A review of the automatic concentration of DNA extracts using Microcon® Centrifugal Filter Devices: Options for QPS consideration.

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Document Details

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1. Abstract

All casework DNA extracts that underwent a concentration step using the 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 findings of this evaluation are presented for the Queensland Police Service to advise on whether they would prefer their Priority 2 samples to continue with the 'auto-microcon' process, or to cease this automatic step and notify the laboratory if particular samples are requested to be reworked.

These options relate to Priority 2 (Major Crime) samples only, as the process developed in 2012 for Priority 3 (Volume Crime) samples will be reinstated with the operationally-required move to process these samples using PowerPlex® 21 system (PP21).

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 and other casework samples. 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 and other casework samples. 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 ≤35μL for amplification with PowerPlex® 21 system.

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 (Quantification <0.0088ng/uL) 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) for Priority 2 samples.

A workflow for Priority 3 samples remained within active Standard Operating Procedures to have the DNA extracts not amplified, nor automatically concentrated with Microcon® filters, but to be held after Quantification and QPS informed that low levels of DNA were obtained that were insufficient for further processing at that stage [3][4].

Anecdotally, the suitability to provide QPS with DNA profile Intelligence from extracts that have been concentrated has been noted to be limited, and added to scientist's time and availability to direct resources to samples with more DNA detected.

4. Data interrogation

The 'auto-microcon' 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.

5. 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.

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

Intent
Evaluate the difference between the Quantification values obtained for samples prior to the 'auto-microcon' step, and then after the 'auto-microcon' process. This is to assess, through the Quantification data, the effectiveness of the Microcon® step in concentrating the DNA extract.

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

Data Analysis

The samples applicable to this experiment had Quantification values above 0.001ng/μL and less than 0.015ng/μL where the final result was 'success'.

This range was considered by the author to be able to provide a sufficient demonstration of the trend of the data (N=278 samples).

7. Results and Discussion

7.1 Assessment of 'auto-microcon' results

There were N=1449 samples in the 'auto-microcon' Quantification range, excluding certain samples as per Section 5.

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/μL (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

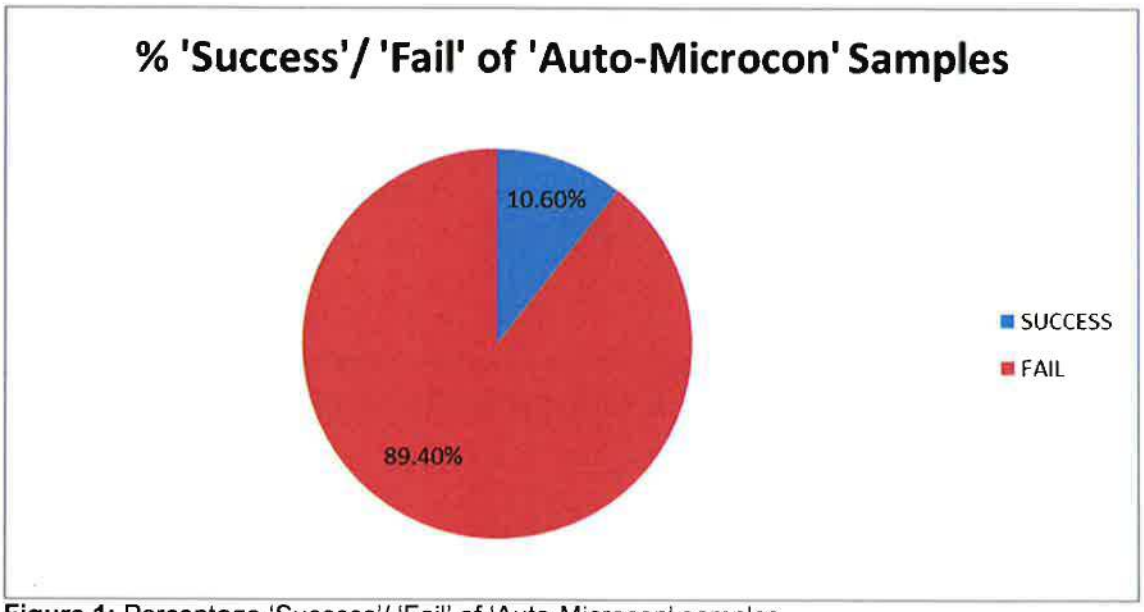

Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

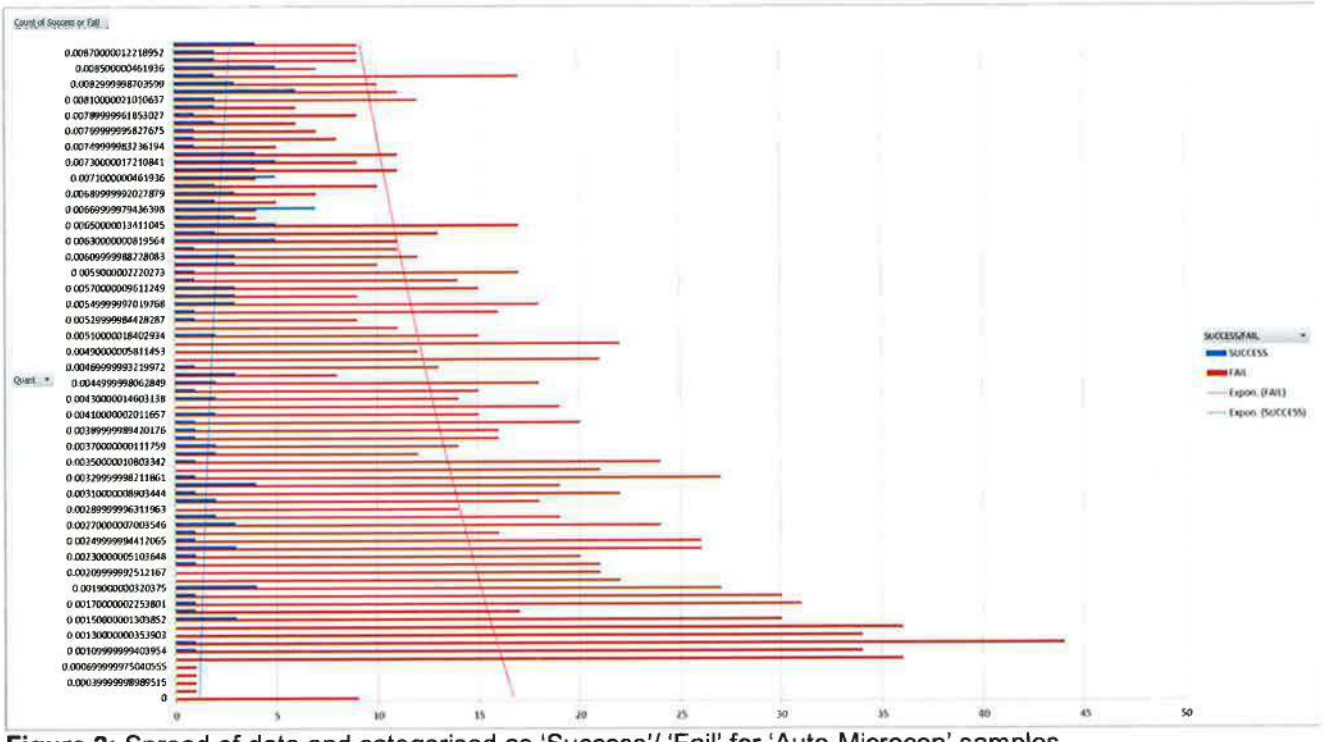

Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

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. 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 (Fig 3)..

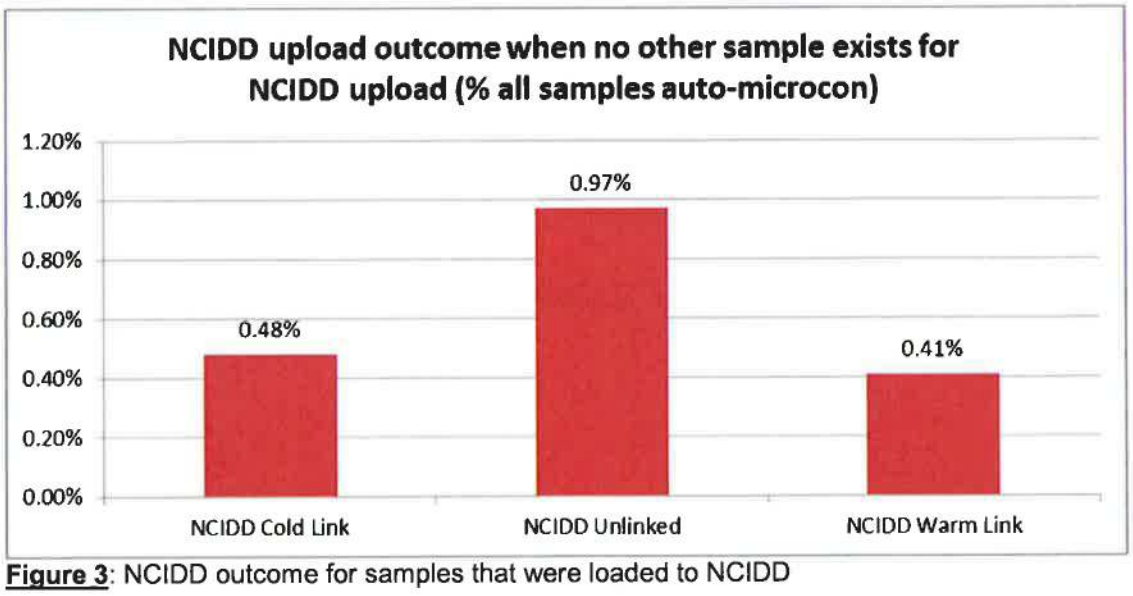

Figure 3: NCIDD outcome for samples that were loaded to NCIDD

This 1.45% of 'auto-microcon' samples is considered to be the pertinent value for the client to assess if the 'auto-microcon' process was not performed.

7.2 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'.

As the Microcon® process concentrates the DNA extract from approximately 100uL to approximately 35μL, in theory it would be a reasonable expectation to obtain approximately two to three-fold increases in DNA Quantification after concentration. Figure 4 shows the plot of the differences found for samples that resulted in 'success'.

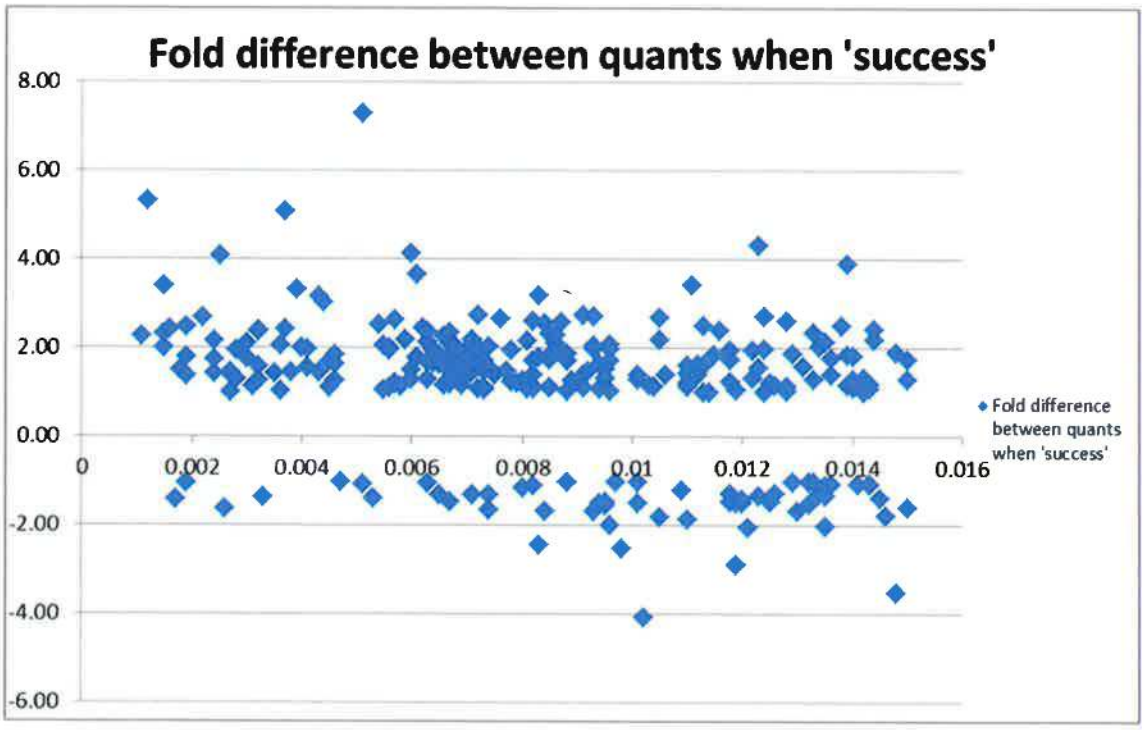

Figure 4: Quantification differences pre and post concentration

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 4 where the Quantification values decreased after concentration (below the horizontal axis). 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. Options for consideration

The options to consider are:

1. Continue with 'auto-microcon' process for Priority 2 (Major Crime) casework; or,

2. Cease the 'auto-microcon' process for Priority 2 (Major Crime) casework and report the exhibit result of 'DNA insufficient for further processing' based on Quantification result.

    a. Priority 1 samples could proceed with the 'auto-microcon' process. If a DNA concentration rework is required, the Microcon® process can be ordered manually by the scientist.

In considering continuing or discontinuing the automatic concentration of DNA extracts for Priority 2 (Major Crime) samples, some key elements to consider include, but are not limited to:

- The opportunity to link DNA profiles on NCIDD would not be initially possible (without automatic concentration) for approximately 1.45% of samples that would qualify for this process. Of the 'auto-microcon' data set (N=1449 samples) evaluated, 1.45% equates to 21 samples;

- Time and cost for processing all samples in the 'auto-microcon' range, including batch preparation, Quality checking and control;

- Time and cost for processing these samples further with additional rework options, as one would expect with low levels of DNA detected initially;

- The ability to potentially reallocate staff time currently allocated to processing, interpreting and reporting 'auto-microcon' samples, to samples with higher DNA yield, thus improving the turnaround time for results on these samples;

- The opportunity to conserve DNA extract for further processing with other technologies should that be considered (eg. Y-STR analysis, Low Copy Number analysis);

- The improved ability to provide quick results to QPS (using the Forensic Register at Quantification stage) indicating low levels of DNA detected, thus enabling QPS to employ further strategies at their discretion (eg. further sampling of items, request the rework);

- The continued ability to process the DNA extract upon client request or depending on priority (eg Priority 1 – Critical Priority).

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] QIS 23008v15 – Explanation of EXR/EXH Results

[4] QIS 24012v13 – Miscellaneous Analytical Section Tasks