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Evaluation of the Efficacy of a Post-Extraction Concentration Step Using the Microcon® Centrifugal Filter Devices in Yielding DNA Profile Intelligence.

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Project Proposal #184 Evaluation of the Efficacy of a Post-Extraction Concentration Step Using the Microcon® Centrifugal Filter Devices in Yielding DNA Profile Intelligence.

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1. Abstract

All samples that underwent a Microcon® process were evaluated and categorised into whether there was meaningful information obtained or not. This evaluation primarily focussed on samples that underwent an 'auto-microcon' process in 2016. The results suggest there to be arguably minimal value in performing the 'auto-microcon' process due to the limited meaningful DNA Intelligence obtained from these samples. Given this, further streamlining of workflow processes could be implemented that would provide significant efficiencies such that these efforts could be better placed in processing higher DNA-yielding samples.

2. Definitions

DNA Profile Intelligence: DNA profile information available for interpretation by Forensic DNA practitioners that is able to be provided to clients.

Fail: In this report, this is DNA profile information that was not suitable for comparing to reference DNA profiles. This word was used to filter the data into two possible outcomes (fail/success).

NCIDD: National Criminal Investigation DNA Database.

QPS: Queensland Police Service.

Success: In this report, this is DNA profile information that was obtained that was suitable for comparing to reference DNA profiles. This word was used to filter the data into two possible outcomes (fail/success).

3. Introduction

Microcon® Centrifugal Filter Devices desalt and concentrate macromolecular solutions such as DNA-containing solutions. They employ Amicon's low binding, anisotropic, hydrophilic regenerated cellulose membrane [1].

The use of Microcon® filters to concentrate extract has been a standard post-extraction process within Forensic DNA Analysis to reduce the volume of extract from approximately 100uL to ≤20µL for amplification with AmpFℓSTR® Profiler Plus®, and to ≤35µL for amplification with PowerPlex® 21 system (PP21).

Since the implementation of PP21 amplification kit within Forensic DNA Analysis for casework samples in December 2012, extracts with low Quantification values were recommended to be concentrated. Templates of

<0.132ng were found to exhibit marked stochastic effects after amplification [2]. Consequently, a workflow that directed extracts automatically to a concentration step based on Quantification value was implemented ('auto-microcon' process).

Anecdotally, the suitability to provide QPS with DNA profile Intelligence from extracts that have been concentrated has been noted to be limited. Furthermore, extracts that are of low quant value that have been automatically concentrated have been observed to rarely yield DNA information for QPS.

NB. Project #163 – *Assessment of results obtained from 'automatic-microcon' samples* [3] was conducted to evaluate the results of samples that were processed with the 'auto-microcon' process. A recommendation of this project was to re-evaluate after the introduction of the Forensic Register in conjunction with the use of Quantifiler® Trio DNA Quantification Kit.

This recommendation was based on the perceived ease of retrieving data from the FR as opposed to AUSLAB, and with the thought that the FR would soon be implemented. For the purposes of this project, it is not considered essential to have the FR implemented if the data can be retrieved from AUSLAB. However, it is considered important that the data be spanning a sufficient period of processing, and be based on the same Quantification system namely the Quantifiler® Trio DNA Quantification Kit.

The purpose of this project is to evaluate the suitability for interpretation of DNA profiles that may be obtained after the post-extraction concentration step using the Microcon® centrifugal filter devices. This evaluation includes an assessment of those samples that underwent the 'auto-microcon' process. This evaluation is based on a data mine of extracts in the year 2016 that were concentrated with Microcon® centrifugal filter devices, and assesses the 'suitability' of PP21 profile outcomes as a function of quant values obtained from using the Quantifiler® Trio DNA Quantification Kit.

This evaluation looks at two data sets as a function of the Quantification value:

1. PP21 DNA profile outcomes from extracts that were processed through the 'auto-microcon' process;

2. PP21 DNA profile outcomes from all extracts that were concentrated with the Microcon® filter devices.

4. Resources

The following resources were required for this validation/project:

Forensic DNA Analysis staff and computer time to retrieve data from AUSLAB and to use Microsoft Excel.

5. Methods

5.1. Data retrieval from AUSLAB (LIMS)

Data was retrieved from AUSLAB using Extended Enquiries. Data was searched for samples that had a testcode of 'XPLEX' and 'MCONC1' ordered in the year 2016 in Forensic DNA Analysis. These were High Priority (P2) samples.

The data was output with the corresponding Quantification value and the reported DNA profile interpretation (Exhibit Report Line in the Exhibit Report (EXH)) for that particular barcode. If the barcode was a sub-sample, the corresponding EXH line for the sub-sample was output.

For ease of data interrogation, the RAW data (I:\Change Management\Proposal#184 - Evaluation of the efficacy of Microcons\Data\RAW Data from AUSLAB) had a column added to describe whether the sample underwent the 'auto-microcon' process ('AUTO' = 0.001ng/μL<Quant <0.0088ng/μL) or not ('MANUAL' = Quant >0.0088ng/μL). Another column was added to describe whether there was a Quantification value returned in the data collation ('TRUE' = Quant value obtained), or not ('FALSE' = no Quant value obtained (ie. 0 ng/μL).

The data excluded samples that had not returned a DNA profile result, Quality samples (including environmental monitoring samples), have no quant value in the data export, or have quality issues noted.

5.2. Data interrogation

The data was interrogated by assessing the DNA profile outcome results reported as Exhibit Report lines as a function of the Quantification value.

The Exhibit lines were interrogated and grouped into two interpretation outcomes as follows:

1. 'Fail': DNA profile interpretation outcomes of 'Complex unsuitable for interpretation', 'No DNA profile', 'Partial unsuitable for interpretation', 'No DNA Detected';

2. 'Success': All other DNA profile outcomes including single source DNA profiles matching assumed known contributors or different reference DNA profiles, mixtures that were suitable for comparison to reference DNA profiles, DNA profiles that were suitable for loading to NCIDD.

NB. These descriptions were used to filter the data. A 'fail' does not mean there was a Quality failure in the process; a 'success' does not necessarily mean a DNA match.

6. Experimental Design

6.1. Experiment 1: Assessment of 'auto-microcon' results

Intent
Evaluate the 'success' or 'fail' outcomes for PP21 samples that were processed in 2016 through the 'auto-microcon' workflow.

Data Analysis
The samples applicable to this experiment had Quantification values in the range 0.001ng/μL to 0.0088ng/μL, and a total number of samples that were processed this way was determined. This total number excluded environmental samples, samples without Quantification values, samples not requested for further work, samples where quality flags were raised, and samples that had not returned results at the time of data collection.

DNA profile interpretation outcomes were grouped into either 'success' or 'fail' as a function of the Quantification value. A percentage of samples that fell into these categories was determined.

The 'auto-microcon' data could be expressed as a function of Quantification value.

The percentage of samples that had an 'auto-microcon' process and led to an NCIDD upload was obtained. This data could be filtered further into the outcome from the NCIDD load, at the time of data collection.

Data on the DNA profile outcomes for various suspected biological types was obtained. Furthermore, data on the profile outcomes for various substrate types was obtained.

6.2. Experiment 2: Assessment of all DNA profile results from extracts that have had a concentration step.

Intent
Evaluate the 'success' or 'fail' outcomes for PP21 samples that were processed in 2016 and underwent a post-extraction concentration step using Microcon® centrifugal filter devices.

Data Analysis

The samples that were applicable to this experiment had Quantification values above 0.001ng/μL, and underwent the Microcon® process. This included the 'auto-microcon' samples, and those that had a Microcon® rework performed (termed 'manual'). This combination of data was termed 'combined data'.

A total number of samples that were processed this way was determined. This total number excluded environmental samples, samples without Quantification values, samples not requested for further work, samples where quality flags were raised, and samples that had not returned results at the time of data collection.

DNA profile interpretation outcomes were grouped into either 'success' or 'fail' as a function of the Quantification value.

The percentage of samples that fell into these categories ('manual' and 'combined') was determined. 'Manual' referred to the samples beyond the 'auto-microcon' range that were reworked with the Microcon® process, and 'combined' referred to all samples ('auto-microcon' and 'manual').

There was a point where the number of 'success' samples was approximately the same as the number of 'fail' samples when the Microcon® process was performed. This appeared to be approximately Quant = 0.02ng/uL. Therefore, the data was interrogated further at a Quantification value lower than this mark to determine what percentage of samples in certain ranges led to DNA profile interpretation outcomes of 'success'.

From this data, a sub-section of samples was interrogated further to evaluate the effect on DNA Intelligence that was obtained. A range of samples with Quantification range up to 0.015ng/uL was chosen and a total number of samples was determined. This Quantification value was chosen as it was the approximate value where all samples below this value that underwent a Microcon® process, led to an approximate, round figure of 85% 'failure'.

The percentage of samples that were in this Quantification range and led to an NCIDD upload was determined. This data could be filtered further into the outcome from the NCIDD load. This data could then be used to evaluate the

potential for samples to not provide meaningful DNA Intelligence to QPS if the Microcon® process was re-defined in some way. By 'meaningful DNA Intelligence', this means DNA profile information that can be provided to the client that could lead to an identification of a person potentially associated to the alleged matter.

6.3. Experiment 3: Datamine of the difference in pre- and post-Microcon® Quantification values

Intent
Evaluate the difference between the values obtained from the Quantification process in samples that have had a Microcon® concentration step applied.

As this is purely a datamining experiment, only the samples that have yielded a result of 'success' was examined.

Data Analysis

The samples applicable to this experiment had Quantification values above 0.001ng/μL where the final result was 'success'.

The range was further refined as per Section 5.2, such that samples that had Quantification values between 0.001ng/μL and 0.015ng/μL were examined.

This range was considered by the author to be able to provide a sufficient demonstration of the trend of the data.

7. Results and Discussion

7.1 Assessment of 'auto-microcon' results

For samples in the 'auto-microcon' Quantification range, the total number of samples that were processed this way (excluding certain samples as per Section 5.1) was N= 1449 samples.

The percentage of samples that resulted in a determination of 'fail' was 89.4% (Fig 1). As expected, the number of 'fails' increased when the Quantification decreased and approached the Limit of Detection of Quantification ie. 0.001ng/uL (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

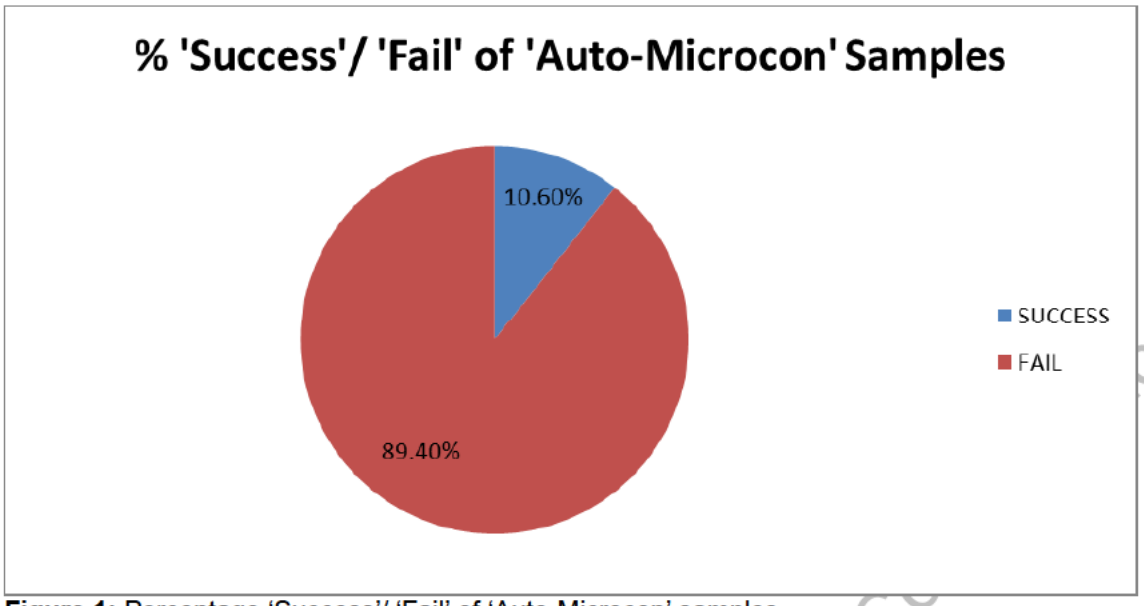

Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

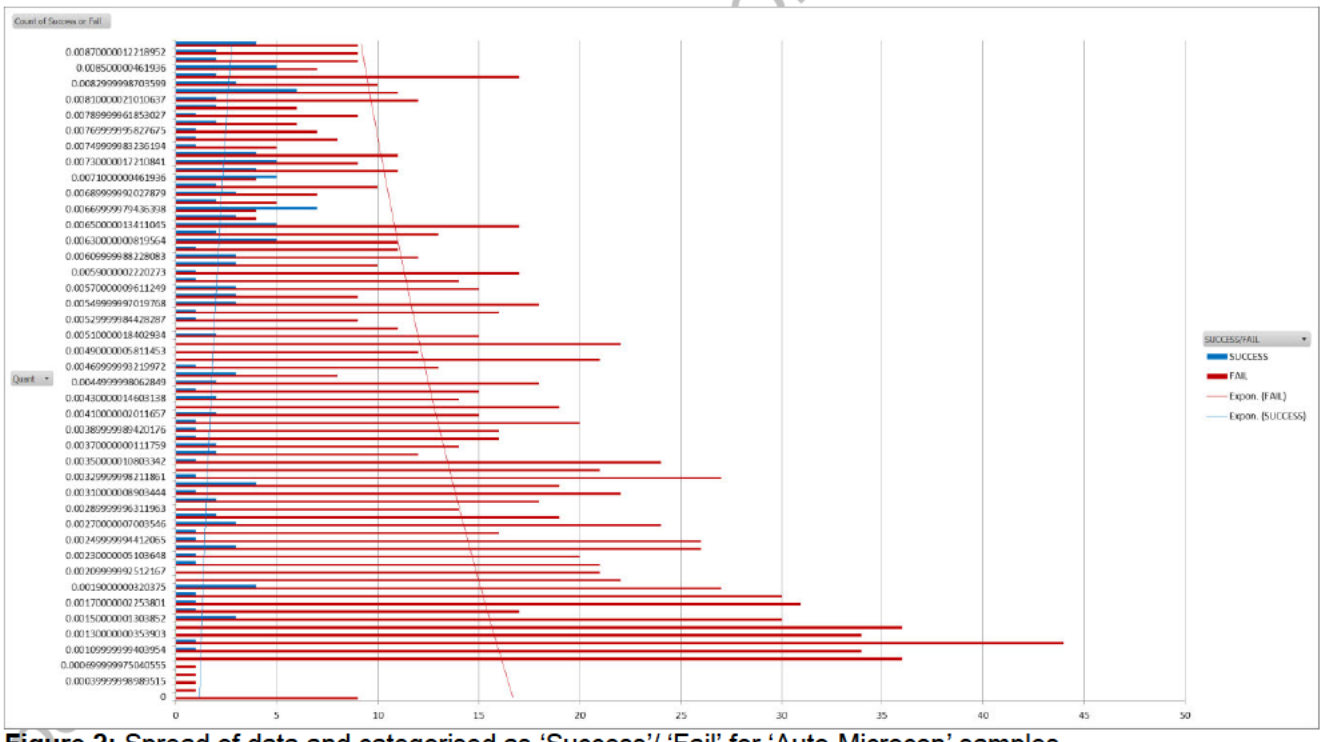

Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

If samples were not processed through the 'auto-microcon' process, what DNA Intelligence would the client miss out on? To evaluate this, the 'success' data was drilled down to the samples that had some NCIDD interaction and in particular, where they were the only samples in the case that were NCIDD-suitable for that particular profile (Fig 3). This represented 1.86% of all 'auto-microcon' samples. In looking at samples that provide *new* Intelligence, that is

DNA information available for future linking, or has provided a cold-link, this equated to 1.45% of all 'auto-microcon' samples.

This 1.45% of samples would be the pertinent value for the client to consider if the 'auto-microcon' process was not performed. In considering this, it would be important to evaluate the time and cost for processing, and the opportunity to concentrate efforts on other higher yielding samples. In saying this, with the ease of communication through the Forensic Register, these samples could process if the client has no other Forensic Intelligence assisting the matter, or if the item is considered to be of critical priority.

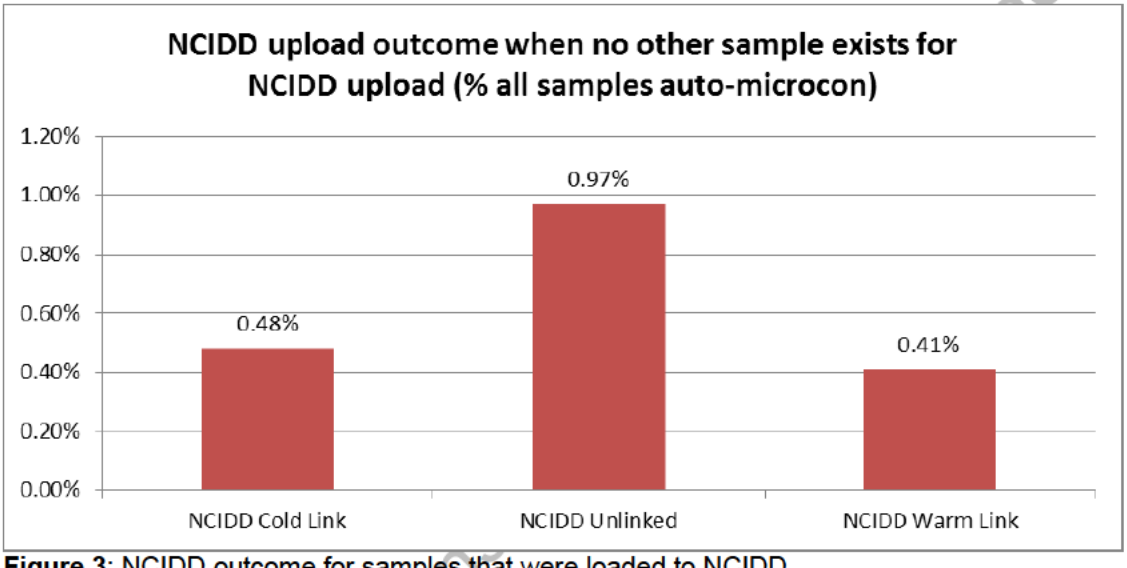

Figure 3: NCIDD outcome for samples that were loaded to NCIDD

The 'success' data was further evaluated to see if any particular substrate type or possible biological source, was more likely to lead to meaningful interpretations after an 'auto-microcon'. The data set for this evaluation was N=154 samples. These samples were broken down into three general interpretation outcomes:

- Profiles matching assumed known contributors. These were either single source DNA profiles, or mixed DNA profiles where the profile was conditioned with no information available for comparison in the remaining contribution (ie. peaks visible sub-threshold or the profile has allelic imbalance suggesting a mixture);

- Single source. These were DNA profiles that were attributed to unknowns, or matched reference DNA profiles, or were from items where ownership could not be confirmed; and,

- Mixtures where no statistical interpretation (NSIP) was performed or were suitable for comparison to reference DNA profiles for Likelihood Ratio (LR) purposes.

Figures 4 displays the DNA profile outcome as a function of the possible biological type, and Figure 5 displays the DNA profile outcome as a function of the substrate.

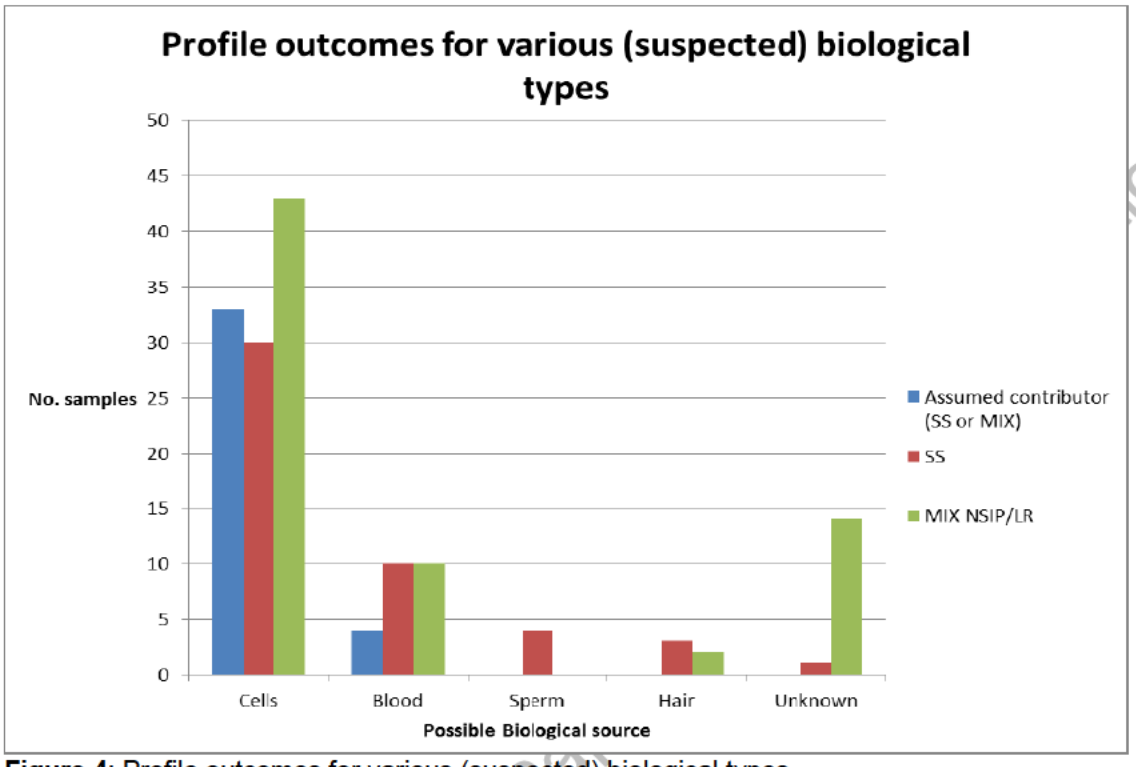

Figure 4: Profile outcomes for various (suspected) biological types

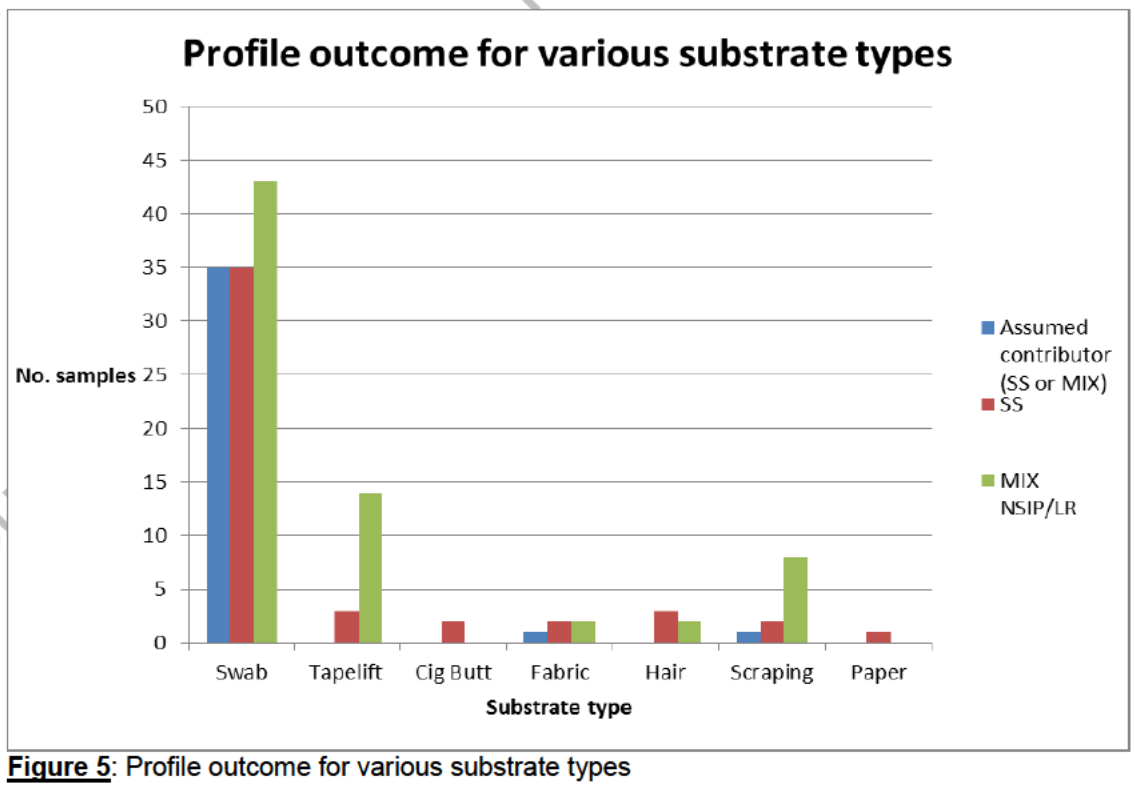

Figure 5: Profile outcome for various substrate types

Figures 4 and 5 show that there do not appear to be any obvious trends in the data. It is not unexpected to have a variety of DNA profile outcomes for different biological source types, and not unexpected for a variety of DNA profile outcomes for different substrate types. Interestingly, the number of 'assumed known contributors' is almost one-third of DNA profile outcomes for the most numerous suspected biological type (cells), and substrate type (swab). It could be argued that this DNA profile outcome is not meaningful to the client as the results are not unexpected.

What this means is that if the client requested a Microcon® process on a particular sample that was initially in the 'auto-microcon' Quantification range, there does not appear to be a predictive element to the likely success of the microcon rework for a particular biological source type, nor substrate type.

Ultimately, for approximately 90% of samples that underwent an 'auto-microcon' process, there is arguably negligible DNA profile Intelligence for the client. If the 'auto-microcon' was not applied as a streamlining strategy, there would be the following advantages, including but not limited to:

-the potential to make available at least 1449 processing positions for other samples including further available positions that would have been used for reworks. It must be noted that it is not unusual for low-quantification samples to reworked further before determining if the profile is suitable for comparison to reference DNA profiles.

-the lack of a need for the considerable efforts required to prepare and process Microcon® (and further rework) batches for this number of samples,

-consumable and labour savings in the end-to-end processing of these samples, and

-time and effort could be redirected in the laboratory workflow to other activities including service extensions like Y-STR profiling.

7.2 Assessment of all DNA profile results from extracts that have had a concentration step.

All samples from 2016 that had a Microcon® process were determined. The total number of samples was N= 2201 samples, excluding certain samples as per Section 5.1.

The percentage of samples that resulted in a determination of 'fail' was 78.5% (see Fig 6). As expected, in looking at the spread of the 'combined' data, the number of 'successes' increased when the Quantification increased (Fig 7).

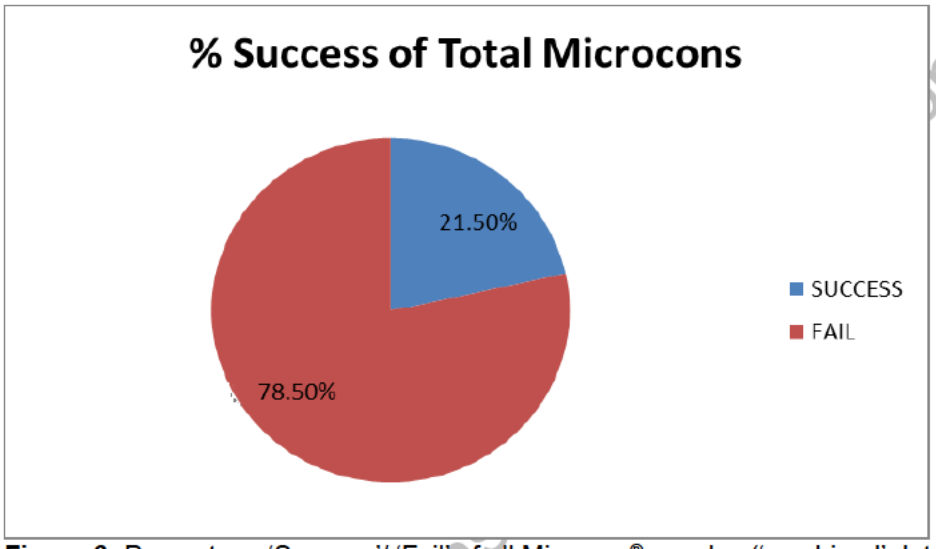

Figure 6: Percentage 'Success'/ 'Fail' of all Microcon®samples ('combined' data).

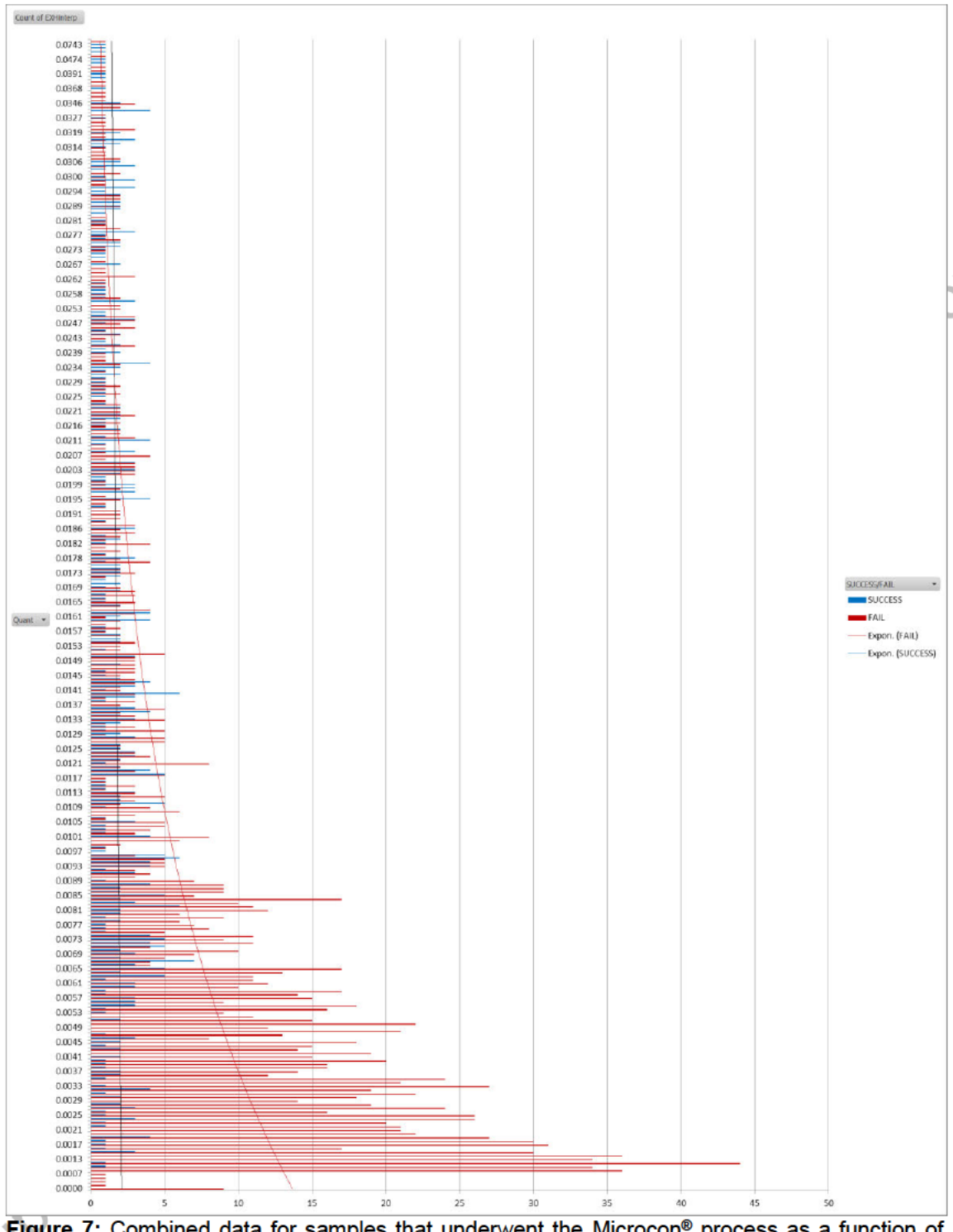

Figure 7: Combined data for samples that underwent the Microcon® process as a function of Quantification value.

As mentioned in Section 5.2, the Quantification value where there was roughly the same number of 'success' and 'fail' samples was approximately 0.02ng/uL. It must be noted that this is a rough estimate *at this* particular Quantification value, and it is based on limited samples that returned that Quantification value. It can be argued that taking a range of Quantification values to look at the overall success/fail percentages could provide the client with approximate likelihoods of obtaining meaningful DNA Intelligence.

A number of ranges were looked at to determine the percentage 'success' of samples with Quantification values in various ranges (Fig 8). The ranges were established up to the highest Quantification value of 0.02ng/uL. As expected, the percentage 'success' increased as the Quantification increased due to the higher amount of DNA in the extract available to be concentrated.

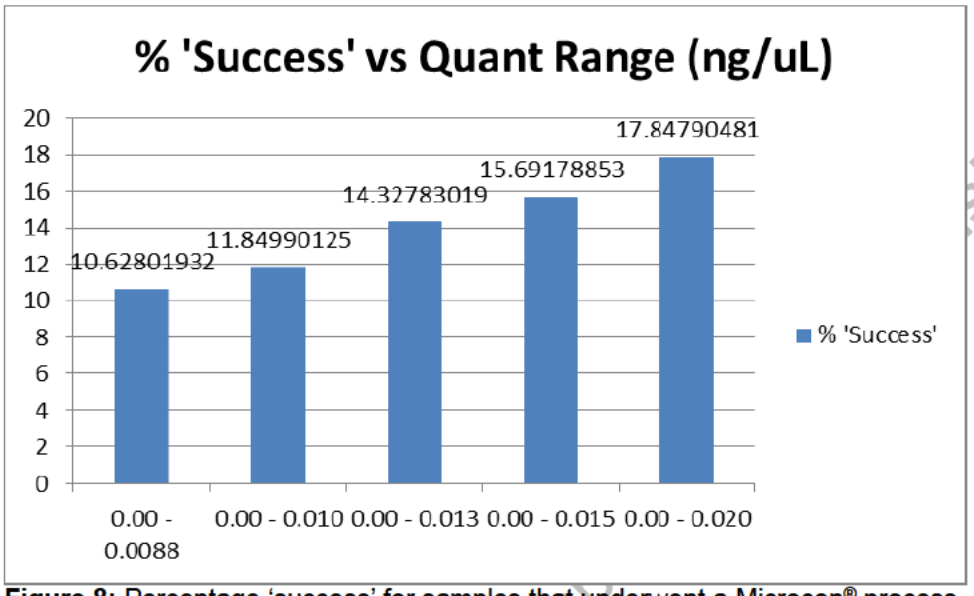

Figure 8: Percentage 'success' for samples that underwent a Microcon® process

In viewing the data in Fig 8, a limitation is that all samples that fell in the 'auto-microcon' range, had a Microcon® process performed, whereas there are samples that are in higher Quantification ranges that might not have required a Microcon® concentration rework step to yield useful DNA profiles. These samples were not evaluated.

A lower Quantification value to where the number of 'successes' roughly equalled the 'failures' was chosen to be the upper end of data ranges that were evaluated further. The value chosen was 0.015ng/uL. Table 1 and Figure 9 describe the risk to NCIDD upload for samples in these ranges if Microcon® concentration steps were not performed.

Table 1: NCIDD outcome for samples that were loaded to NCIDD in various Quant ranges

	% No other samples to Upload in Quantification ranges (Q)		
	Q = 0.00ng/uL to 0 01ng/uL (total samples in range = 1519)	Q = 0.00ng/uL to 0 0133ng/uL (total samples in range = 1696)	Q = 0.00ng/uL to 0.015ng/uL (total samples in range = 1778)
NCIDD Cold link	0.92	0 88	1.01
NCIDD Unlinked	0.53	0.77	1.24
NCIDD Warm Link	0.46	0 83	0.90

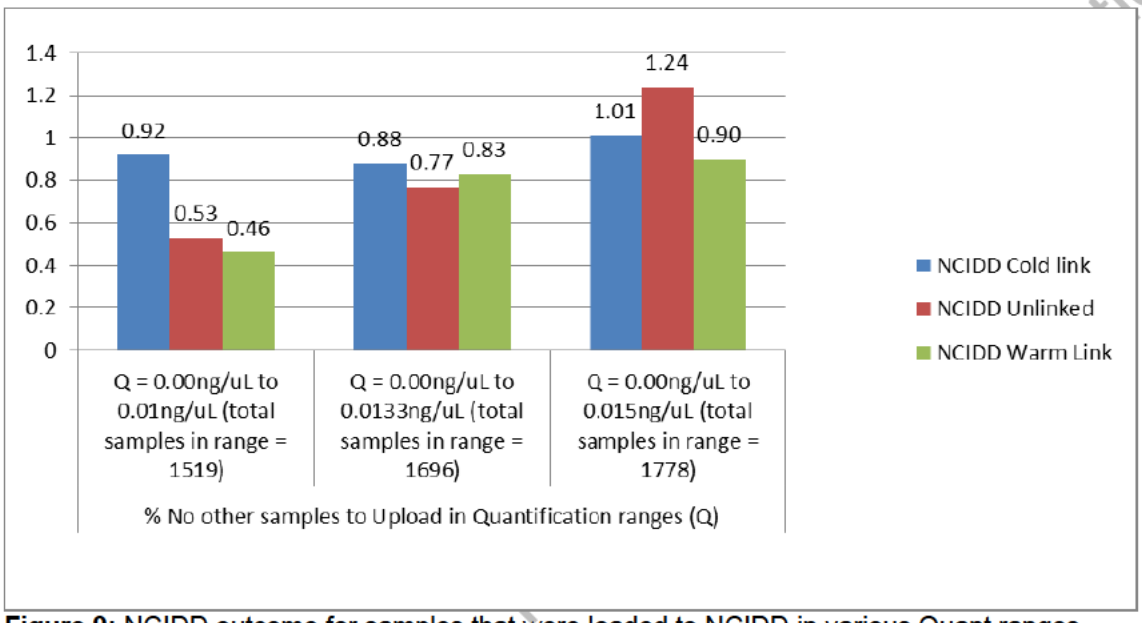

Figure 9: NCIDD outcome for samples that were loaded to NCIDD in various Quant ranges

Approximately 1.45% of samples in the Quantification range up to 0.01ng/uL resulted in 'new' DNA Intelligence. This percentage is the same as that found in the 'auto-microcon' range. This percentage increased to 1.65% and 2.25% for the Quantification ranges up to 0.0133ng/uL and 0.015ng/uL respectively.

7.3 Datamine of the difference in pre- and post- Microcon® Quantification values

The samples applicable to this experiment had Quantification values above 0.001ng/µL where the final result was 'success'. The range was further refined as per Section 5.2, such that samples that had Quantification values between 0.001ng/µL and 0.015ng/µL were examined.

As the Microcon® process concentrates the DNA extract from approximately 100uL to approximately 35uL, in theory it would be a reasonable expectation to obtain approximately two to three-fold increases in DNA Quantification after

concentration. Figure 10 shows the plot of the differences found for samples that resulted in 'success'.

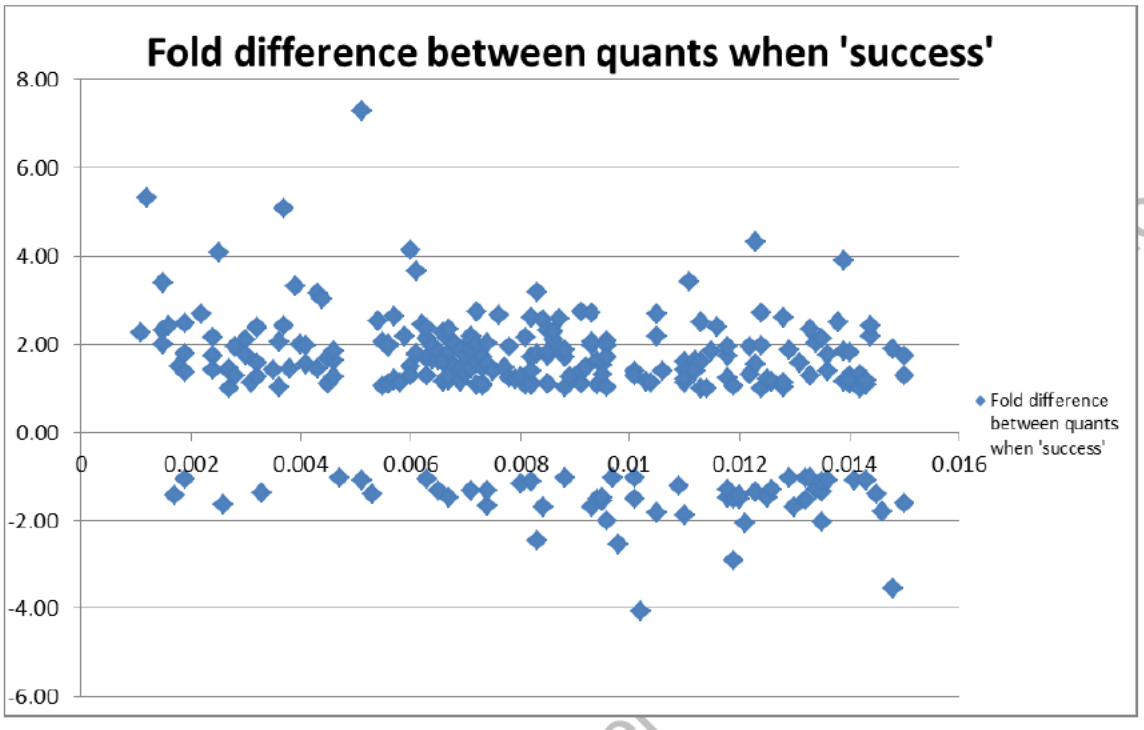

Figure 10: Quantification differences pre and post concentration

The findings are not unexpected as the scatter focusses mostly around two-fold increases in Quantification. It was also not unexpected to observe the variable results. Anecdotally, variability in success rates is found at profile management stage when assessing results of samples that have had this concentration step.

DNA can be lost in the process as seen in Fig 10 where the Quantification values decreased after concentration. Variability in results could be attributed to a number of things, including but not limited to the slight differences between operators and instrumentation, the differences in substrate type and level of degradation, and the variability in Quantification result.

8. Conclusion and Recommendations

The data analysis demonstrated that there was arguably minimal value in performing the 'auto-microcon' concentration step. This opinion was formed by analysing the data from 2016 where it was found that for all samples that underwent the 'auto-microcon' step, 89% did not yield results suitable for meaningful interpretation (or 'success' in this report).

It was found that in considering *all* samples that underwent a Microcon® step at some stage in 2016, 78.5% did not yield results suitable for meaningful interpretation. As expected, when the Quantification value increased, the percentage of meaningful results increased. However, it was also demonstrated in the data analysis that the Quantification values did not always improve after Microcon®, but where they did, the magnitude of change was roughly equivalent to the change in volume (from neat to concentrated sample).

Based on the data analysis, the following recommendations are offered:

1. Cease 'auto-microcon' (Quant range: 0.001ng/uL to 0.0088ng/uL) processing for all samples of Priority 2 and 3 requested to be amplified with PowerPlex 21, with the following exceptions:

    a. Priority 1 samples (Critical Priority); and

    b. Coronial/DVI samples where profiles are mostly single-source. Quite often incomplete profiles may be enough to provide Intelligence on possible identity.

2. Automatically send result information via the Forensic Register to QPS at Quantification stage for samples in the Quant range: 0.001ng/uL to 0.0088ng/uL. This result information is recommended to be the exhibit result line of 'DNA Insufficient for Further Processing'. This recommendation is an extension to the current 'No DNA Detected' process, which looks at Priority 2 samples yielding Quantification results of less than the Limit of Detection (0.001ng/uL).

3. After a six month period of processing, re-analyse samples that have had a Microcon® process performed and were in the initial Quantification range greater than 0.0088ng/uL, to evaluate whether the range from Recommendation 1 can be extended.

4. Communicate the change in process to QPS and ensure that QPS are aware that for samples in the range mentioned in Recommendations 1, that they could be requested for Microcon® concentration steps at any point in time. This request can be made via the Forensic Register after they have received the 'DNA insufficient…' result line.

9. References

[1] QIS 19544v11 – Concentration of DNA Extracts Using Microcon Centrifugal Filter Devices

[2] PowerPlex® 21– Amplification of Extracted DNA Validation. Megan Mathieson, Thomas Nurthen, Cathie Allen. December 2012. Forensic DNA Analysis.

[3] Project #163 - Assessment of results obtained from 'automatic-microcon' samples. Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, Cathie Allen. August 2015. Forensic DNA Analysis.