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Forensic and Scientific Services

Considerations for a new DNA profiling kit for use on casework samples in Forensic DNA Analysis

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25 October 2016

Background

- ANZPAA Board approved 18 DNA markers (STR loci) to be the new core DNA markers for Australia in April 2012.
    - This was prior to implementation of the available kits that met the 18 loci mark
    - The ANZPAA Board noted that a staged implementation may be effective in some jurisdictions and there would be costing implications for laboratories
- Volume Crime (Low Priority) and Major Crime (High Priority) samples commenced profiling with PP21 in December 2012 after implementation for reference samples in September 2012.
- Volume Crime cases reverted to Profiler® Plus (P+) in May, 2013.
    - The main reasons for movement back to P+ were based on:
        - workflow (P+ did not require STRmix™ which was a bottleneck at the time)
        - suitability to continue to match historic P+ samples on NCIDD
        - quicker turnaround times (TAT) with binary interpretation methods, and suitability to progress to profile interpretation without reworks.
    - P+ samples would be available for upgrade to PP21 if QPS requested based on case circumstance or for international searching purposes.
- Thermo Fisher Scientific advised on 17 July, 2016 that P+ will be discontinued.
    - FSS requested enough kits to enable processing for 12 months
- QLD moved to open sharing of DNA profile information with New Zealand (NZ) via Australian Federal Police (AFP) in 2016.
- The NZ lab, Institute of Environmental Science and Research (ESR), reverted from Globalfiler® (GF) back to Identifiler® (ID) in 2015 in order to improve TAT expectations
    - Communication with ESR Technical Leader in 2016 indicated no prospect of moving away from ID due to ability to output results with reliability, and that if they were to implement a DNA profiling kit now, it would be Identifiler® Plus (ID+)
- The Victorian Institute of Forensic Medicine (VIFM) is the only lab within the Biological Specialist Advisory Group (BSAG) cohort that currently used ID+
- Forensic Science South Australia (FSSA) did not implement a kit with more loci than P+ until GF became available and was validated and implemented in 2014.

Considerations

- Volume Crime processing should be intelligence-focussed in that the cases are largely non-suspect cases and therefore, profiles to NCIDD with quick TAT should be the service delivery aim
- Volume Crime samples comprise approximately 50% of samples received at FSS
- In July 2008, QPS requested no reworks on Volume Crime samples (unless not amplified at max and not enough alleles for NCIDD upload) in order to assist in generating quick intelligence through NCIDD interaction
- PP21 was implemented in December 2012 for casework samples and was immediately seen to be highly sensitive, more time consuming to interpret especially with the use of STRmix™, and not ideal to process without reamplification which may include a concentration step (Microcon)
    - Higher rate of spurious peaks in controls was observed nationally [1]
    - Due to the stochastic effects observed through validation and post-implementation, mixtures for Volume Crime were not able to be interpreted with a single amplification. This meant only single-source and 'complex unsuitable' profiles were able to confidently be reported.
        - Volume Crime reverted to P+ processing in order to improve TAT from 6 May 2013, and the mixed DNA profiles (PP21) that were outstanding were largely reported in an agreed approach (with QPS) of informing them that a mixture was obtained but that no further work was conducted; further work could be performed upon request.
        - This agreed approach served to provide some intelligence in the sense that QPS were informed that a mixed DNA profile was obtained, but failed to enable profiles to proceed through to NCIDD and generate 'real' intelligence of a DNA profile load and potential link.
    - No. of Contributor guidelines developed in 2015 determined that the best way to interpret the majority of mixtures was through the processing of three amplifications in total [2]
        - This approach confirmed a single amplification for mixtures would not be ideal in interpreting DNA mixtures with PP21 and therefore, confirmed that processing VC with PP21 without reworks for mixed DNA profiles was not going to be a viable processing option.
- All loci of P+ are incorporated into ID+, PP21, GF and Fusion (the most viable profiling kit options for general casework).
- With more loci available for comparison in PP21 and GF, there is greater discrimination power in these kits than ID+ which could be useful in cases where relatedness may be an issue, and in Identification cases (eg. Disaster Victim Identification (DVI) matters, coronial cases and paternities).
- The Statistics Scientific Working Group worked with the BSAG and in 2012, BSAG agreed that if Likelihood Ratios were to be truncated, they should be truncated to 100 billion.
    - Further work from Forensic DNA Analysis has shown that conservatively, a Likelihood Ratio of 100 billion can be achieved confidently with 32 alleles including correction factors [3].
    - The actual LR values are not reported beyond 100 billion, so the discriminatory power of the current PP21 kit is not always reflected in the final LR value.

- DNA profile sharing ability with NZ improved in 2016
    - Upon request, any samples that have been previously profiled in P+ in QLD have been upgraded to PP21 to facilitate the most complete comparison with NZ profiles
    - With the end of P+, any kit with more loci (at least the 15 autosomal loci within ID+) will enable direct transfer of DNA data to NZ without upgrade (as long as originally profiled with the 'higher' kit)
- Personal communication with other laboratories is that whether it is GF or PP21, there is a general requirement to perform reworks to confirm alleles present.
- Having core loci (18 STR loci) opens up the potential for sharing DNA information with USA and Europe. Since the implementation of PP21 (which contains the 18 core loci) in 2012, there have been at least 27 PP21 profiles shared to Interpol (via AFP) as per personal communication with QPS.
    - Concordance with other kits and standard set of loci is tabulated (Table 1). It is advantageous to have as many loci in common with international laboratories to facilitate intelligence sharing.
- A move to any new kit should be decided in consultation primarily with the QPS, with the key areas of consideration to include, but not limited to:
    - TAT and effect on workflow
    - Intelligence power and international sharing capability
    - Discrimination power
    - Further technology capability
    - Reporting methods
    - Financial implications

Loci	Profiler Plus	Identifiler Plus	Powerplex 21	Globalfiler	European Standard Set (expanded) (2009)	Interpol Standard Set (2010)	CODIS core loci (2015)	CODIS core loci (Jan 2017)	Core Loci Australia (2012)
D3S1358									
vWA									
FGA									
D8S1179									
D21S11									
D18S51									
D5S818									
D13S317									
D7S820									
TH01									
TPOX									
CSF1PO									
D16S539									
D19S433									
D1S1656									
D6S1043									
Penta E									
Penta D									
D2S1338									
D12S391									
D22S1045									
SE33									
D10S1248									
DYS391									
Y indel									
D2S441									
Amelogenin									

Table 1: Concordance of key kits and core loci

Controls and Kit Sensitivity

- A marked increase in the observation of spurious peaks in control samples was observed after the Forensic DNA Analysis laboratory implemented PP21. This observation was also found in other jurisdictions as per personal communication and publication [1].
    - In 2016 (to date), there have been 53 instances where three or more peaks have appeared above the Limit of Detection (16 RFU) in negative extraction controls (during casework processing) where the peaks could not be attributed to any source. The observation of these spurious peaks necessitates reworking to attempt to improve the observation of number of alleles to enable more meaningful Quality searching.
    - Beside the processing cost to these samples, there is a significant time cost in staff (most commonly senior staff) investigating, troubleshooting, discussing and documenting these observations.
    - There is an additional opportunity cost with the inability to process other samples as the processing 'spots' have been occupied by the reworking controls
    - There is a time cost to samples from batches that were processed alongside the controls in that the reporting of these results are quarantined until determinations are made on the quality of the control results.
- In 2013, a number of observations of peaks in controls led the Management Team to decide that a 'line in the sand' had to be drawn and this was that an investigation would be conducted if there were 3 peaks observed above the LOD. This increase in observations is most likely due to the increased sensitivity experienced with PP21 profiling, and is consistent with observations elsewhere in Australia [1].
- Personal communication with VIFM on rate of spurious peaks in controls when using ID+ is in the order of less than 1%, compared to the rate with GF at 2-3% (soon to be implemented).

Costings

- As of 09 September, 2016 the current cost schedule for likely kits on the market are tabulated (Table 2), without factoring in deals with the manufacturers and assuming full volume reactions.

Kit	Kit Cost	Rxns/Kit	Cost per Rxn ($)
P+	3196.69	100	31.9669
GF	15000.94	1000	15.00094
ID+	4152.36	200	20.7618
PP21	11200	800	14

Table 2: Costing schedule

- The approximate costs of VC and MC samples for various kits at full volume, using the most expensive of the two kits not currently validated in the laboratory (ID+), and the current

two kits is tabulated below (Table 3). This cost is based on the number of samples processed in the period 01 January to 09 September 2016 and simulates the approximate cost for the workflow type (with respect to reworks) and kit combination.

- The data was obtained from AUSLAB Extended Enquiries and excludes project samples that were processed with XPLEX testcode, and excludes samples that are finalised pre-amplification.
- Some elements not quantified include the time for creating batches, cost for other consumables and reagents, cost for assessment of DNA profiles including use of GMID-x.
- A significant assumption was made for the second last line of Table 3 which is based on a rework rate of half the current rate for MC samples. This rework rate cannot be known unless the technology was implemented.
- Personal communication with ESR is that the approximate rework rate for ID is in the order of 10-15%.

Details	Cost per reaction ($)
Assuming no change to approach and ability to obtain profile with ID+ for VC	139394.7252
Assuming no change to approach and ability to obtain profile with ID+ for MC	248150.4317
Assuming rework numbers for MC halve with ID+	207021.3059
Total : P+ for VC and PP21 for MC*	381957.4066
Total: ID+ for VC and PP21 for MC	306726.3652
Total: ID+ for VC and MC (same rework rate)	387545.1569
Total: ID+ for VC and less reworks with MC#	346416.0311
Total: PP21 for VC and MC (same rework rate)	302172.64
* Current approach	
# assuming half the current reworks	

Table 3: Cost of processing casework samples YTD

- Current reworking figures used in the costing schedule in Table 3, has MC with approximately 8000 amplifications (with XPLEX) and approximately 4000 rework testcodes.
- Currently, Forensic DNA Analysis use a binary approach to the interpretation of DNA profiles from VC samples. In combination with PP21 and the continuous approach to interpretation of MC samples with the use of STRmix™, the approximate cost of current processing has been $381957.
  - If ID+ was implemented for only VC (the minimum requirement given the discontinuation notice), the cost if these samples were processed alongside PP21 for MC would be $306726. This alone provides a cost saving compared to current processing costs.
  - If ID+ was implemented for VC and MC with the current reworking rates, the cost would be $387545. This cost is approximately the same as the current costs for processing (~1.5% increase).

- Additional processing considerations that affect costing:
    - ID+ may not be as sensitive as PP21 or GF, but it is believed to be robust and reproducible and anecdotally is not thought that reworks will be required to the extent that has considered necessary with PP21 (or GF) processing.
        - This could mean more extract remaining for further processing requests eg. Y-STR profiling
    - If ID+ was implemented for VC and MC with half the reworks, the cost would be $346416. This cost is approx. $35000 cheaper than the status quo. It is approximately $40000 more expensive than if PP21 was used for MC with ID+ for VC.
        - Note this is an assumption of rework rates – the true value cannot be known without implementation

Interpretation Methods

- In 2012, a statistical solution to standardise interpretation was implemented in the Forensic DNA Analysis laboratory. This solution was the newly developed STRmix™ program.
- Major Crime is currently using PP21 with STRmix™. Volume Crime is processed with P+ and a binary approach to interpretation with statistics reported using the Random Match Probability and at times, Likelihood Ratios generated with CODIS Popstats. P+ processing includes the use of terminology such as 'major profile' and 'minor profile'.
- STRmix™ uses more information within the DNA profile in the deconvolution and statistical assessment when generating Likelihood Ratios. It has the ability to interpret up to five contributors (up to 3 contributors has been validated in Forensic DNA Analysis).
    - The STRmix™ approach to interpretation is thought to satisfactorily account for stochastic effects regularly seen within PP21 DNA profiles.

Current Turnaround times

- The approximate turnaround times for profile type are tabulated below (Table 4) for the period November 2014 – March 2016, measured by work days from receipt to result release. The profile type is indicative as it is determined by plate reader and may not be the final result for the DNA profile.

	Major Crime Profile Type				Volume Crime Profile Type				
	No DNA Detected	SS	MIX	Complex	No DNA Detected	NSD	SS	MIX	Complex
Total Averages (work days)	7.94375	26.91875	38.56875	22.1	10.60625	17.41875	21.675	23.15625	19.15625

Table 4: Approximate turnaround times

- Important to note that the TAT for Volume Crime (excluding NSD and undetermined quantification) is relatively consistent at approximately 4 weeks. This has been stable for the entire period of data collection.

- Important to note that the TAT for Major Crime is higher than Volume Crime for single source samples, likely due to some minor need for reworks and comparison to reference samples (less likely with VC profiles).
- Furthermore, the TAT for mixtures is approximately twice the time for the average of Volume Crime samples (excluding NSD and undetermined quantification) due to the complexity of the profiles, the requirement for reworks, the comparison to reference profiles, and the difficulty in determining the likely number of contributors.

Key Considerations and Recommendations

It is non-negotiable that a replacement needs to be found for Volume Crime processing given the information from Thermo Fisher Scientific that they will be discontinuing production of the P+ kit. It is important to focus on the ability to enable quick interpretation and release of information to the client, including interaction with NCIDD. A quick TAT for Volume Crime processing was not being achieved by the laboratory when PP21 was introduced for all casetype. If PP21 was reintroduced for Volume Crime samples, the experience of the laboratory is such that in order to have confidence in determining the likely number of contributors to mixed DNA profiles, for a large percentage of samples, reworks would be required in line with our interpretation guidelines. Reworks take additional time and resources to process, more controls to be processed alongside the samples, less processing spots for other samples, and decreases the amount of extract available for further testing eg. Y-STR, mtDNA, sequencing, defence testing. Reworking can sometimes not lead to a final meaningful result for the client, and anecdotally, this has been noted to affect staff morale. Reworking Volume Crime samples and potentially leading to TAT similar to current Major Crime samples, also defeats the aim of attempting to get DNA profiles to NCIDD quickly for the client to aid in their investigations. As O'Malley points out (2015):

> Rapid forensic analysis has the potential to direct investigations from an early stage, potentially providing significant savings for investigations and ultimately making the community safer by resolving crimes in a timely manner and reducing recidivism. By reducing end-to-end timeframes, the true intelligence value of forensic evidence can be realised. It can direct investigations rather than later simply confirming police suspicions.[4]

It is not recommended that a binary approach to interpreting DNA profiles be used with PP21 given the sensitivity and stochastic behaviour experienced with this kit. It is not thought that this would be any different with GF given the number of loci within GF and the marketed increased sensitivity, and personal communications with laboratories who have used/are using GF [5]. A binary approach to interpretation may work with ID+, but this would need to be assessed as the interpretation methods incorporated into STRmix™ have gathered momentum in the forensic community and have sound literature support. Given this, the most appropriate step for enabling consistency in interpretation approach within Australia moving forward is to use STRmix™ for all casetype.

While the cost of PP21 and GF is cheaper than ID+ per full volume reaction, the marketed increased robustness and sensitivity of ID+ (compared to P+ and ID) may mean less reworks will be required for casework samples [6]. This has been supported by personal communication with ESR. Less reworks for casework samples, means there will be more volume remaining to extend the level of service we currently provide to QPS by being able to implement new technology. Not only do less reworks impact the consumable usage and batch processing in the Analytical Team, it also means less time for staff processing, analysing, assessing and reporting profiles, and therefore, more of an ability to redirect human resources to other tasks with the view to improving TAT and expanding services.

The cost, including opportunity cost, in reworking controls that appear to have extraneous peaks needs to be considered as it is not likely that ID+ will have peaks present in controls to the extent currently experienced with PP21. This has been supported by personal communication with VIFM. If more samples are able to be processed in place of reworking controls, this also increases the capacity for processing samples as mentioned above.

It is important to have STR loci that can be used for international sharing of intelligence. Arguably the most important country to share information with is NZ, who currently process samples with ID. Notwithstanding the P+ discontinuation, this can be considered enough justification to profile casework samples with kits equal to or higher than ID.

While the ANZPAA Board approved the adoption of the 18 Marker set as the new core DNA markers in Australia in 2012, it is important to note that not all jurisdictions moved to PP21 (eg. SA), some reverted to other kits (QLD and NZ), and the Tasmanian laboratory did not and still has not adopted STRmix™ which was recommended to be implemented alongside the higher kits. It is also important to note that the ANZPAA approval predated any completed validations and implementations of the higher kits; therefore, the complexities with interpretation and in the processing of controls had not been experienced. Post-implementation feedback is an important component of a change management process, and this needs to be taken into account when the opportunity to revisit suitable amplification kits arises.

While the discrimination power increased with the use of PP21 and GF, it should be noted that likelihood ratios are truncated to 100 billion in order to assist the courts (including the jury) in comprehending large values. This means that while all information within PP21 and GF are used for comparison, they are not necessary to reach a value of 100 billion. One could argue then that having a kit with a number of loci that generate likelihood ratios beyond that which would reach 100 billion confidently is unnecessary for the client's comprehension.

It is with all things considered, the recommendations to the Forensic DNA Analysis Management Team for consultation with QPS hierarchy are as follows:

1. Seek to validate and implement ID+ for Volume Crime casework on the 3500xL Genetic Analyser.
2. Seek to validate and implement STRmix™ for use in interpreting Volume Crime DNA profiles. During validation, compare to a binary approach to interpretation (by assessing TAT, reworks etc) to confirm interpretation approach moving forward.
3. Code the Forensic Register for ID+ batches, plate maps and reporting methods etc.
4. Repeat the study into minimum number of alleles required to reach a Likelihood Ratio of 100 billion using ID+
5. Subject to validation results, process Volume Crime casework samples with ID+ and STRmix™ for a period of 3 months and in combination with validation observations, evaluate (not an exhaustive list):
    i. Rework rate for casework samples
    ii. Rate of extraneous peaks observed in control samples (this will require processing VC extraction negative controls in ID+ for the duration of this assessment period)
    iii. Turnaround times
    iv. Staff morale
6. Assess the above factors and decide if Major Crime samples would benefit in moving to ID+. In assessment, also considering the amount of extract that might be available for further testing regimes eg. Y-STRs, sequencing.
7. Continue with PP21 for Major Crime samples either as the kit of choice (for this crime type), or as a validated kit for Business Continuity purposes pending the assessment in Recommendation 6.
    i. PP21 could always be used to profile samples previously profiled with P+ or ID+ at QPS request for international exchange.
8. Continue to assess GF as a kit for processing reference samples and DVI/Coronial casework, and potentially as the Business Continuity measure in place of PP21 as per Recommendation 7.
9. As a Business Continuity measure, if the laboratory depletes the stock of P+ kits and the ID+ validation is not complete, an interim measure of using PP21 for Volume Crime should be discussed with QPS.
    i. NB. A supply of P+ kits has been ordered to enable the laboratory to get to Quarter 4 of 2017 at least.

NB. These recommendations are based on casework samples only. Reference samples and coronial casework including DVI samples are separately recommended to continue with PP21. This is because identification work benefits from higher discriminatory power, especially in DVI situations where profiling of relatives may be warranted, and the samples are unlikely to yield mixed DNA profiles. Furthermore, it is the author's opinion that GF continues to be assessed for implementation as the preferred kit for DVI and reference sample profiling.

Additional points based on the recommendations

In adopting these recommendations, this would enable one interpretation method and would replace the need for CODIS Popstats to remain in the laboratory. It would allow some time to establish workflow arrangements for Volume Crime samples, and ensure that enough behaviour of ID+ has been experienced in order to evaluate the viability of profiling Major Crime with ID+. It is thought that a move from PP21 to ID+ would be more of a seamless transition than was experienced by the laboratory, and by the other BSAG laboratories, when the higher kits were implemented.

While ID+ has less loci than the current PP21 kit, when the recommended assessment is conducted after 3 months of implementation, it is not the author's opinion that a strategy of moving Major Crime to ID+ would be a 'backward step' as it may open up avenues for further technology, will continue to enable sharing with NZ, won't prevent the opportunity to use PP21 for international transfer if necessary, and will very likely improve TAT. As a result of this, there will be an improved ability to assist QPS in detecting, preventing and resolving criminal activity in QLD. It would therefore align with the FSS Vision (2016) in providing 'specialist analysis and expert, independent advice to improve the health and safety of our community' [7].

References

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