Assessment of results obtained from 'automatic-microcon' samples

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1. Abstract

Since December 2012, casework samples with the parameters of PowerPlex priority 1 or 2, and have yielded a quantification value between 0.00214 ng/µL and 0.0088 ng/µL have been automatically processed with a Microcon Centrifugal Filter Device concentration step.

An assessment of results from these samples has been conducted.

Relevant data was extracted from AUSLAB, sorted, reconciled and interrogated. Broad categories of informative results and non-informative results were used based on result types that the Queensland Police Service consider informative (including single source and interpretable 2 and 3 person mixtures) and non-informative (complex profiles, no DNA detected, no DNA profile obtained).

From 1001 assessable samples, 184 yielded an informative result, with 79 samples being uploaded to NCIDD.

2. Introduction

Currently (and since 19/12/12), any priority 1 or 2 PowerPlex® 21 (PP21) casework samples that produce DNA extracts with a quantification value of between 0.00214 ng/µL and 0.0088 ng/µL are sent automatically for a concentration step using a Microcon® Centrifugal Filter Device. This concentration step was introduced as part of PP21 implementation in an effort to minimise the stochastic effects observed at these lower quantification values and improve the overall quality of the profile.

It has been observed anecdotally within the laboratory, that samples which have been sent automatically for concentration (quantification between 0.00214 ng/µL and 0.0088 ng/µL) often yield a DNA profile result which is unsuitable for interpretation or comparison (deemed 'non-informative). In addition, the timeframe (from quantification to result release) can be seen to be lengthy, in comparison to other samples types, particularly if the sample has required further amplification/s to enhance or confirm the profile result.

As part of the laboratory's commitment to ongoing quality assessment, and improvement of processes and results released, an assessment of samples processed by automatic-microcon has been conducted. This assessment includes observations of the number of samples processed by automatic-microcon that are deemed 'informative' by QPS and the number of samples that have been nominated for uploading to NCIDD. This assessment also outlines possible process alternatives, including risks and benefits, and taking into consideration the opportunity to improve turn around times, laboratory expenditure, the ability to incorporate the recently introduced Number of Contributors Guidelines to a broader range of suitable samples, and improvement of the quality of profiles and results issued.

3. Materials and Methods

3.1 Materials

The following resources have been required for this data mining project:

Staff

Computers (including applications such as Excel and AUSLAB)

PP21 case work samples that have already been processed within the laboratory via the automatic microcon concentration step

3.2 Methods

Extended enquiries functionality in AUSLAB was used to extract data pertaining to all samples with MCONC1 test codes with received dates from 2012 – March 2015 that have a 'parent' EXH (i.e. not sub-samples). This data dump included the following fields:

Sample ID

QP number

Result type (based on EXH lines released)

NCIDD upload

Original quantification value

Additional quantification values

Additional test codes

Sample type

Case type

A worksheet in Excel was created, containing the data from the data dump. This data was further sorted into columns and refined/filtered to produce only concentrated samples within the laboratory's 'automatic-microcon' quantification range.

Samples with 'no further work required' requests were removed from the data set as these samples couldn't be assessed and would otherwise skew the data.

The data was then interrogated in an attempt to observe any trends that may have suggested proposing changes to current laboratory processing rules and workflow.

4. Results and Discussion

4.1 Results

A data set of 1136 samples that had been concentrated via an automated microcon process was obtained. This was reduced to a data pool of 1001 assessable samples (designated as the assessable data pool), once samples with 'no further work required' requests were excluded.

From this data pool, 817 samples yielded a result that was considered non-informative (complex unsuitable, no DNA profile, no DNA detected). This represents ~82% of the assessable data pool.

184 samples yielded a result that was considered informative (single source, 2 person mixed DNA profile, 3 person mixed DNA profile). This represents ~18% of the assessable data pool.

Of the informative results, 127 samples yielded 2 or 3 person mixed DNA profiles and 57 samples yielded single source DNA profiles. Therefore the mixed DNA profile result samples represented ~12% of the assessable data pool, and ~69% of the informative result pool. The single source DNA profile result samples represented ~5% of the assessable data pool, and ~30% of the informative result pool.

79 samples from the assessable data pool obtained profiles that were uploaded to NCIDD. This represents ~8% of the assessable data pool and ~42% of the informative result pool. Some of the profiles uploaded to NCIDD were from sole samples within a case, and some of these NCIDD uploads resulted in 'cold links'.

	Total from assessable pool	Percentage of total	Percentage of informative
Total assessable results	1001	100%	N/A
Informative	184	18%	N/A
Non-informative	817	82%	N/A
NCIDD	79	8%	42%
Single source DNA profiles	57	5%	30%
Informative mixed DNA profiles	127	12%	69%

Table 1 Automatic-microcon category data

Observations can be made from the assessment of the categories of samples against quantification values.

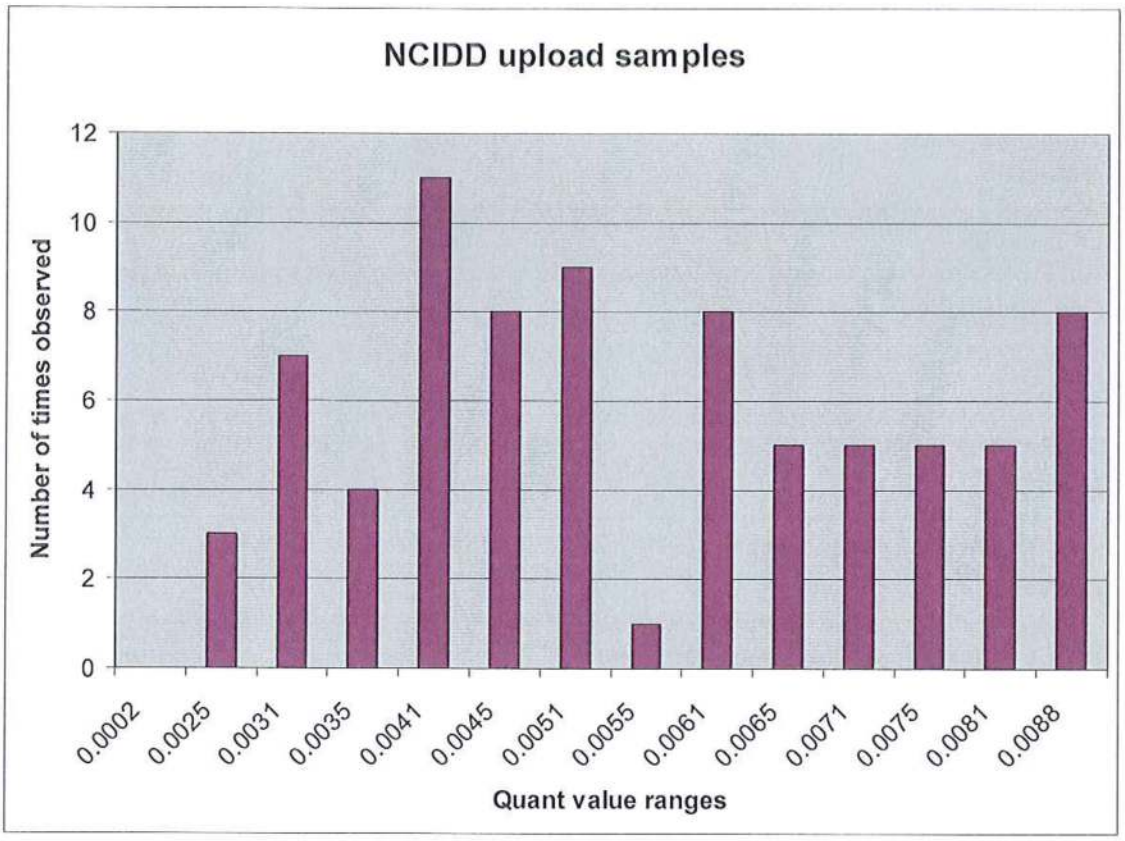

Figure 1 NCIDD upload samples

Automatic-microcon samples uploaded to NCIDD can be observed (see Figure 1) at each of the quant value ranges, with the exception of the range between 0.002 ng/µL and 0.0025 ng/µL and the single NCIDD upload at the quant value range of 0.0055 ng/µL to 0.0061 ng/µL.

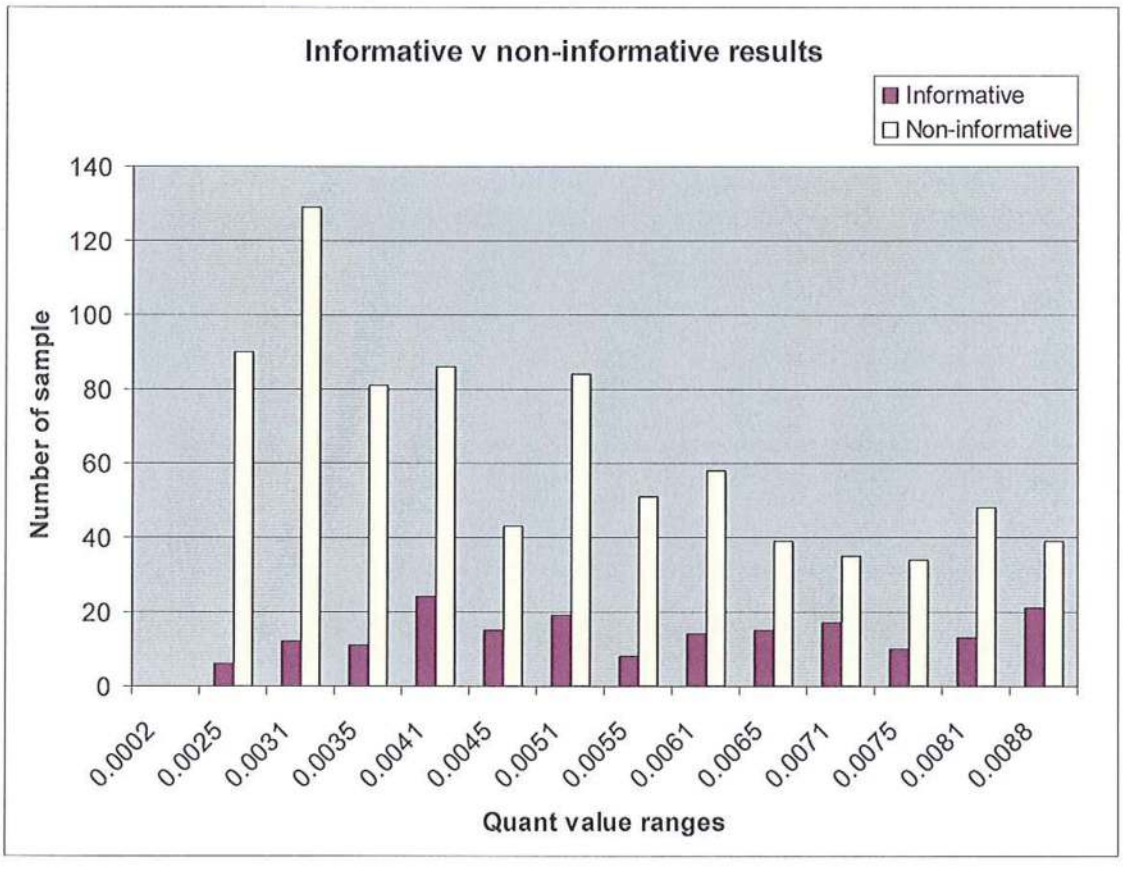

Figure 2 Informative v non-informative results

The number of non-informative results can be observed (see Figure 2) to decrease beyond the quantification value of 0.0035 ng/µL and become closer in occurrence with the numbers observed for informative results.

The number of informative results can be observed to be less than those of non-informative results for the majority of the quantification value ranges and remain fairly consistent across the quantification value ranges.

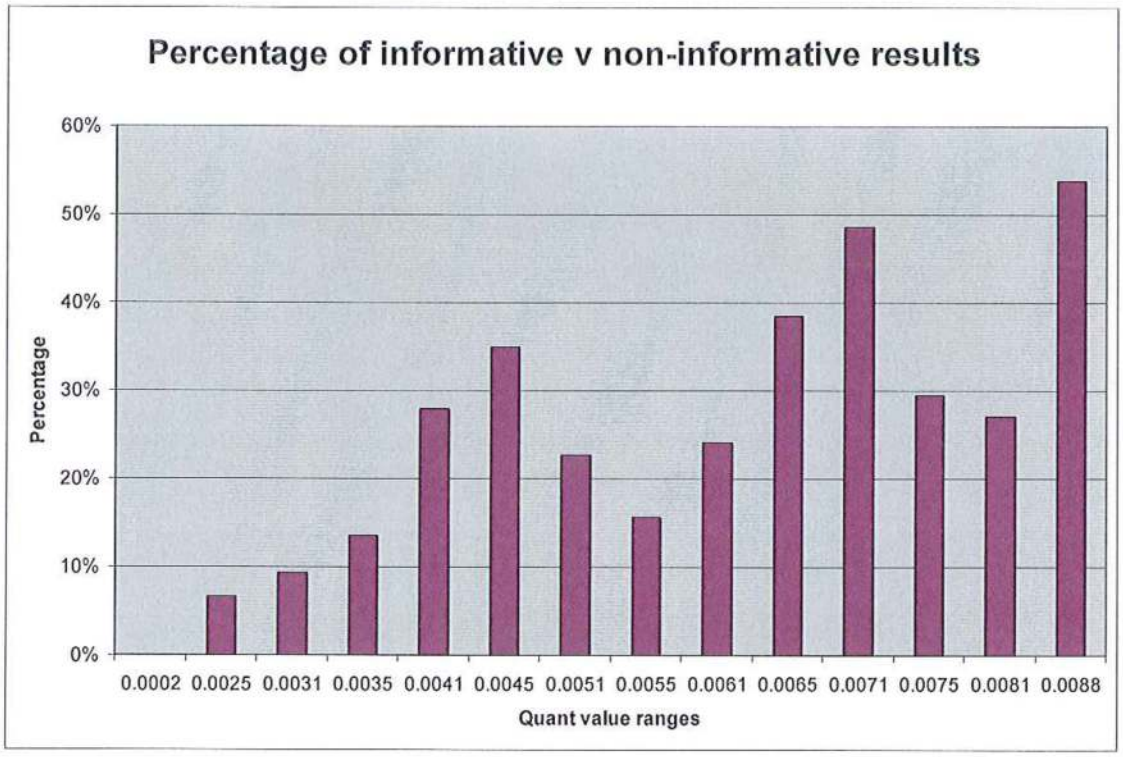

Figure 3 Percentage of informative v non-informative results

The percentage of informative v non-informative results can be observed (see Figure 3) to increase on the whole, with some fluctuation across the quantification value ranges. The lowest percentage of informative v non-informative occurs at the lowest quantification value range and the highest percentage occurs at the highest quantification value range.

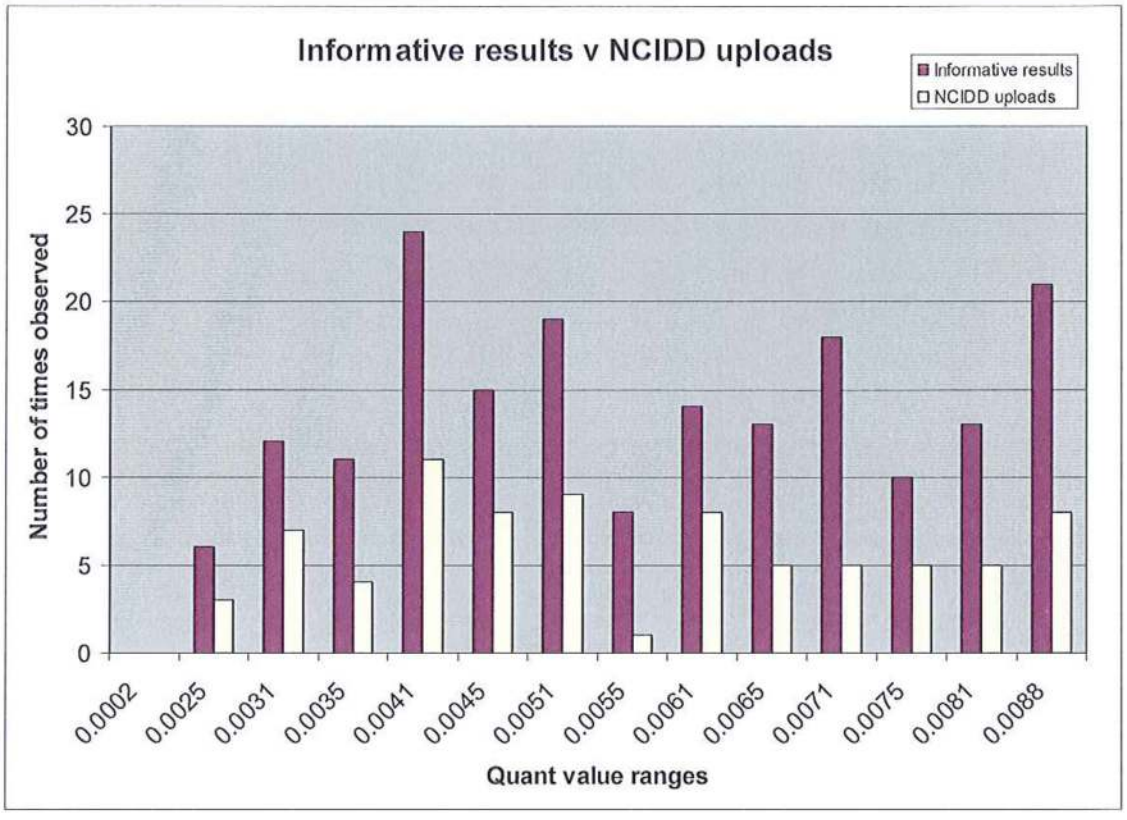

Figure 4 Informative results v NCIDD uploads

The number of samples uploaded to NCIDD can be observed (see Figure 4) to be generally consistent with the informative results and approximately half for each quantification value range. The number of samples uploaded to NCIDD is observed to be highest at the quantification value range of 0.0041 and lowest at the quantification value range of 0.0055 ng/µL.

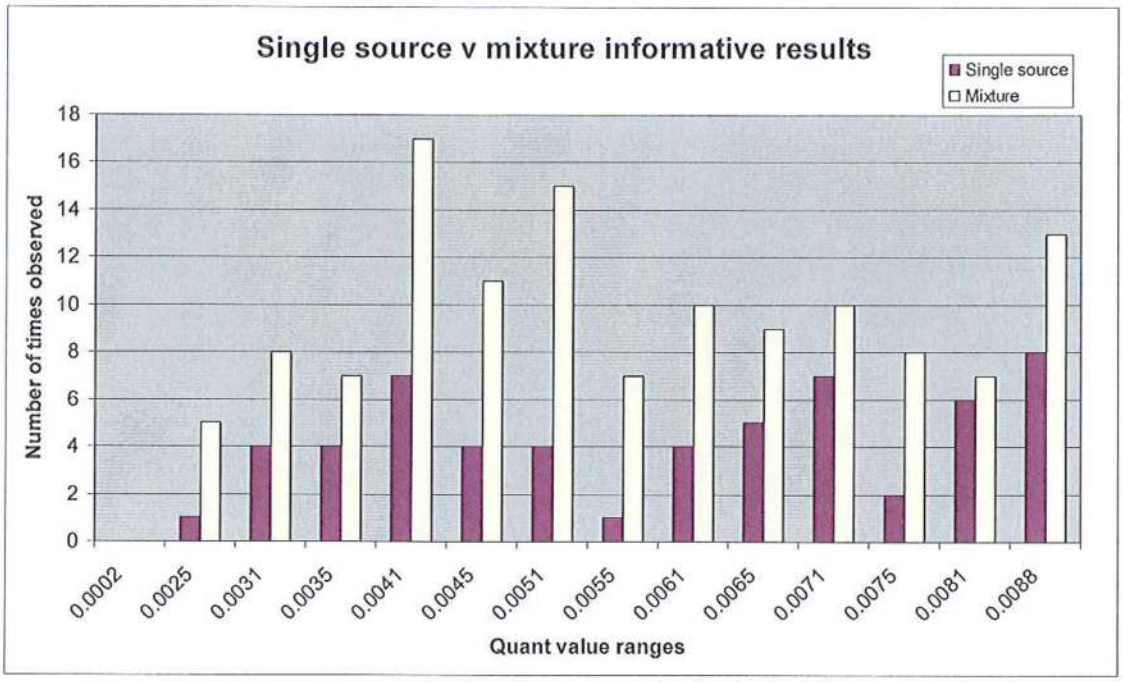

Figure 5 Single source v mixture informative results

The number of mixed DNA profile informative results can be observed (see Figure 5) to be higher than that of single source results. The highest number of informative mixture results can be observed at the quantification value range of 0.0041 ng/µL, and it appears that the bulk of the informative mixed DNA results occur beyond this quantification value range.

The single source informative results can be observed at each of the quantification value ranges and appears to fluctuate across the quantification value ranges.

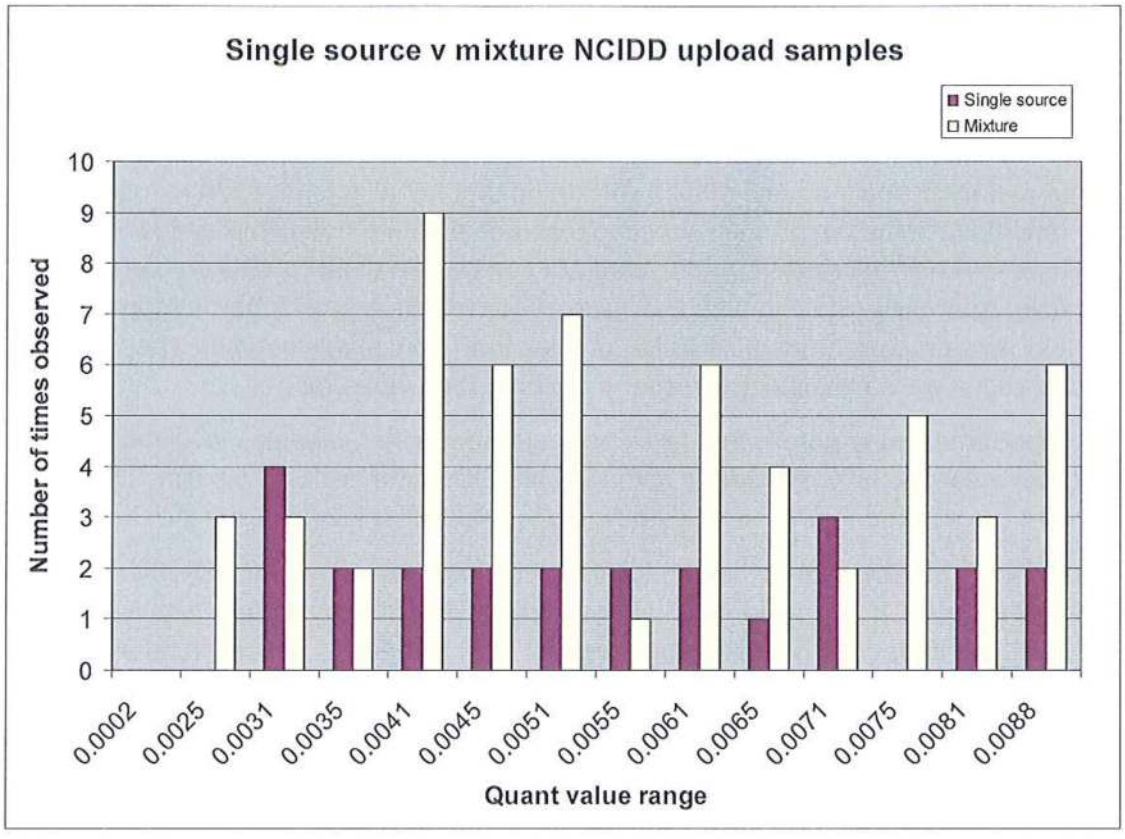

Figure 6 Single source v mixture NCIDD upload samples

The number of mixed DNA profiles uploaded to NCIDD can be observed (see Figure 6) to be highest at the quantification value range of 0.0041 ng/µL and lowest at the quantification value range of 0.0055. It appears that the bulk of uploads from mixed DNA profiles occurs beyond the quantification value range of 0.0041 ng/µL.

The number of NCIDD uploads from single source profiles can be observed to be less than that from mixed DNA profiles and with the exception of no uploads within the quantification value ranges of 0.0025 ng/µL and 0.0081 ng/µL, appears to be fairly consistent within the quantification value ranges.

4.2 Discussion

This data assessment has not been an in-depth study and more detailed statistical analyses was outside the scope, however the data obtained has shown that informative results were obtained across the quantification value ranges within the automatic-microcon process parameters as well as samples uploaded to NCIDD, even at the lowest quantification value ranges.

No real trend was observed for the number of informative results obtained, other than there being informative results and NCIDD uploads across the automatic-microcon quantification range. It appears that across the quantification value ranges, the number of samples loaded for NCIDD was approximately half of the number of informative results obtained and this was generally consistent across the quantification value ranges.

A decline in non-informative results was observed as the quantification value increased. Given the observations in the PP21 validation of greater stochastic effects at lower quantification ranges, this observation is not unexpected.

It was observed that interpretable mixed DNA profiles were obtained and were greater in number than single source results, indicating that not all interpretable results from the automatic-microcon process are single source and that not all mixed DNA profile obtained are non-informative. Additionally, it can be seen that NCIDD uploads were obtained from both single source and mixed DNA results and a higher number of the NCIDD uploads were from mixed DNA profiles than from single source. These observations were consistent across the quantification value ranges.

An important point to note is that there are numerous other variables involved in whether a sample is nominated to upload to NCIDD and therefore, it is difficult to capture the true number of samples suitable for NCIDD uploading from the data pool.

Additionally, there may be a higher significance placed on some of these samples nominated for NCIDD upload, such as a sample being the only sample within the case, the priority and/or case type, and the potential (and actuality) for "cold links" arising from these uploads.

We don't have data from a similar assessment of informative vs non-informative results from samples processed outside the automatic-microcon quantification range to make a comparison. It is possible that what is observed here is similar for all quantification values and therefore these results shouldn't be overstated.

New instruments and processes are soon to be introduced into the laboratory and possibly in the future (Quant Trio, QIAsymphony and Yfiler, for example). These instruments and process may introduce variations to the data observed here and may indicate changes to the processes, irrespective of any possible changes made at this point.

5. Conclusions and Recommendations

This assessment has indicated that there has been value in the automatic-microcon process, with informative results and NCIDD uploads obtained across the quantification value range, including the lowest value ranges, albeit with a high number of non-informative results, which declined as the quantification value increased.

A higher number of informative mixed results were obtained, which also represented the bulk of samples nominated for NCIDD.

NCIDD uploads were obtained across the quantification value ranges and were obtained from both mixed and single source samples and importantly, some of these uploads led to 'cold links' and some were from sole samples within a case.

It is possible that these observations are similar to observations that could be made for samples processed outside of the automatic-microcon process.

Automatic-mirocon process changes, along with introduction of new laboratory instruments may assist in changing the balance of informative to non-informative results.

Based on the analysis of the data, an assessment of current practices and the risks and benefits, two process change options can be considered.

5.1. Process change consideration 1

One possible change to current process could be to submit all samples within the current automatic-microcon quantification range to a half microcon instead of full. Processing as half microcon would provide additional remaining volume to allow for additional amplification runs to enable reproducibility assessments.

Samples falling within this range could be directed to this process step automatically within the Forensic Register.

These samples could then be directed (again by the FR) to a separate CM list, bearing in mind that a large number of these samples may be mixtures and possibly non-informative at first run.

Any samples that can be initially interpreted with a final result could be assessed at this stage, much in the same way that the complex and single source case management lists operate currently.

Profiles that are assessed as requiring additional runs for reproducibility assessments could join the normal CM processing stream after the reworks have been requested.

5.1.1. Benefits

This option seeks to improve upon the already implemented automatic-microcon process, which has shown some success with obtaining informative results and NCIDD uploads from samples within higher stochastic quantification value ranges.

This option presents the least risk with regards to loss of informative results and loss of NCIDD uploads (including cold links).

All samples are given an opportunity for additional processing which may improve the initial result and/or possibly give more confidence with regards to number of contributors present and allowing for interpretation of an informative result.

Additionally, this allows for the use of the newly introduced Number of Contributors Guidelines, being a more consistent approach as with other PP21 samples, as currently the automatic-microcon samples cannot be case managed in this way as there is insufficient remaining volume.

A separate work list for these sample types may result in reduced turn around times for result reporting as some profiles can be reported with final results, with others having their additional runs ordered concurrently at the time of assessment, all from a smaller work list than the general categories in current use.

No additional time awaiting results would be experienced for samples requiring additional runs as both additional runs (XAMP1 and XAMP2) could be requested at the same time as they are likely to be required at full amplification volume.

5.1.2. Risks and disadvantages

The number of samples processed within this category will not be reduced and may in fact, increase with additional runs being requested for reproducibility assessments. The possible additional run (XAMP2) would increase the cost to the laboratory in terms of consumables, staff and time spent on task, including interpretation. This may also increase the turn around time for release of results with the interpretation of an additional profile with a reproducibility calculation.

Additional runs would increase the cost to the laboratory, in terms of staff, consumables and time spent on task (as opposed to other samples).

5.2. Process change consideration 2

An alternative to the above recommendation is to hold all samples within the current automatic-microcon range of 0.002 ng/µL and 0.0088 ng/µL. This would exclude all samples within the automatic-microcon quantification range from processing and case management, with the exception of samples within agreed parameters.

Priority 1 samples and sole samples within a case would be an exception from the hold process and could proceed to a half microcon.

Additionally, there may be an option for held samples to be reactivated if the remainder of samples within the case have yielded non-informative results.

A result line similar to "low DNA" would be sent and either at the discretion of QPS or Forensic DNA Analysis, these samples could be reactivated and proceed to a half microcon with further reworks as required and join the existing case management process.

5.2.1. Benefits

This option would reduce the amount of samples requiring processing (approximately 35 samples per month) and therefore provides the most benefit with regards to turn around times and cost, in terms of consumables, staff and time spent on task.

5.2.2. Risks and disadvantages

Turn around times would increase for reactivated samples, more so than for those requiring additional runs as in Option 1 due to the lag time of reactivation once the initial results have been released and actioned.

This option represents the highest risk for loss of informative results and NCIDD uploads from samples that are not reactivated.

This option gives less of an opportunity for possible improvement of the number of informative results released and uploads to NCIDD as the number of samples being processed by half microcon and with additional runs for reproducibility calculations would be reduced.

Despite the exclusion of Priority 1 samples and sole samples within a case, there remains a risk of possible informative results and NCIDD uploads being lost, with the potential for different informative results and NCIDD uploads not being processed.

Reporting of statements may be affected if reactivation of samples is desired after statement request as there may be limited time for processing and interpretation of samples.

This option represents a higher potential CM burden for analytical staff, with an increased amount of samples requiring validation of "low DNA" results.

5.3. Process change consideration 3

No change to existing process.

5.3.1. Benefits

Samples continue to have an opportunity to have improved results from concentration.

Number of samples requiring this process would not be increased.

No additional cost to the laboratory in terms of staff, time, consumables or funds.

5.3.2. Risks and disadvantages

Number of samples requiring this process wouldn't decrease.

No change in cost to the laboratory in terms of staff, time, consumables or funds.

No opportunity to improve the results for low quant samples.

5.4 Process change consideration 4

Finalise this project at this time, using the concept of this project for an assessment of this process six months post-implementation of the Forensic Register, in conjunction with Quantifiler® Trio DNA Quantification Kit.

5.4.1. Benefits

More effective and efficient use of data with the Forensic Register, with ability to capture additional parameters provided by Quantifiler® Trio DNA Quantification Kit and the Forensic Register including interpretation and Degradation Index.

Data reflective of procedures, instruments and LIMS in use at the time of data capture.

Better opportunity to suggest process improvements conducive to the technology, workflow and LIMS in use at that time.

5.4.2. Risks and disadvantages

Number of samples requiring this process wouldn't decrease for the short-term at least.

No change in cost to the laboratory in terms of staff, time, consumables or funds in the short-term.

No opportunity to improve the results for low quant samples in the short-term.

5.5. General recommendations and considerations

It is recommended that this project be finalised at this point and a new project commence approximately six months after the introduction of the Forensic Register; in conjunction with the use of Quantifiler® Trio DNA Quantification Kit. The concept of this project would be used to guide the new project in terms of a starting point for data mining and parameters of interest.

6. Abbreviations

CM	Case management
DNA	Deoxyribonucleic Acid
NCIDD	National Criminal Investigation DNA Database
QPS	Queensland Police Service
FR	Forensic Register

7. References

1 Nurthen, T, Mathieson, M and Allen, C, *PowerPlex 21 – Amplification of Extracted DNA Validation v2.0.* Forensic DNA Analysis, Forensic & Scientific Serves, 2013