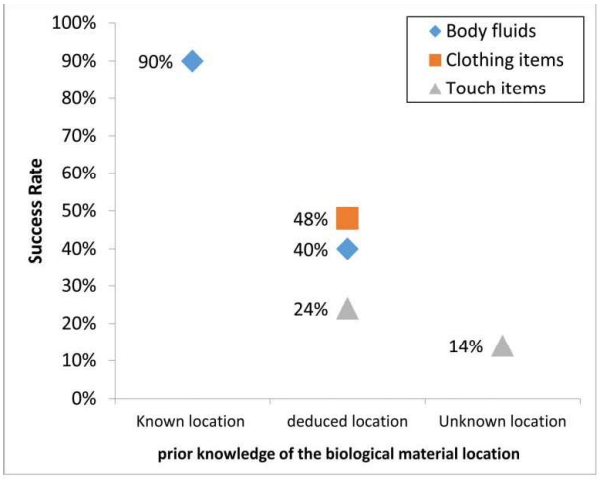

Figure 2. The success rate with body fluids, clothing items and touch items, in relation to the confidence of the biological material location on an item. Known location: the location of the biological material is identified visually or by presumptive tests. Assumed location: the location of the biological material is unknown but can be deduced according to the common use of the item (bottle neck or shirt for example). Unknown location: the location of the biological material is unknown and the sampling area was selected randomly (towels or paper sheets for example).

For touch items for which the biological material location was assumed (tools or weapons), the success rate was 24% but in cases where the location was unknown (cloth, towels, etc.) the items were sampled randomly and the success rate was reduced to 14%.

For clothing items, the location of the biological material is always deduced based on the examiner's experience and common sense (van Oorschot, Ballantyne, and Mitchell 2010). The success rate differences in the assumed category between body fluids (40%) clothing items (48%) and touch items (24%) might be explained by the DNA quantity deposited on those items.

Factors impacting the amount of work invested

When examining the efficiency of DNA profiling per item type, the success rate is not enough. One should also consider the work required to receive a meaningful result. In order to quantify the work invested in an item, the AWU and RWU measures were calculated, as detailed in the Materials and methods section and Table 2.

Several steps of DNA profiling have similar AWU score for all items (i.e., opening and describing an item) and therefore have no significant effect on their RWU score. Two of the steps have a major impact on the RWU score. First, the number of samples taken from each item varies according to the items properties. The more samples taken, the higher is the AWU score and consequently the RWU score increases. Second, the profile complexity influences the work invested during the expert analysis. For example, the analysis of complex mixtures requires more work than a single profile as reflected by the AWU score; the more complex mixtures obtained the higher the RWU score is.

The average number of samples per item

The average number of samples per item type is shown in Table 3. Several factors influence the number of samples taken from an item. The morphology and size of an item dictates the number of potential samples; some items such as a button can be sampled only once and others such as shirts can potentially be sampled multiple times from different locations. Prior knowledge of the biological material location can also affect the number of samples. Cigarettes have a well-defined location of the biological material and therefore have an average of one sample per item. Weapons on the other hand, are sampled on average more than three times since the location of the biological material is uncertain.

Beyond the considerations mentioned above, other factors may influence the number of samples taken from each item category. For instance, the forensic circumstances; the number of individuals suspected of touching an item and other evidence, such as security camera video, pointing to a specific location on the item where the suspect's DNA might be found. In addition, the number of samples taken from an item is dependent on the expert's experience and judgment.

Sample quantification and genotyping

Body fluids stains usually originate from a single DNA donor, which explains the low mixture percentage (5%; see Table 3). Touch items on the other hand, are often used by multiple individuals and contain a higher percentage of complex mixtures (36%).

Negative quantification results prevent the downstream DNA profiling processing steps (see Table 3), thus reducing the RWU score of an item. Adhesive tapes and blood stains had a similar number of samples per item (1.27 and 1.31, respectively) and a mixture percentage of 39% and 2%, respectively. The difference between the RWU score for those items (1 and 1.36, respectively) can be explained by the significant difference of their negative quantification results (64% and 8% respectively), which lead to many adhesive tape samples not being amplified, thus requiring less work.

The efficiency of DNA recovery from case items (PI)

As discussed above, the PI grade takes into account the success rate and the amount of work invested (RWU) and reflects the cost benefit ratio, thus providing an assessment tool of items efficiency. Considering the success rate as the only parameter for assessing efficiency of items can be misleading. As shown in our results (Table 3), the success rate alone does not explain the variation of the PI score.

As expected, body fluids which have a high success rate were the most efficient items (6.2 PI) while clothing and touch items were less efficient (2.7 and 1.6, respectively).

Cigarettes, which have a high success rate (89%) and a 1.03 RWU (1 sample per item in average, 1%

mixtures, and do not require presumptive tests), are the most efficient items with 8.6 PI. Blood stains which had the highest success rate (90%) had a lower PI (6.7), due to an average of 1.3 samples per item and the need for presumptive tests which increase the amount of RWU invested.

Drinking items (i.e., bottle necks and drinking cans) have a low PI grade (2.9) as a result of their low success rate (35%) and a 1.21 RWU score. Saliva items (i.e., chewing gum and other Phadebas positive items), on the other hand, have a high success rate (79%) yet their PI grade is only 4.3 due to a relative high RWU score (1.83). This score stems from the need of saliva presumptive test (Phadebas) and other factors detailed in Table 3.

Clothing category which has a 48% success rate had only 2.7 PI grade. These items are routinely sampled more than twice (2.3 in average) since the biological material location is deduced. The mixture percentage of these items is substantially higher compared to body fluids (38% and 5%, respectively) since they can be used by multiple individuals. As a result, the RWU increases to 1.8, decreasing the PI.

Touch items have a high percentage of negative quantification results (64%) as reflected by a relatively low RWU (1.06). Combined with a low success rate (17%) their PI grade (1.6) is the lowest among all items type categories.

Cellphones and cable ties have different usage characteristics than other touch items. Cellphones are repeatedly used and may contain saliva traces. Cable ties are often used to restrain a victim thus increasing the probability of obtaining a DNA profile. Therefore, their PI grade (3.9 and 2.9, respectively) stand out compared to grades among other touch item category (1.1–1.6). However, it should be noted that in the majority of cases involving cable ties, the victim profile would be the evidentiary significant profile.

Number of samples per case item

When sampling case items, the examiner has to decide how many times to sample each case item. Here the marginal benefit of each additional sample to the probability of obtaining at least one DNA profile suitable for DNA database inclusion was examined.

To this end, a dataset of 100 case-items which were sampled at least five times (Figure 3) was analyzed. A single sample from a case-item results in a success rate

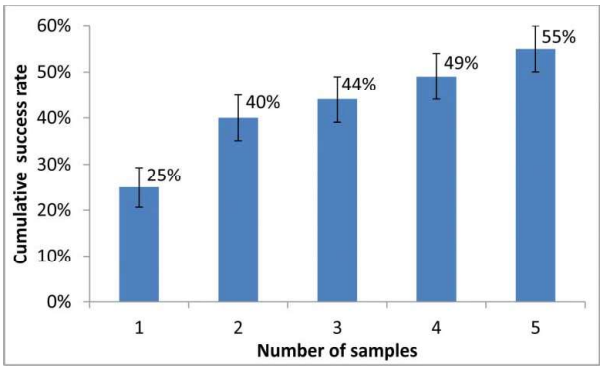

Figure 3. The cumulative rate of recovery of at least one DNA profile suitable for DNA database inclusion as a function of the number of samples taken from a case-item (n = 100 in each group). The error bars represents a 0.9 a confidence interval for the estimate of success proportion.

of 25%. The addition of a second sample increased the success rate by 15%. Adding a third, fourth, or fifth sample moderately increased the success rate (by approximately 5% each).

The decrease in the marginal benefit beyond two samples indicates that additional samples beyond two have a low chance of producing a profile. This may be explained by the distribution pattern of DNA on different case items. If sufficient DNA is present throughout a case item, success is expected within first few samples. On the other hand, items with minute amounts of DNA or with DNA concentrated in small, unknown locations are not expected to produce a successful profile even after numerous samples. These results are applicable only when a single DNA contributor is sought after. When DNA of more than one individual is expected on the item (according to the case information), more samples are required to increase chances of obtaining two or more different DNA profiles.

Obviously, each additional sample requires additional work to process and analyze so as the marginal contribution to success of each additional sample decreases, the efficiency (as reflected by the PI grade) decreases as well.

Number of items per case

The previous sections dealt with efficiency per case-item. Ultimately, the aim of the forensic examination is to produce sufficient evidence in each case as a whole. Therefore, in this section a success of a case is defined by the number of different profiles submitted to the DNA database.

To reduce the overall workload, it is important to examine the efficiency of case processing. The number of items sent to the DNA laboratory varies greatly between cases. In our dataset, 35.4% of the cases had 1 item, 32.1% had between 2–3 items, 12.1% had between 4–5 items, 11.5% had between 6–9 items, 7.3% had between 10–20 items, and 1.6% had more than 21 items. As the number of items rises, the number of different profiles added to the database (NDP – number of different profiles) increases moderately while the rise in the RWU is more significant (Figure 4). The RWU increase is constant since each additional item adds a similar amount of work. As more items are examined, the possibility of receiving at least one profile increases while the second profile has a higher probability to be identical to the first one. Therefore, the probability of obtaining different profiles increases with the number of tested items until reaching a plateau at the 6–7 item group.

In order to reduce the overall workload in our lab the optimal number of items for examination per case was searched by calculating the ratio between the NDP and the RWU. This ratio represents the cost benefit between the total amount of work invested in a case (RWU) and the number of different profiles added to the database (NDP). The ratio increases until reaching a peak at the 4–5 item group and then declines with the addition of items.

Our results indicate that testing more than 5 items per case is unproductive and increases the overall workload and thus should be limited. This limitation will encourage the case investigators to prioritize the items submitted to the laboratory according to their relevance and success potential as previously suggested (Mapes et al. 2016). As a result, the laboratory workload will decrease and resources will be freed to handle more cases. In a situation where the investigators are not limited to a certain number of items per case, irrelevant case-items are submitted but returned without examination (17% of all received items in the dataset analyzed in this work, data not shown).

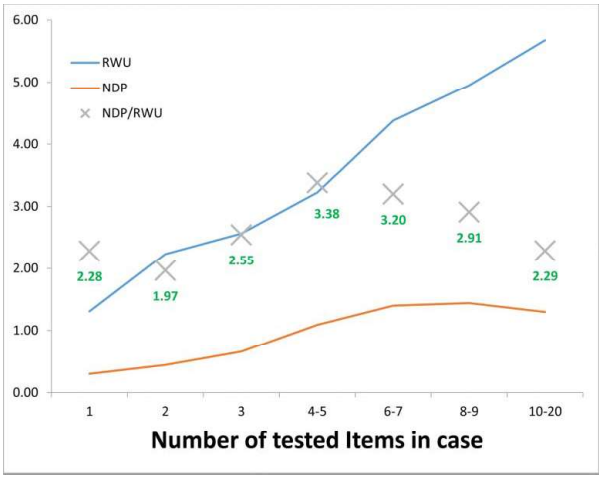

Figure 4. RWU and NDP as a function of the number of tested items per case. RWU – relative working units. NDP – Number of Different Profiles submitted to the database. The dataset consists of 463 profiles obtained from 644 cases.

Conclusions

In order to reduce the workload in forensic DNA labs we recommend the following guidelines:

1. Prioritizing items based on efficiency criteria, such as the PI proposed here. This criterion takes both the work investment and the success rate into consideration.
2. Limiting the number of samples per item. In general, the benefit from additional samples beyond two samples per items is fairly low.
3. Limiting the number of items sent per case. Our results indicate that limiting the number of items to 4–5 does not substantially reduce the number of profiles included in the DNA database. Investigative units should be encouraged to send the items based on their relevance and efficiency (PI).

In each case the investigator should consider not only these recommendations but also the unique circumstances of the case such as the number of suspects and the relevance of each item to the forensic question, as well as the severity of the investigated crime.

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