HealthSupport Queensland

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. Purpose and Scope

1.1. Background

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 the Queensland Police Service (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.

1.2. Purpose

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 will include an assessment of those samples that underwent the 'auto-microcon' process.

1.3. Scope

This evaluation will be based on a data mine of extracts in the year 2016 that were concentrated with Microcon® centrifugal filter devices, and will assess the 'suitability' of PP21 profile outcomes as a function of quant values obtained from using the Quantifiler® Trio DNA Quantification Kit.

This evaluation will look 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.

1.4. Definitions

Auto-microcon: Samples with extracts quantified in the range 0.001ng/μL to 0.0088ng/μL that were automatically processed for a concentration step using Microcon® centrifugal filter devices.

NCIDD: National Criminal Investigation DNA Database

2. Governance

Project Personnel

- Project Manager: Justin Howes – Team Leader, Forensic Reporting and Intelligence Team.

Decision Making Group

- The Management Team (including the Project Manager), are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the

experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and retained with the original approved Experimental Design.

Reporting
The Project Manager will provide a fortnightly project status update to the Decision Making Group at the Management Team meetings and by exception as required.

3. Resources

The following resources are required for this validation/project:

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

4. Methods

4.1. Data retrieval from AUSLAB (LIMS)

The data date range is to encompass all samples quantified with Quantifiler® Trio DNA Quantification Kit in 2016, with the following criteria:

1. Quantification value above the Limit of Detection (Quantification) for the Quantifiler® Trio DNA Quantification Kit (ie. 0.001ng/μL);

2. Extracts to be from samples with DNA Priority 2 (High Priority – PP21 amplification kit);

3. Extracts to have undergone a concentration step using Microcon® centrifugal filter devices;

4. Exhibit report outcome (interpretation).

Data will be exported to Microsoft Excel for interrogation.

4.2. Data interrogation

The data will be interrogated by assessing the DNA profile outcome results reported as Exhibit Report lines (from AUSLAB) as a function of the quantification value.

The data will exclude samples that have 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.

The DNA profile outcome will be assessed as either 'fail' or 'success' with the following definitions:

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.

5. Experimental Design

5.1. 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 will have quantification values in the range 0.001ng/μL to 0.0088ng/μL.

DNA profile interpretation outcomes will be grouped into either 'success' or 'fail' as a function of the quantification value.

A percentage of samples that fall into these categories will be determined.

Of the DNA profile interpretation outcomes of 'success', the type of outcome will be broken down further to determine:

1. The percentage of these samples that were reworked; and,

2. The percentage of samples that led to an upload of DNA information to NCIDD, including the amount of reworking required for this upload.

Assessment Criteria
The Decision Making Group will determine if the percentages obtained are significant enough to inform a new workflow strategy in consultation with QPS.

Factors to consider include, but not limited to:
1. Effect on turnaround time for samples in this range considering the success/fail results;

2. Effect on intelligence provision that is provided to QPS from DNA profiles uploaded to NCIDD for samples in this range;

3. Cost of processing samples (including reworks where appropriate) including staff and consumables considering the success/fail results;

4. Opportunity cost of not being able to process other samples that could lead to meaningful information for QPS, including processing with further techniques (eg. Y-STR profiling).

5.2. 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 applicable to this experiment will have quantification values above 0.001ng/μL.

DNA profile interpretation outcomes will be grouped into either 'success' or 'fail' as a function of the quantification value.

A percentage of samples that fall into these categories will be determined.

Of the DNA profile interpretation outcomes of 'success', the type of outcome will be broken down further to determine:

1. The percentage of these samples that were reworked; and,

2. The percentage of samples that led to an upload of DNA information to NCIDD, including the amount of reworking required for this upload.

Assessment Criteria
The Decision Making Group will determine if the percentages obtained are significant enough to inform a new workflow strategy in consultation with QPS.

Factors to consider include, but not limited to:
1. Effect on turnaround time for samples in this range considering the success/fail results;

2. Effect on intelligence provision that is provided to QPS from DNA profiles uploaded to NCIDD for samples in this range;

3. Cost of processing samples (including reworks where appropriate) including staff and consumables considering the success/fail results;

4. Opportunity cost of not being able to process other samples that could lead to meaningful information for QPS, including processing with further techniques (eg. Y-STR profiling).

5.3. 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' will be examined.

Data Analysis

The samples applicable to this experiment will have quantification values above 0.001ng/μL where the final result has been 'success'.

Assessment Criteria
The Decision Making Group will determine if the differences are satisfactorily correlating with expectations.

The Decision Making Group may use this information to decide if the Microcon® concentrating step is worthwhile in keeping as a post-extraction processing step, or to look into other providers of similar technology.

6. Results and Data Compilation

The assessment criteria for each experiment will be used to make an overall assessment as to whether there is sufficient information to inform a new workflow strategy for low quant samples.

The decision points will be based on two data groups:
1. Samples in the 'auto-microcon' range;

2. Samples in an extended quant range (and what that extended range may be).

The Decision Making Group is responsible for assessing the need for further work to assist in making a decision, and will inform the Project Manager.

A final report will be produced which will compile all analyses, conclusion and recommendations.

7. 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.