Concentration of DNA Extracts using Microcon[®] Centrifugal Filter Devices

1 **PURPOSE & SCOPE**

This method describes the use of Microcon® Centrifugal Filter Devices to concentrate extracted DNA. It is used for samples where the yield of DNA is not sufficient to obtain a DNA profile using the AmpF{STR[®] Profiler Plus[®] system or PowerPlex[®]21 system.

Concentrating the DNA extract using Microcon[®] devices typically reduces the volume from approximately 150μ L to less than 20μ L.

2 PRINCIPLE

Microcon[®] Centrifugal Filter Devices are centrifugal filter devices that employ an Ultracel[®] low-binding regenerated cellulose membrane to desalt and concentrate macromolecular solutions such as DNA-containing solutions.

The low-adsorption characteristics of the Ultracel® membrane and the device's component parts, together with an inverted recovery spin, combine to yield high recovery rates (typically >95% of the sample, with concentration factors as high as 100x).

3 **REAGENTS & EQUIPMENT**

3.1 Reagents

- TE⁻⁴ Buffer (10mM TRIS.HCl, 0.1mM EDTA, pH 8.0).
- Nanopure water (Milli-Q) ٠

Reagent components are stored within the following designated locations:

Table 1. Reagent storage locations.

Reaction Component	Storage Location
TE ⁻⁴ buffer	Room 3189
Nanopure H ₂ O	Room 3188

3.2 Equipment

Any of the following equipment can be used for this method:

Table 2. Equipment used and location.						
Equipment	Asset No.	Location				
Eppendorf 5424	30433322	Room 3189				
Eppendorf 5424	30433323	Room 3189				
Eppendorf 5424	30433324	Room 3189				
Eppendorf 5424	10233209	Room 3189				
Microcon [®] Centrifugal Filter Devices	N/A	Room 3189				

4 SAFETY

As per the procedures in the QIS document "Anti-contamination procedure" (QIS 22857), PPE is to be worn by all staff when performing this procedure.



5 SAMPLING & SAMPLE PREPARATION

Samples that have been extracted are stored in freezers as described in Table 3.

Table 3. Sample	storage locations.
-----------------	--------------------

Sample type	Device	Asset No.	Location
DNA extracts	Freezer (Orford 3-door)	10238431	Room 3194 A
DNA extracts	Freezer (Westinghouse 1-door)	30512883	Room 3194 A

QC samples

A Microcon[®] batch has one negative control consisting of 100µL of nanopure water. This is registered in AUSLAB using the appropriate UR number for negative extraction controls for the current year. The registration of control samples is covered in the DNA Analysis Workflow Procedure (QIS <u>24919</u>)

Create the Batch

Creation of batches is covered in the DNA Analysis Workflow Procedure (QIS 24919).

Locating Samples

Determine the storage locations of the required samples using the Batch Creation table print out. Locate and remove samples from storage according to Storage Guidelines for DNA Analysis (QIS document <u>23959</u>)

6 **PROCEDURE**

Microcon[®] Concentration of DNA Extracts

- 1. Aliquot nanopure water into a 1.5mL or 2mL tube using the Milli-Q in room 3188 for the negative control before proceeding to room 3189.
- 2. Thaw samples, vortex briefly and pulse spin (maximum 5 sec at 1,000 x g).
- 3. For each sample, assemble a Microcon[®] tube and reservoir together, checking the membrane of the Microcon[®] tube is intact before use. Also prepare a separate Microcon[®] reservoir (without Microcon[®] tube) per sample. At each step during the procedure check the membranes to ensure they are still intact (See **Figure 1**).

The membrane should be uniform and complete.		If the membrane is not uniform or it looks as though it has pulled away from the sides – do not use.

Figure 1 - Membrane.

- 4. Label the top of the Microcon[®] tube/reservoir and separate reservoir with the batch position number and label the sides with the laboratory number label.
- 5. Label final 1.0mL Nunc[®] Bank -It[®] storage tubes with the laboratory number label.
- 6. Have a second operator perform a manual sequence check and add the sequence check details into AUSLAB. This is done by accessing the batch in AUSLAB and pressing [F5] Sequence Check, Scan in the Microcon® batch ID barcode



7. Transfer the extract into Microcon[®] tubes:

Microcon[®] to Full or a Target volume

- Using a pipette, measure the initial volume (up to 500μL) into the Microcon[®] reservoir without touching the membrane.
- Record the volume in the Initial volume column next to the lab number on the worksheet. Ensure no Chelex beads are pipetted into the column.
- Seal with the attached cap.
- To achieve the "final target volume" of concentrated DNA sample, calculate the amount of "expected flow through volume" using Table 4 as a guide.
- **Note:** Allow for 5 10μ L unrecoverable volume trapped beneath membrane.
- **Note:** 100uL of nanopure water is added to the negative control and processed as a Microcon to 30uL.

	Initial volume (µL)	Target volume (μL)	Unrecoverable trapped volume (5-10µL)	Flow through volume (µL)
Example	Α	B	С	A – (B+C)
Negative Control	100	30	5	65
Microcon to Full	80	10	5	65
Microcon to Half	80	40	5	35
Microcon to 20 µL	80	20	5	55

Table 4. Example of "Flow through" calculations required to achieve the desired "Target volume"

8. Place the assembly into the centrifuge and spin $Microcon^{(i)}$ tubes at 500 x g. (2 minute intervals is optimal, however can be spun for less time at operator's discretion) :

Microcon[®] to Full

- Spin at 500 x g and periodically measure the flow through volume.
- This volume should be the calculated flow through volume.
- The target volume to aim for is between 5-20µL.

Microcon[®] to a Target volume

- Spin at 500 x g and periodically measure the flow through volume.
- This volume should be the calculated flow through volume.
- •

CAUTION: DO NOT SPIN OVER A TOTAL OF 12 MINUTES.

- 9. Once the calculated flow through volume has been achieved, remove the assembly from the centrifuge and separate the sample reservoir from the tube.
- 10. Place the sample reservoir upside down into the separate labelled Microcon[®] reservoir. With the tube cap open carefully place the assembly into the centrifuge. Spin for 3 minutes at 1,000 x g.
- 11. Carefully remove sample from the centrifuge. Separate the reservoir from the tube and place back into the original flow through tube.
- 12. Accurately measure the final sample volume using a pipette and record in the Final Volume column on the worksheet. Indicate whether or not the sample is to go to Quantitation or Amplification.



- 13. Transfer the final volume to a labelled 1.0mL Nunc[™] Bank-It[™] tube.
- 14. For samples that are <20µL, add an appropriate volume of TE⁻⁴ (for AmpF{STR[®] Profiler Plus[®] samples) or Amplification Grade water (for PowerPlex[®]21samples) to the Nunc[™] Bank-It[™] tube , so that the final volume is 20µL.
- 15. Ensure that the reagents used are recorded in AUSLAB.
- 16. Record the final volumes in the Microcon[®] results file I:\Results\Mres\(Batch ID...)
- 17. Highlight the file named the same as the Batch Id and right click
- 18. Select "Open with" then point to "Microsoft Excel"
- 19. Record the final volumes in the final volume column.
- 20. For AmpF{STR[®] Profiler Plus[®] samples, if the Final volume is less than or equal to 26μL, no Quantitation step is required and the sample is progressed straight through to Amplification. Enter the appropriate sample volume (without units) to be amplified in SV1. As the TE⁻⁴ has already been added to the sample, the SV1 volume will = 20, and the TV1 volume will = 0. Add zero (0) values in SV2 and TV2.
- 21. If the final volume is >26µL, then enter zero values (0) in SV1,TV1,SV2 & TV2
- 22. For PowerPlex[®]21 samples, all samples will be quantified. Enter zero values (0) in SV1,TV1,SV2 & TV2.

NOTE: All negative controls should be quantified irrespective of the final volume.

- 23. Click the Save icon, Answer "Yes" appropriately to all prompts by Windows.
- 24. Store the samples in Freezer 6117-3 as described in Storage Guidelines for DNA Analysis (QIS document <u>23959</u>). Discard the sample flow through and reservoir.
- 25. File the completed Microcon worksheet in the Pre-PCR Sorting Area on the shelf above the printer (Room 3194 A).

7 TROUBLE SHOOTING

7.1 Volumes >500µL

 If volumes greater than 500µL are encountered then pipette in the same manner but split the respective sample over more Microcon[®] centrifugal filter devices (pool after completion of subsequent steps) and write a comment into the audit trail, specimen note, and staff communication list.

7.2 Initial Spin sees ALL liquid pass through the membrane

 If all liquid has passed through the membrane after the first spin this may indicate a membrane failure. In this case the filtrate should be transferred to a new Microcon[®] filter and the following recovery procedure performed.

Recovery (required when over spinning/sample dryness has occurred) -



- ii. Add $10\mu L$ of TE buffer to the sample reservoir
- iii. Agitate gently for 30 seconds
- iv. Continue with the recovery of the sample DNA as above.

7.3 No Fluid appears to be passing through the filter

In the case of no fluid passing through filter, the filter is to be inverted into a new collection tube and spun at 1,000 x g for 3 minutes. The sample is then to be transferred to a new Microcon assembly, the procedure will continue as normal and a specimen note & batch audit entry is to be made in AUSLAB.

7.4 Excess Samples remaining after Microcon[®] Procedure

 Should there be excess sample left after a Microcon[®] to Full, indicate on the worksheet so that a specimen note indicating that there is sample remaining can be sent to the case scientist.

8 RESULTS & ANALYSIS

Importing Microcon[®] Results into AUSLAB

- Log into AUSLAB and import the results file as covered in the Batch functionality in AUSLAB (QIS 24469). The file will be imported into AUSLAB and appear in the DNA file table.
- 2. Highlight the result file and press **[Enter]**, User is taken into the DNA results table.
- 3. Page down through the table and check that all sample results have been imported.
- 4. Press **[SF8]** *Table sort*, this sorts the table so any samples that have failed Autovalidation are sorted to the top of the table. Highlight the first entry that has failed and press **[Enter]**.
- Confirm the reason for the failure by checking the Finvol value (>26µL). Press [Esc] to exit back to the DNA results table. Repeat until all entries that failed Autovalidation have been checked. Ensure all samples requiring PowerPlex[®]21 are toggled to No.
- 6. Return any samples requiring Quantification to the appropriate Quantification batch allocation list. For more details, refer to the Batch functionality in AUSLAB (QIS <u>24469</u>).
- 7. Complete the batch. All of the samples with a **Yes** in the Accept column are transferred to the relevant Amplification batch allocation lists. For more details, refer to the Batch functionality in AUSLAB (QIS 24469).

9 CALCULATIONS

All volumes recorded on the Microcon[®] worksheet and in the Microcon[®] results file should be whole numbers in microlitres (μ L).

10 QUALITY ASSURANCE/ACCEPTANCE CRITERIA

A negative control consisting of 100μ L of nanopure water is included with each batch of extractions. This negative control is processed as a normal sample (Microcon to 30μ L) through to completion. If any results indicating the presence of DNA are obtained from this



sample, either at the quantification or the CE QC check, then the possible source of the contamination is investigated. The samples extracted with this control are thoroughly checked and repeated if possible.

11 **REFERENCES**

Millipore 2005 MICROCON[®] Centrifugal Filter Devices User Guide. 99394, Rev. M, 06/05. Millipore 2007. <u>http://www.millipore.com/creative_solutions</u>. Accessed 2007.

12 ASSOCIATED DOCUMENTS

QIS 22857 Anti-contamination Procedure QIS 24919 DNA Analysis Workflow Procedure QIS 23959 Storage Guidelines for DNA Analysis QIS 24469 Batch Functionality in AUSLAB

13 STORAGE OF DOCUMENTS

All worksheets are stored in the Pre-PCR Sorting Area on the shelf above the printer (Room 3194 A).

14 AMENDMENT HISTORY	AMENDMENT HISTO	ORY
----------------------	-----------------	-----

Version	Date	Author/s	Amendment
R0	23 Aug 2002	V lentile	New Issue.
R1	04 May 2005	M Gardam	Requirements for Microcon [®] to Full/Half and Nominated. Volumes were outlined. Preparation of samples prior to use was also added. Table amended to record spin times for each sample.
R2	07 Nov 2005	M Gardam	Added recovery instructions, enlarged table, added new columns and added diagrams of the membrane.
R3	12 Sep 2006	M Gardam	Minimum value for quant ≥26μL.
R4	09 Feb 2007	T Nurthen, V Hlinka	Reformat and LIMS integration data.
R5	31 Mar 2008	R Smith C Revera M Harvey	New Template; Added section on "Troubleshooting"; Table 4 QC position for Microcon [®] batches- UR number for Neg Ctl changed; Removed Appendices (AUSLAB Masks); Revised Associated documents.
R6	13 March 2009	QIS2 Migration	Revision changed to version and incremented on migration to QIS2
7	15 May 2009	A McNevin K Lancaster	Removed details of registration of controls and storage of samples as this is covered in appropriate SOP's, corrected spelling errors, changed nanopure water from autoclaved nanopure water, updated approver to C Allen. Corrected numbering of bullet points. Referenced importing results and creating batches to relevant SOP's. Minor changes as per comments
8	25 Nov 2010	E Leckenby	Updated Reagents, Equipment and sample/worksheet Storage to reflect move from block 6 to 3, including updating/changing equipment and freezer asset numbers, minor changes to procedure (added steps to aliquot nH20 in 3188, note to m'con



Concentration of DNA Extracts using Microcon® Centrifugal Filter Devices

Version	Date	Author/s	Amendment
			neg ctrl to 30uL), updated and fixed hyperlinks and associated documents
9	18 Jul 2012	Amy Cheng / Allan McNevin	Minor formatting & wording changes.
10		Amy Cheng	Included processing changes for the new microcon membranes and PowerPlex [®] 21 samples.



Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Version Status: Active

🛋 Add 🖷 View Document 📄 Print Comments 🚱 History								
General Reviews and Ap	provals Noti	ifications C	Comments	Controlled Copies	s Version History	Associations	Records	
Workflow								
Comments By	Comm	ent Date	Res	ponse By	Response Date	Comm	ent Noted	
Adam KAITY	12/04/2022					Not Require	ed	
Luke RYAN	07/06/2022					Not Require	ed	
Luke RYAN	11/07/2022					Not Require	ed	
Luke RYAN	12/07/2022					Not Require	ed	
Luke RYAN	13/07/2022					Not Require	ed	
Luke RYAN	25/08/2022					Not Require	ed	
Lisa FARRELLY	16/08/2022					Pending		
Comments		to include a s	of additiona	l troubleshooting in n numbers and SI	structions is very hel; unit (1 ul not 1ul). on as per version 6.	oful. For next ve	ersion, check	

Response

Created on 12/04/2022 7:16 AM by Adam KAITY

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Version Status: Active

📑 Add 📄 View Document	Print Comments 🥙	History				
General Reviews and App Workflow	rovals Notifications C	omments C	Controlled Copies	s Version History	Associations	Records
Comments By	Comment Date	Respo	nse By	Response Date	Comm	ent Noted
Adam KAITY	12/04/2022				Not Require	d
✓ Luke RYAN	07/06/2022				Not Require	d
Luke RYAN	11/07/2022				Not Require	d
Luke RYAN	12/07/2022				Not Require	d
Luke RYAN	13/07/2022				Not Require	d
Luke RYAN	25/08/2022				Not Require	d
Lisa FARRELLY	16/08/2022				Pending	
Comments	Workflow arra - samples will be amplified a - case manag amplification. standard wor - samples cur microcon pro - no change t	I not have DIFP after Quant. Thi gers can assess This does not a kflow arrangem rrently reported cedure.	samples as of 6 added to results is applies to P2 a samples for rew apply to P3 samp ents. I as DIFP that are ow where sampl	s in the quant range 0	de a microcon a sed without rewo tarted by QPS w	fter the first ork as per rill undergo a

Response

Created on 7/06/2022 9:25 AM by Luke RYAN

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler $\ensuremath{\mathbb{R}}$ Trio DNA Quantification Kit

Version Status: Active

		History			
General Reviews and Ap	oprovals Notifications C	omments	Controlled Copie	s Version History	Associations Records
Workflow					
Comments By	Comment Date	Res	ponse By	Response Date	Comment Noted
Adam KAITY	12/04/2022				Not Required
Luke RYAN	07/06/2022				Not Required
Luke RYAN	11/07/2022				Not Required
Luke RYAN	12/07/2022				Not Required
Luke RYAN	13/07/2022				Not Required
Luke RYAN	25/08/2022				Not Required
Lisa FARRELLY	16/08/2022				Pending
Comments		tep 38. Add r ove by either	new step re remov	ing bubbles from quant on the bench or by flick	plates. If bubbles are ing individual wells using a
Response					

Created on 11/07/2022 9:49 AM by Luke RYAN

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Version Status: Active

Add 🖳 View Document 📄 Print Comments 🥙 History General Reviews and Approvals Notifications Comments Controlled Copies Version History Associations Records							
Workflow							
Comments By	Comment Date	Response By	Response Date	Comment Noted			
Adam KAITY	12/04/2022			Not Required			
Luke RYAN	07/06/2022			Not Required			
Luke RYAN	11/07/2022			Not Required			
✓ Luke RYAN	12/07/2022			Not Required			
Luke RYAN	13/07/2022			Not Required			
Luke RYAN	25/08/2022			Not Required			
Lisa FARRELLY	16/08/2022			Pending			
Comments 12/07/2022 7:06:07 AM Luke RYAN: Quant Trio user guide recommends centrifuging at 3000 rpm to remove bubbles from the qua plate. Current SOP lists speed as 2000 rpm. Update to 3000 rpm as this is more effective to remove bubbles.							

Response

Created on 12/07/2022 7:06 AM by Luke RYAN

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler $\ensuremath{\mathbb{R}}$ Trio DNA Quantification Kit

Version Status: Active

General Reviews and A Workflow	pprovals Notifications	Comments Controlled Cop	vies Version History A	Associations Records
Comments By	Comment Date	Response By	Response Date	Comment Noted
Adam KAITY	12/04/2022			Not Required
Luke RYAN	07/06/2022			Not Required
Luke RYAN	11/07/2022			Not Required
Luke RYAN	12/07/2022			Not Required
✓ Luke RYAN	13/07/2022			Not Required
Luke RYAN	25/08/2022			Not Required
Lisa FARRELLY	16/08/2022			Pending
Comments <u>13/07/2022 7:35:51 AM Luke RYAN:</u> This comment from 12/07/2022: "Quant Trio user guide recommends centrifuging at 3000 rpm to remove bubbles from the quant plate. Current SOP lists speed as 2000 rpm. Update to 3000 rpm as this is more effective to remove bubbles." was implemented on 12/07/2022.				

Response

Created on 13/07/2022 7:35 AM by Luke RYAN

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Version Status: Active

🚔 Add 🔄 View Document 📄 Print Comments 🧭 History							
General Reviews and Ap	provals Notifications	Comments	Controlled Copie	es Version History	Associations Records		
Workflow							
Comments By	Comment Date	Res	sponse By	Response Date	Comment Noted		
Adam KAITY	12/04/2022				Not Required		
Luke RYAN	07/06/2022				Not Required		
Luke RYAN	11/07/2022				Not Required		
Luke RYAN	12/07/2022				Not Required		
Luke RYAN	13/07/2022			Not Required			
✓ Luke RYAN	25/08/2022				Not Required		
Lisa FARRELLY	16/08/2022				Pending		

Comments

25/08/2022 2:06:04 PM Luke RYAN:

The Quant transition rules have been updated as per the DG's request in the relevant memo. Quant transition rules in FR have been changed to:

Priority 1 and 2 samples – transition to microcon when in 0.001 – 0.0088 ng/µL.

Priority 3 samples – transition to Amp when equal to or above 0.001 ng/µL.

All priority samples below 0.001 ng/µL reported as No DNA Detected.

Response

Created on 25/08/2022 2:06 PM by Luke RYAN

Document Management: 34045 - V7.0 - Quantification of Extracted DNA using the Quantifiler® Trio DNA Quantification Kit

Version Status: Active

💣 Add 🛛 🖭 View Docum	ent 📔 Print Comments 🕙	History				
General Reviews and A	pprovals Notifications C	omments Controlled Co	pies Version History	Associations Records		
Workflow						
Comments By	Comment Date	Response By	Response Date	Comment Noted		
Adam KAITY	12/04/2022			Not Required		
Luke RYAN	07/06/2022	2 Not Required				
Luke RYAN	11/07/2022			Not Required		
Luke RYAN	12/07/2022			Not Required		
Luke RYAN	13/07/2022			Not Required		
Luke RYAN	25/08/2022			Not Required		
Lisa FARRELLY	16/08/2022			Pending		
I6/08/2022 1:27:33 PM Lisa FARRELLY: As per Analytical meeting Step 12 of Section 8 "Batch Finalisation" is amended to send >5ng/u samples with IPCCT failures to dilution first instead of Nucleospin. Section 8 Step 12 is: For samples that are displayed in the IPCCT row on the QC summary page of the Results PDF (Figure 24): If Quantification Value is >5 ng/µL proceed with the default Technique "Post Extraction" and						

 If Quantification Value is >5 ng/µL proceed with the default lechnique "Post Extraction" and Method "Dilution" on the Quant Results page in FR as per Step 11 - In addition, an Analytical Note is to be manually added to the sample in FR stating "IPCCT result indicates possible inhibition, however SAT result is >5 ng/µL therefore dilution has been ordered. Please consider nucleospin if dilution strategy is unsuccessful"

nucleospin if dilution strategy is unsuccessful" • If Quantification Value is ≤5 ng/µL and if the sample has come from either a Microcon® or Nucleospin® batch, contact the case scientist or Analytical HP5 before changing the Technique and Method on the Quant Results page in FR.

and Method on the Quant Results page in FR. • If Quantification Value is ≤5 ng/µL and if the sample has come from any other batch type including extraction and dilution batches, change to "Post-Extraction" Technique and "Nucleospin" Method on the Quant Results page in FR.

Response

Created on 16/08/2022 1:27 PM by Lisa FARRELLY

Document Management: 33773 - V3.0 - Procedure for Profile Data Analysis using the Forensic Register

Version Status: Active

🚔 Add 🕙 View Document 📄 Print Comments History							
General Reviews and Approvals Notifications Comments Controlled Copies Version History Associations					ciations Records		
Comments By	Comment Date	Response	By Re	sponse Date	Comment Noted		
Justin HOWES	04/04/2022	Angelina KELLER	05/04/20	022 Not	ted		
Justin HOWES	07/06/2022	Angelina KELLER	27/06/20	022 Not	ted		
Angelina KELLER	21/07/2022			Not	t Required		
Angelina KELLER	10/03/2022	Angelina KELLER	10/03/20	022 Not	t Required		
Comments 4/04/2022 3:17:14 PM Justin HOWES: In case managing cases with samples from v2.0, the information surrounding as the sole version is contained in: I:\Change Management\Minor Change Fo completed\STRmix versions and retention\STRmix versions doc_09062020.dd				je Forms -			
Response	sponse 5/04/2022 7:09:16 AM Angelina KELLER: Noted for next revision						

Last Modified at 5/04/2022 7:09 AM by Angelina KELLER, Created on 4/04/2022 3:17 PM by Justin HOWES

Document Management: 33773 - V3.0 - Procedure for Profile Data Analysis using the Forensic Register

Version Status: Active

🚔 Add 👜 View Document 📄 Print Comments 🤣 History							
General Reviews and App	rovals Notificati	ons Comments	Controlled Copie	s Version History	Associations Records		
Workflow							
Comments By	Comment D	ate Res	ponse By	Response Date	Comment Noted		
Justin HOWES	04/04/2022	Angelina K	ELLER 05	5/04/2022	Noted		
Justin HOWES	07/06/2022	Angelina K	ELLER 27	//06/2022	Noted		
Angelina KELLER	21/07/2022				Not Required		
Angelina KELLER	10/03/2022	Angelina K	ELLER 10)/03/2022	Not Required		
Comments	Worl - sar will t - cas amp stan - sar micr - no	22 Angelina KELLER 10/03/2022 Not Required 7/06/2022 8:17:25 AM Justin HOWES: Workflow arrangements for samples as of 6 June, 2022: - samples will not have DIFP added to results in the quant range 0.001-0.0088ng/uL. These will be amplified after Quant. This applies to P2 and P3 samples. - case managers can assess samples for rework which could include a microcon after the first amplification. This does not apply to P3 samples which are processed without rework as per standard workflow arrangements. - samples currently reported as DIFP that are requested to be restarted by QPS will undergo microcon procedure. - no change to the P1 workflow where samples in the quant range 0.001-0.0088ng/uL will undergo a microcon prior to amplification.					
Response		6/2022 8:43:54 AM Ar d. To be added to SC		sion.			

Last Modified at 27/06/2022 8:43 AM by Angelina KELLER, Created on 7/06/2022 8:17 AM by Justin HOWES

Document Management: 33773 - V3.0 - Procedure for Profile Data Analysis using the Forensic Register

Version Status: Active

🖆 Add 🔄 View Document 📄 Print Comments 🧭 History								
General Reviews and A Workflow	pprovals Noti	fications Co	omments	Controlled Co	pies	Version History	Associations	Records
WORKHOW								
Comments By	Comme	ent Date	Res	ponse By		Response Date	Comm	ent Noted
Justin HOWES	04/04/2022		Angelina K	ELLER	05/0	4/2022	Noted	
Justin HOWES	07/06/2022		Angelina K	ELLER	27/0	6/2022	Noted	
✓ Angelina KELLER	21/07/2022						Not Require	ed
Angelina KELLER	10/03/2022		Angelina K	ELLER	10/0	3/2022	Not Require	ed
Comments		<u>21/07/2022 4</u> : Document still SOPs 33773, 3	l contains in	nages/tables fro	m FR	version 1 with upda	tes to be com	pleted once
lesponse								

Created on 21/07/2022 4:23 PM by Angelina KELLER

Document Management: 33773 - V3.0 - Procedure for Profile Data Analysis using the Forensic Register

Version Status: Active

🚔 Add 🛛 💾 View Document	Print C	comments 🥝	History					
General Reviews and App	rovals No	tifications Co	omments	Controlled Cop	pies	Version History	Associations	Records
Workflow							_	
Comments By	Comm	ient Date	Res	ponse By		Response Date	Comme	ent Noted
Justin HOWES	04/04/2022		Angelina K	ELLER	05/0	4/2022	Noted	
Justin HOWES	07/06/2022		Angelina K	ELLER	27/0	6/2022	Noted	
Angelina KELLER	21/07/2022						Not Required	d
Angelina KELLER	10/03/2022		Angelina K	ELLER	10/0	3/2022	Not Required	d
Comments		2 Angelina KELLER 10/03/2022 Not Required 10/03/2022 8:55:05 AM Angelina KELLER: 9/03/2022 2:40:39 PM Justin HOWES: 9/03/2022 2:40:39 PM Justin HOWES: When FSS creates a processing barcode to be used as a Reference sample in a case and th profile for the barcode has been approved by QPS to be used in another QP, Team LEader i to email QPS DNA Mgt with the actual barcode to request it to be associated to the second (more) QP. This will facilitate the ability to see the barcode in the CPT for both cases to be u for reporting purposes. Note: when creating the reference profile in the FR, ensure on the P page that the profile is transcribed and this transcription is checked by a second operator erif aliquot 2 from Bone is to be used as a ref, when creating the Reference sample, transcrib the alleles into the Profile Record and have this checked.					n LEader is e second (or es to be used e on the PDA operator eg.	
Response		<u>10/03/2022 8:55:43 AM Angelina KELLER:</u> To be incorporated into next version						

Last Modified at 10/03/2022 8:55 AM by Angelina KELLER, Created on 10/03/2022 8:55 AM by Angelina KELLER

Queensland Government



Documents	Document Manage	ment: 17117 - V2	21.0 - Procedure fo	r Case Manageme	ent				
Summary	Version Status: Active								
Search									
Notification Search	💣 Add 🖷 View Document	🚔 Add 🖻 View Document 🖹 Print Comments 🔗 History							
Resources									
Favourites	General Reviews and App	rovals Notifications Cor	nments Controlled Copies	Version History Associat					
Reports	Comments By	Comment Date	Response By	Response Date	Comment Noted				
PD	Justin HOWES	07/04/2021			NotRequired				
OQIS	Justin HOWES	04/04/2022			NotRequired				
Audits	Justin HOWES	19/04/2022			Not Required				
Calibration	Justin HOWES	13/05/2022			NotRequired				
Reminders	Justin HOWES	07/06/2022			Not Required				
Support	Justin HOWES	13/05/2021	Justin HOWES	10/03/2022	Not Required				
Reports	Kylie RIKA	19/07/2021	Justin HOWES	20/07/2021	Noted				
My QIS	Kylie RIKA	10/06/2021	Justin HOWES	20/07/2021	Not Required				
	Cassandra JAMES	22/07/2021	Justin HOWES	10/03/2022	Noted				
	Angela ADAMSON	09/08/2021	Justin HOWES	10/03/2022	Noted				
	Change page: < Prev 1 <u>2 Next ></u> Displaying page 1 of 2, items 1 to 10 of 12. Change page: 1 Go Page size: 10 Change								
	Comments	7/05/2022 8:17:	SIAM Justin HOWES;						
		- samples will n amplified after (case manager amplification, T workflow arran samples curre procedure.	ently reported as DIFP that are to the P1 workflow where samples	n the quant range 0.001-0.00 3 samples. rk which could indude a micr is which are processed withou requested to be restarted by	ocon after the first ut rework as per standard QPS will undergo a microcor				
	Response		10						

HealthSupport Queensland



Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, Cathie Allen

August 2015



Great state. Great opportunity.

Assessment of results obtained from 'automatic-microcon' samples

Published by the State of Queensland (Queensland Health), July 2015



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2015

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Department of Health, GPO Box 48, Brisbane QLD 4001

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

Document details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact: Contact officer: Josie Entwistle Title: Scientist – Forensic Intelligence & Reporting Phone: { USERADDRESS "+61 7" * MERGEFORMAT } Email:

Version history

Version	Date	Changed by	Description
0.1		Josie Entwistle	First Draft

Document sign off

This document has been approved by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist		

This document has been endorsed by:

Name	Position	Signature	Date
Justin Howes	Team Leader FRIT		
Luke Ryan	A/Team Leader ER & Q		
Sharon Johnstone	Senior Scientist Intel Team		
Kirsten Scott	Senior Scientist Q & P		
Allan McNevin	Senior Scientist ER		
Megan Matheison	A/Senior Scientist Analytical		
Amanda Reeves	Senior Scientist Reporting 1		
Kylie Rika	Senior Scientist Reporting 2		

Contents

1	Abstract	
2	Introduction	3
3	Materials and Methods	4
	3.1 <u>Materials</u>	4
	3.2 <u>Methods</u>	4
4	Results and Discussion	5
5	Conclusions and Recommendations	14
6	Abbreviations	
7	References	18

Figures

	4
tive samples	5
v non-informative	
ads	6
formative samples	
CIDD samples	8
f	ive samples v non-informative ads

Tables

Table 1	Automatic-microcon category data	3
---------	----------------------------------	---

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/ μ L and 0.0088 ng/ μ L 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/ μ L and 0.0088 ng/ μ L) 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

Assessment of results obtained from 'automatic-microcon' samples – Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, Cathie Allen

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

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.

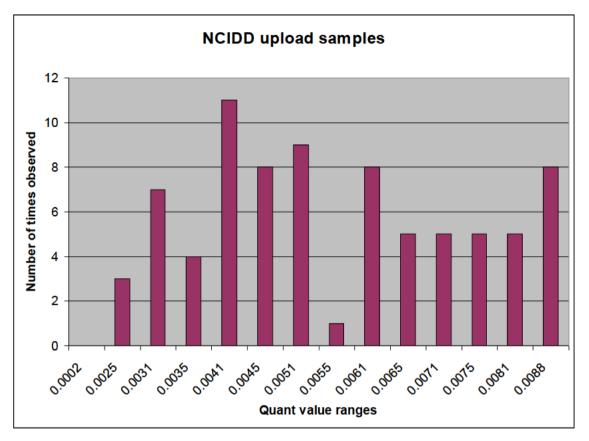


Figure 1 NCIDD upload samples

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/ μ L and 0.0025 ng/ μ L and the single NCIDD upload at the quant value range of 0.0055 ng/ μ L to 0.0061 ng/ μ L.

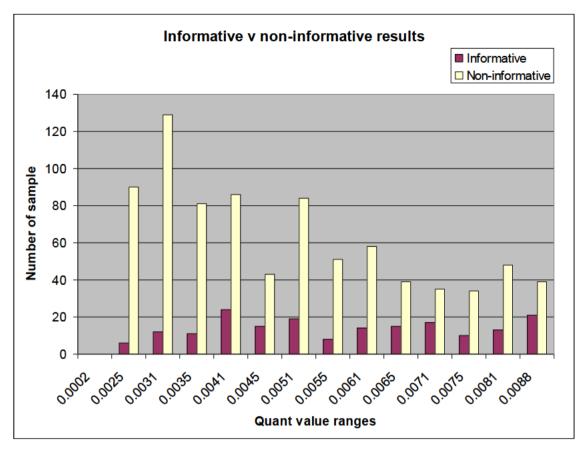


Figure 2 Informative v non-informative results

The number of non-informative results can be observed (see Figure 2) to decrease beyond the quantification value of $0.0035 \text{ ng/}\mu\text{L}$ 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 noninformative results for the majority of the quantification value ranges and remain fairly consistent across the quantification value ranges.

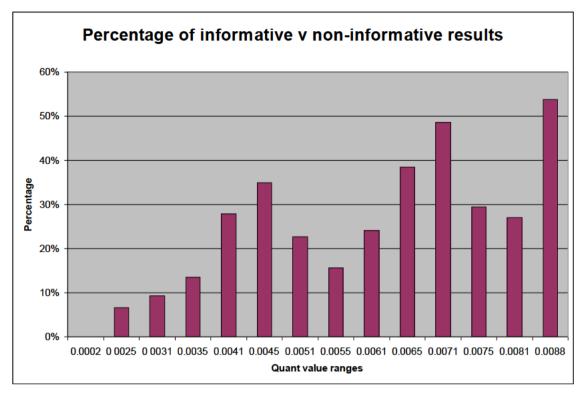


Figure 3 Percentage of informative v non-informative results

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.

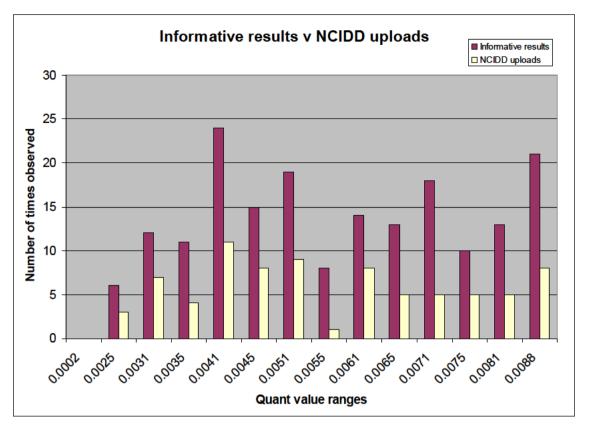


Figure 4 Informative results v NCIDD uploads

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/ μ L.

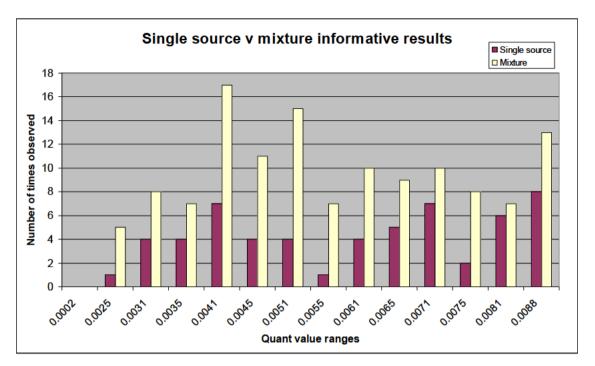


Figure 5 Single source v mixture informative results

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/ μ L, 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.

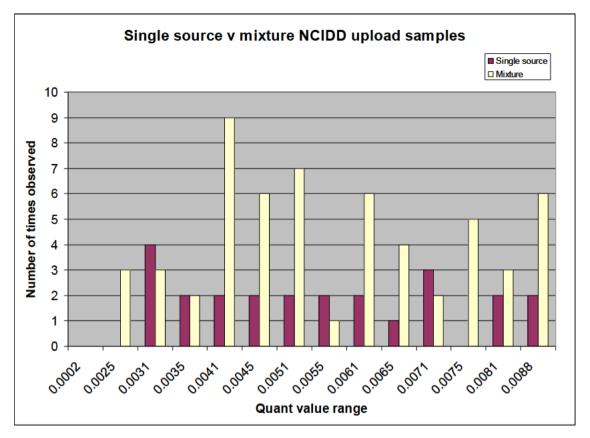


Figure 6 Single source v mixture NCIDD upload samples

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/ μ L 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/ μ L.

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/ μ L and 0.0081 ng/ μ L, appears to be fairly consistent within the quantification value ranges.

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.

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.

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.

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).

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.

Assessment of results obtained from 'automatic-microcon' samples – Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, Cathie Allen

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.

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.

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.

General recommendations and considerations

It is recommended that the quality of profiles obtained from samples processed at half microcon be explored, either in conjunction with the verification and implementation of new laboratory instruments, or as a stand alone assessment, based on existing data.

As the introduction of new instruments and processes have the capacity to vary these observations, it may be prudent to re-assess samples within this quantification range and/or being processed in this way, or by a changed process (as described above).

It would be possible, if desired, to expand this study to include additional parameters, for example, NCIDD upload details, and/or statistical analyses.

Communication with the QPS regarding any possible changes to our current process, including the risk and benefits is advised.

6. Abbreviations

СМ	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

HealthSupport Queensland



obtained from 'automaticmicrocon' samples

Josie Entwistle, Allison Lloyd, Kylie Rika, Thomas Nurthen, Cathie Allen

August 2015



Great state. Great opportunity.

Assessment of results obtained from 'automatic-microcon' samples

Published by the State of Queensland (Queensland Health), July 2015



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2015

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Department of Health, GPO Box 48, Brisbane QLD 4001

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

Document details

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact: Contact officer: Josie Entwistle Title: Scientist – Forensic Intelligence & Reporting Phone: {USERADDRESS "+61 7" * MERGEFORMAT }

Version history

Version	Date	Changed by	Description
0.1	11/11/2015	Josie Entwistle	First Draft
0.2	04/12/2015	Josie Entwistle	Second Draft

Document sign off

This document has been approved by:

Name	Position	Signature	Date
Cathie Allen	Managing Scientist		

This document has been endorsed by:

Name	Position	Signature	Date
Justin Howes	Team Leader FRIT		
Luke Ryan	A/Team Leader ER & Q		
Sharon Johnstone	Senior Scientist Intel Team		
Kirsten Scott	Senior Scientist Q & P		
Allan McNevin	Senior Scientist ER		
Megan Matheison	A/Senior Scientist Analytical		
Amanda Reeves	Senior Scientist Reporting 1		
Kylie Rika	Senior Scientist Reporting 2		

Contents

<u>1.</u>	Abstract	. 5
<u>2.</u>	Introduction	. 5
<u>3.</u>	Materials and Methods	. 6
	<u>3.1</u> <u>Materials</u>	6
	<u>3.2</u> <u>Methods</u>	6
<u>4.</u>	Results and Discussion	. 7
	<u>4.1</u> <u>Results</u>	7
	<u>4.2</u> <u>Discussion</u>	14
<u>5.</u>	Conclusions and Recommendations	16
	5.1. Process change consideration 1	16
	5.1.1.Benefits	17
	5.1.2. Risks and disadvantages	17
	5.2. Process change consideration 2	17
	5.2.1.Benefits	
	5.2.2. Risks and disadvantages	18
	5.3. Process change consideration 3	18
	5.3.1.Benefits	
	5.3.2. Risks and disadvantages	19
	5.4 Process change consideration 4	19
	5.4.1.Benefits	
	5.4.2. Risks and disadvantages	
	5.5. General recommendations and considerations	
<u>6.</u>	Abbreviations	20
<u>7.</u>	References	20

Figures

Figure 1 NCIDD upload samples	9
Figure 2 Informative v non-informative results	10
Figure 3 Percentage of informative v non-informative results	11
Figure 4 Informative results v NCIDD uploads	12
Figure 5 Single source v mixture informative results	13
Figure 6 Single source v mixture NCIDD upload samples	14

Tables

able 1 Automatic-microcon category data8

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/ μ L and 0.0088 ng/ μ L 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/ μ L and 0.0088 ng/ μ L) 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.

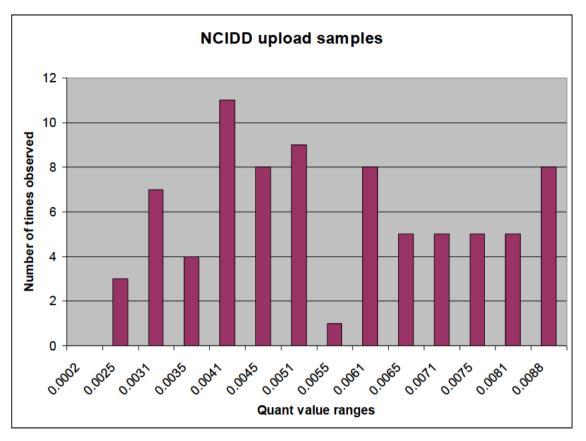


Figure 1 NCIDD upload samples

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/ μ L and 0.0025 ng/ μ L and the single NCIDD upload at the quant value range of 0.0055 ng/ μ L to 0.0061 ng/ μ L.

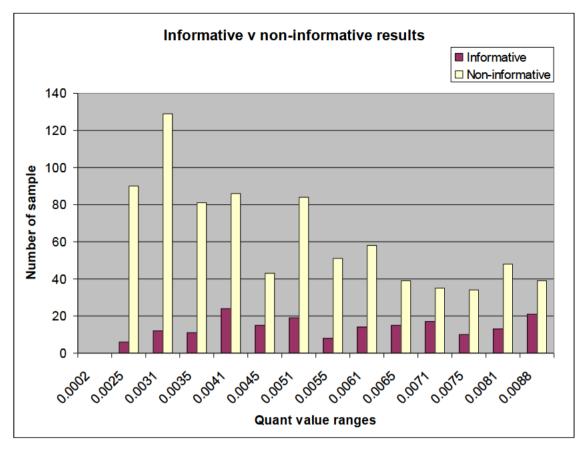


Figure 2 Informative v non-informative results

The number of non-informative results can be observed (see Figure 2) to decrease beyond the quantification value of $0.0035 \text{ ng/}\mu\text{L}$ 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 noninformative results for the majority of the quantification value ranges and remain fairly consistent across the quantification value ranges.

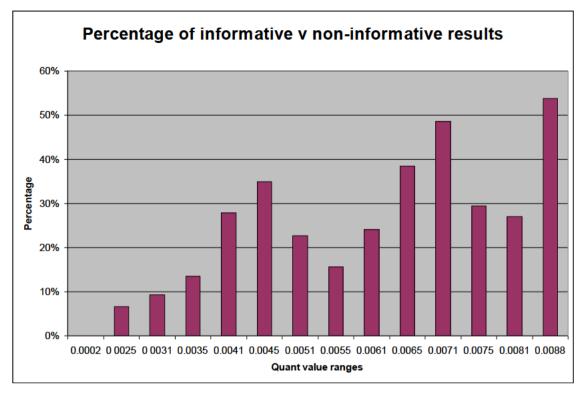


Figure 3 Percentage of informative v non-informative results

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.

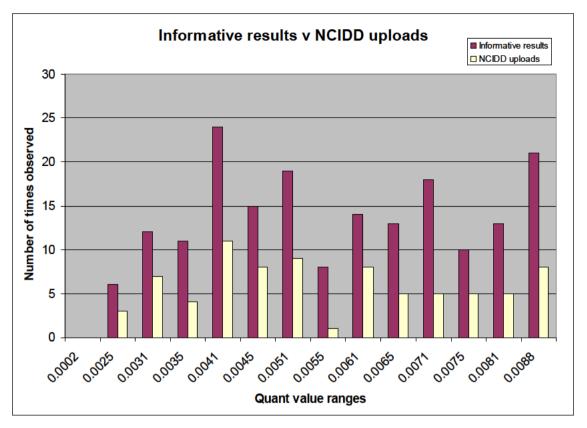


Figure 4 Informative results v NCIDD uploads

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/ μ L.

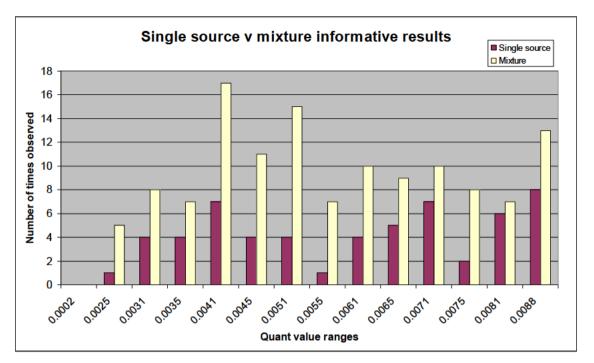


Figure 5 Single source v mixture informative results

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/ μ L, 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.

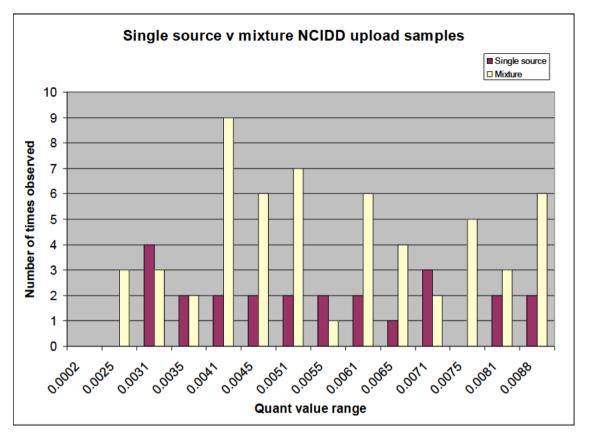


Figure 6 Single source v mixture NCIDD upload samples

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/ μ L 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/ μ L.

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/ μ L and 0.0081 ng/ μ L, 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/ μ L and 0.0088 ng/ μ L. 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

СМ	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

FW: Options Papers - First one and Draft of Second

From:	Lara Keller <	
To:	Megan Fairweather <	COI_DNA <
Date:	Mon, 08 Aug 2022 14:12:51 +1000	
Attachments: #184 Review of Microcon Options pa		S (Final report).pdf (633.18 kB); Assessment of low quant
	DNA Samples.docm (56.75 kB); Email advis	ce Supt Frieberg on Options Paper_Feb 2018.pdf (1.19 MB)

 From: Lara Keller

 Sent: Thursday, 2 June 2022 2:39 PM

 To: Petra Derr

 Fairweather

 Subject: FW: Options Papers - First one and Draft of Second

FYI All

Confidential and subject to legal privilege

Provided to Minister and DG at their request. Note that we had not issued the assessment report to QPS based upon previous legal advice.

Further information will be sent regarding:

- * Timeline for QPS approach re thresholds → draft report
- * Estimated number of QPS requests for further concentration of DNA where < 0.088 and insufficient DNA reported
- * Challenge re QPS data (Cathie working on this already)

DG will write to other state CEs to ask for their threshold data and analyser platforms to compare and contrast.

Thanks and Kind Regards

Lara
Lara
Lara
Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Lara Keller Sent: Thursday, 2 June 2022 2:33 PM M CC: Subject: FW: Options Papers - First one and Draft of Second

Good afternoon All

Papers attached as discussed.

2018 options paper : 1.86% were suitable to be uploaded to the National Criminal Investigation DNA database 2022 review paper: 5.3% " (but note smaller number assessed)

Thanks and Kind Regards



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Cathie Allen < Sent: Thursday, 2 To: Lara Keller < <u>d.gov.au</u>> Subject: Options Papers - First one and Draft of Second

Hi Lara

The first options paper is the pdf doc = #184 review of Microcon Options paper QPS. Attached email from Supt Frieberg advising her authorisation to proceed with the 'DNA Insufficient' process (dated Feb 2018).

I'll work on the rest and send as it's done.



*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Cathie Allen

From: Sent: To: Cc: Subject: Alison Slade Friday, 3 June 2022 4:38 PM Lara Keller Cathie Allen FW: Data and costs

Hi Lara

See below

Cheers

Option 1 – Preferred:

Revert to pre 2018 workflow – which is where all samples above a quant value of 0 are **processed through** to DNA profiling. Samples that are identified as being beneficial for concentration can be based on the DNA profile achieved, item criticality and case context.

Consumable costs (non-labour):

Under this change, approx 2,200 additional samples would be have to be **processed through** to DNA profiling in a 6 month period (based on sample volumes from 2021 calendar year). Additional costs of reagents would be: Profiling Kits: \$55,000.

Labour costs:

- Note: It takes 12 months to fully train a DNA scientist to report results and provide a Statement of Witness
 and give court evidence, however this option would not deliver timely assistance in managing the
 immediate additional workload created by reverting to the pre-2018 workflow.
- An alternative option to full-capability training: **Recruit 7 x HP3 Scientists** to work across a limited number of tasks to target high-volume and 'bottle-neck' processes, allowing fully-trained scientists to remain focussed on core responsibilities. The training required for this type of work could be completed within 14 weeks.

Option 2 – Least preferred:

Discontinue 2018 workflow and <u>concentrate</u> all samples with a quant value between 0 and 0.0088ng/uL and then <u>process through</u> to DNA profiling stage. Note, the concentration step creates a risk of there being no DNA sample available for testing by other technologies not undertaken in Queensland, future technologies or testing requested by Defence. In previous discussions, the QPS did not support an automatic concentration process, as the sample hadn't been assessed in the context of the case and may leave no sample remaining for future testing.

Costs: As per Option 1 plus \$20,000 for concentration kits.

Risks:

- Option 1: The DNA concentration step requires significant manual labour any significant volume increase for this part of the process could result in manual injury to staff (WH&S), fatigue and increase in lab errors.
- Both Options: Additional cost in overtime is highly likely in order for scientists to manage increased throughput, particularly until new additional HP3 scientists are adequately trained.

- Both Options: Increase in TAT for results to the QPS (adding approximately 1+ month's work to a 6 month period ie 7+ months' work to process in 6 months) which may equate to an increase of at least 1 week TAT increase from 2 weeks to 3+ weeks.
- Without additional staffing, the increase in TAT will likely create a backlog situation.
- Note also, there can be a decrease in throughput during training as competent staff members are producing less work due to the training burden.



Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

a 39 Kessels Road, Coopers Plains, QLD 4108 e w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Fwd: Forensic DNA testing impacts



Get Outlook for iOS

From: Lara Keller < Sent: Friday, June 3 To: Shaun Drummond <Shaun.Drum Subject: Forensic DNA testing impacts

Good afternoon Shaun

Kindly find below two options for the term-of-review process. Please note that these figures are estimates only.

Option 1 – Process Only (Preferred)

Revert to pre 2018 workflow – which is where all samples above a quant value of 0 are <u>processed through</u> to DNA profiling. Samples that are identified as being beneficial for concentration can be based on the DNA profile achieved, item criticality and case context.

Will increase TAT to report, plus generate approx. 6 weeks backlog per 6 months Estimated cost of kits plus IT = \$60K Overtime likely

Option 2 - Concentrate and Process (Least Preferred)

Discontinue 2018 workflow and <u>concentrate</u> all samples with a quant value between 0 and 0.0088ng/uL and then <u>process through</u> to DNA profiling stage.

<u>Risks</u>:

- 1. 1. concentration step creates a risk of there being no DNA sample available for testing by other technologies not undertaken in Queensland, future technologies or testing requested by Defence.
- 2. 2. in previous discussions, the QPS did not support an automatic concentration process, as the sample
- hadn't been assessed in the context of the case and may leave no sample remaining for future testing.
- 3. 3. concentration step is a manual process so will impact labour and TAT

Will increase TAT to report, plus generate approx. 3 months backlog per 6 months Estimated cost of kits plus IT = \$80K Overtime likely

To address subsequent backlog will require 5+ HP3 staff, noting that achieving minimum competency takes 3 months, full competency takes 12 months.

Thanks and Kind Regards



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Fwd: Forensic DNA testing impacts



Get Outlook for iOS

From: Lara Keller Sent: Friday, June 3, 2022 5:09:48 PM To: Shaun Drummond < Subject: Forensic DNA testing impacts

Good afternoon Shaun

Kindly find below two options for the term-of-review process. Please note that these figures are estimates only.

Option 1 - Process Only (Preferred)

Revert to pre 2018 workflow – which is where all samples above a quant value of 0 are **processed through** to DNA profiling. Samples that are identified as being beneficial for concentration can be based on the DNA profile achieved, item criticality and case context. Will increase TAT to report, plus generate approx. 6 weeks backlog per 6 months Estimated cost of kits plus IT = \$60K Overtime likely

Option 2 - Concentrate and Process (Least Preferred)

Discontinue 2018 workflow and <u>concentrate</u> all samples with a quant value between 0 and 0.0088ng/uL and then process through to DNA profiling stage.

<u>Risks</u>:

- 1. 1. concentration step creates a risk of there being no DNA sample available for testing by other technologies not undertaken in Queensland, future technologies or testing requested by Defence.
- 2. 2. in previous discussions, the QPS did not support an automatic concentration process, as the sample hadn't been assessed in the context of the case and may leave no sample remaining for future testing.
- 3. 3. concentration step is a manual process so will impact labour and TAT
- Will increase TAT to report, plus generate approx. 3 months backlog per 6 months Estimated cost of kits plus IT = \$80K Overtime likely

To address subsequent backlog will require 5+ HP3 staff, noting that achieving minimum competency takes 3 months, full competency takes 12 months.

Thanks and Kind Regards



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

FW: Documents - timeline and number of requests

From:	Lara Keller <	
То:	Megan Fairweather <	COI_DNA <
Date:	Mon, 08 Aug 2022 14:13:32 +1000	
Attachments:	Timeline of communcations between QPS and QHFSS.docm (76.05 kB); Requests for processing_2021 2022.docm (57.13 kB); DNA insuff samples further processed_Sexual Offences.xlsx (16.59 kB)	

From: Lara Keller Sen To: Subject: d number of Importance: High
Good afternoon Apologies as the initial emails were rejected. Kindly find resend below and attached. Kind regards Lara
From: Lara Keller Sent: Thursday, 2 June 2022 3:47 PM Shaun Drummond < <u>Shaun Drumm</u>
CC: FSS Como < Subject: FW: Do ts Importance: High
Good afternoon All
 As requested, kindly find attached: 1. 1. Timeline re QPS and FSS engagement regarding thresholds 2. 2. Number of requests for further concentration of samples reported as "Insufficient DNA Detected) Note: We are unable to readily identify outcomes of the requests without full case file reviews for each request. This would require a number of staff to go offline for some days as we do not have the capability via the IT platform to mine this data. 3. 3. Cathie Allen's start of her review to challenge/confirm the findings put forward by QPS. This is a laborious case file review process as well.
Thanks and Kind Regards Lara
Lara Keller B App Sc (MLS), Grad Cert Health Mgt, MAIMS, CMgr FIML A/Executive Director Forensic and Scientific Services Prevention Division, Queensland Health
Administration, Level 1, 39 Kessels Road, Coopers Plains, QLD, 4108 w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Cathie Allen <
Sent: Thursday, 2
To: Lara Keller <
<u>d.gov.au</u>>
Subject: Docume mber of requests
Importance: High

Attached is the Timeline of communications, and a collation of the number of requests for processing of DNA Insufficient samples for 2021 and 2022.

Attached is the excel spreadsheet that I've been working on – reviewing whether the processing of a DNA insufficient gave a new DNA profile that hadn't been seen before (given we don't know how the QPS are making decisions on what to process). I haven't finished but here's what I've got so far.





Fwd: Options Papers - First one and Draft of Second

From:	Shaun Drummond <
To:	yvette.
Date:	Mon, 06 Jun 2022 09:41:11 +1000
Attachments:	#184 Review of Microcon Options paper QPS (Final report).pdf (633.18 kB); Assessment of low quant DNA Samples.docm (56.75 kB); Email advice Supt Frieberg on Options Paper_Feb 2018.pdf (1.19 MB)

Get Outlook for iOS

From: Lara Keller <				
Sent: Thursday, Jun				
To: Shau	m			
<yvette< td=""><td></td><td></td><td>4</td><td></td></yvette<>			4	
Matthew				
Cc: FSS Corro <		v.au>		
Subject: FW: Op		Second		

Good afternoon All

Papers attached as discussed.

2018 options paper : 1.86% were suitable to be uploaded to the National Criminal Investigation DNA database 2022 review paper: 5.3% " " (but note smaller number assessed)

Thanks and Kind Regards
_ara
Lara Keller B App Sc (MLS), Grad Cert Health Mgt, MAIMS, CMgr FIML
A/Executive Director
Forensic and Scientific Services
Pr <u>evention Division, Queensland He</u> alth
a Administration, Level 1, 39 Kessels Road, Coopers Plains, QLD, 4108
e www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Cathie Allen < Sent: Thursday, 2 To: Lara Keller < dot for the second d.gov.au> Subject: Options Papers - First one and Draft of Second

Hi Lara

The first options paper is the pdf doc = #184 review of Microcon Options paper QPS. Attached email from Supt Frieberg advising her authorisation to proceed with the 'DNA Insufficient' process (dated Feb 2018).

I'll work on the rest and send as it's done.

Cheers Cathie

Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the

Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022 **Police Services Stream, Forensic & Scientific Services** Prevention Division. Queensland Health

p a <u>39 Kessels Road, Coopers Plains, QLD</u> 4108

e

w <u>www.health.qld.gov.au/fss</u>

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





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

January 2018 Justin Howes and Cathie Allen



Great state. Great opportunity.

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

Published by the State of Queensland (Queensland Health), January 2018



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2018

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

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

Document Details

.

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

Contact officer:	Justin Howes
Title:	Team Leader - Forensic Reporting and Intelligence Team
Phone:	+61 7
Email:	

Contents

Doc	ument Details
1.	Abstract
2.	Definitions
3.	Introduction
4.	Data interrogation
5.	Assessment of 'auto-microcon' results
6. Quai	Datamine of the difference in pre- and post- Microcon [®] ntification values
7.	Results and Discussion
7.1 A	Assessment of 'auto-microcon' results
7.2 Quai	Datamine of the difference in pre- and post- Microcon [®] ntification values
8.	Options for consideration
9.	References

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 postextraction process within Forensic DNA Analysis to reduce the volume of extract from approximately 100uL to $\leq 35\mu$ L 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 0.001 ng/ μ L to 0.0088 ng/ μ L, 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 0.001 ng/µL and less than 0.015 ng/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/ μ L (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

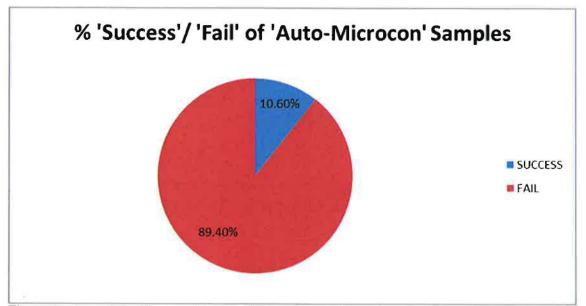


Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

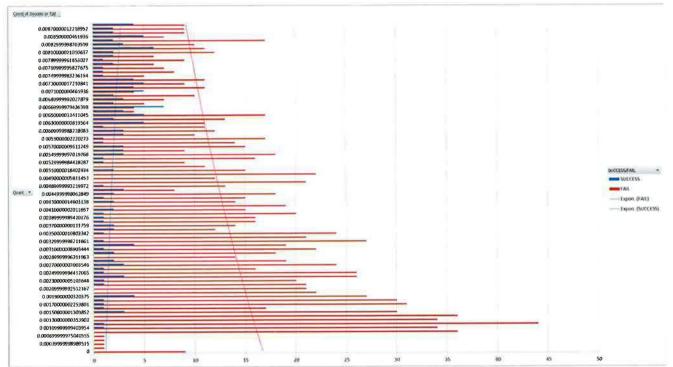


Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

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)..

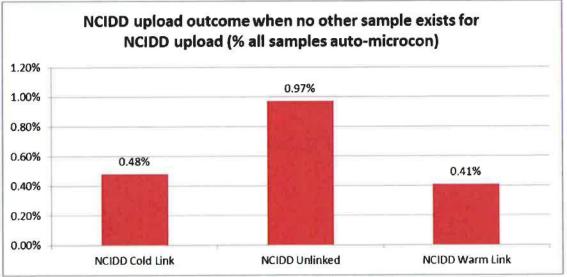


Figure 3: NCIDD outcome for samples that were loaded to NCIDD

A review of the automatic concentration of DNA extracts using Microcon[®] Centrifugal Filter Devices: Options for QPS consideration. 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 0.001 ng/µL where the final result was 'success'.

As the Microcon[®] process concentrates the DNA extract from approximately 100uL to approximately 35 μ L, 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'.

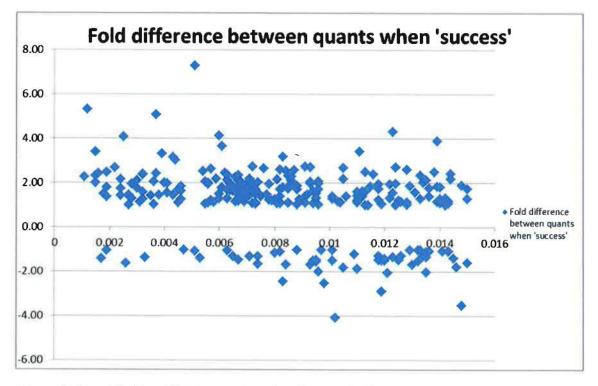


Figure 4: Quantification differences pre and post concentration

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,
- 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);

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

- 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

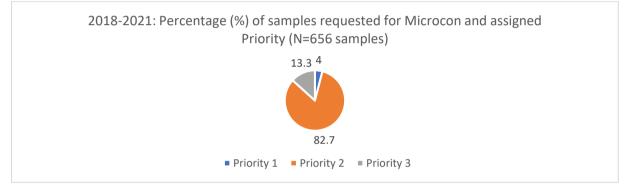
- [1] QIS 19544v11 Concentration of DNA Extracts Using Microcon Centrifugal Filter Devices
- [2] PowerPlex[®] 21– Amplification of Extracted DNA Validation. Megan Mathieson, Thomas Nurthen, Cathie Allen. December 2012. Forensic DNA Analysis.
- [3] QIS 23008v15 Explanation of EXR/EXH Results
- [4] QIS 24012v13 Miscellaneous Analytical Section Tasks

Assessment of Low Quantification Value DNA Samples

Authors: Cathie Allen, Justin Howes and Paula Brisotto

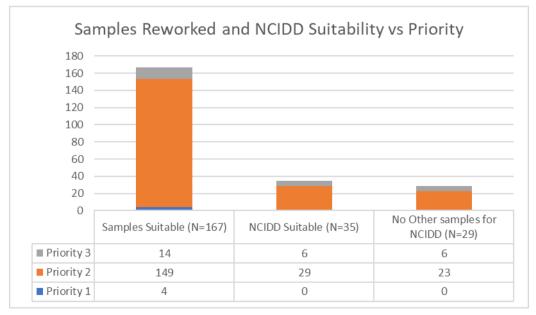
Executive Briefing:

An assessment of all casework DNA samples, with the following criteria was conducted: an initial quantification result of between zero and 0.0088ng/µL, underwent a concentration step and reported results produced between 2018 and 2021. This equated to an assessment of 656 DNA samples. The reported DNA result, which may have been completed after one or more amplifications steps, was categorised into two broad categories - 'suitable for comparison purposes' or 'unsuitable for comparison purposes'.



167 DNA samples (25.5%) were categorised as 'suitable for comparison purposes', with most of these samples being major crime samples. 456 DNA samples (74.5%) were categorised as 'unsuitable for comparison purposes' after concentration and amplification processes.

Of the 167 DNA samples categorised as 'suitable for comparison purposes', 35 DNA samples were able to yield a profile suitable for uploading and searching of the National Criminal Investigation DNA Database (NCIDD). This represents 5.3% of total samples selected for processing.





Please note the current dataset is different to the previous dataset due to, but not limited to: implementation of the statistical interpretation of four-person mixtures, all DNA samples were selected in this dataset (previously the dataset only included DNA samples assigned to Major Crime cases), active selection of samples for processing by either the Queensland Police Service or Forensic DNA Analysis staff members based on the context of the case or scientific knowledge with respect to the associated parameters from the quantification process, and new instrumentation implemented over that period.

Forensic staff are mindful of consuming all DNA extract when requesting a concentration step. Future technologies may be applied to DNA extracts, however if all extract has been exhausted (through concentration and amplifications processes), no extract will be available for these technologies.

Observations:

Review of quantitation parameters, other than quantitation value, did not yield a trend, however further monitoring of these parameters will be conducted.

The value of 0.0088ng/µL is based on assessment of the data (and equates to 132 picograms). The value of 0.0067ng/µL is based on equating to 100 picograms, and not based on assessment of data.

Options for Consideration:

- 1. Continue with the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL (132 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received.
- 2. Amend the current workflow: **RISKS**
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of 0.001 ng/µL to 0.0067ng/µL (100 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received. DNA samples above 0.0067ng/µL will be processed as per routine and will not be subject to a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.
- 3. Amend the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of either 0.001ng/µL to 0.0088ng/µL or 0.001ng/µL to 0.0067ng/µL and processed upon request. Priority 3 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088 ng/µL or 0.001ng/µL to 0.0067ng/µL will be amplified without a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.



Cathie Allen

From:	Frieberg.DaleJ[OSC] <
Sent:	Friday, 2 February 2018 3:38 PM
To:	Cathie Allen; O'Malley.TroyS[OSC]; Taylor.EwenN[OSC]
Cc:	Paul Csoban
Subject:	RE: Options Paper for consideration

Hi Cathie and Paul,

Thank you for your time this afternoon and for discussion around this options paper. Thank you also to both Troy and Ewen with your assistance and expertise/advice around the paper.

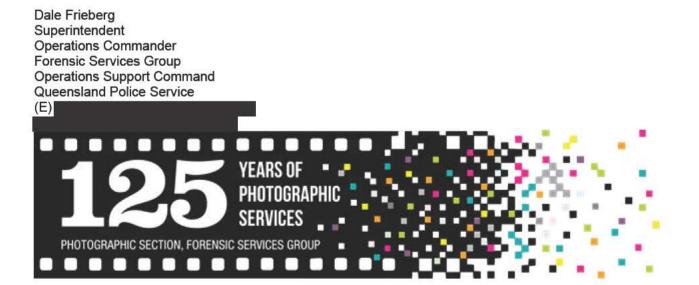
As discussed, I am in agreement that:

- There is clear data that it is not an efficient use of time and resources to continue with the 'auto-microcon' process for Priority 2 (*Major Crime*) samples.
- Option 2. "Cease the 'auto-microcon' process for Priority 2 casework...." Would appear to be a more productive & efficient choice.
- Scientists time and resources would be better spent working samples with a higher DNA yield and more
 potential.
- It would be beneficial to amend the Forensic Register to provide an automated Q-Prime update advising the Investigators of the option to request further 'Auto-microcon' processing for those samples for unsolved crime, which may prove worthwhile.
- DNA staff can request this additional processing if/when a request is received from the investigators.

I trust this is of assistance.

Kind regards,

Dale.



From: Cathie Allen [mailto:		
Sent: Tuesday, 30 January 2018 4	:56 PM	
To: Frieberg.DaleJ[OSC] <		O'Malley.TroyS[OSC]
<	Taylor.EwenN[OSC] <	
Cc: Paul Csoban <		

Subject: Options Paper for consideration

Hi Dale

Please find attached an Options paper regarding concentration of major crime samples that we have prepared for your consideration. I'd like to discuss this on Friday with you.

Cheers Cathie



Cathie Allen

Managing Scientist – Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health



HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

This email, including any attachments sent with it, is confidential and for the sole use of the intended recipient(s). This confidentiality is not waived or lost, if you receive it and you are not the intended recipient(s), or if it is transmitted/received in error.

Any unauthorised use, alteration, disclosure, distribution or review of this email is strictly prohibited. The information contained in this email, including any attachment sent with it, may be subject to a statutory duty of confidentiality if it relates to health service matters.

If you are not the intended recipient(s), or if you have received this email in error, you are asked to immediately notify the sender by telephone collect on Australia +61 1800 198 175 or by return email. You should also delete this email, and any copies, from your computer system network and destroy any hard copies produced.

If not an intended recipient of this email, you must not copy, distribute or take any action(s) that relies on it; any form of disclosure, modification, distribution and/or publication of this email is also prohibited.

Although Queensland Health takes all reasonable steps to ensure this email does not contain malicious software, Queensland Health does not accept responsibility for the consequences if any person's computer inadvertently suffers any disruption to services, loss of information, harm or is infected with a virus, other malicious computer programme or code that may occur as a consequence of receiving this email.

Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest

immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error, please inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

FSS Threshold words

From:	Simon Zanatta <	
То:	Shaun Drummond < <	Jasmina Joldic
Date:	Mon, 06 Jun 2022 09:42:07 +1000	
Attachments:	FSS thresholds.docx (14.52 kB)	

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.



Simon Zanatta Chief of Staff Office of the Hon. Yvette D'Ath MP Minister for Health and Ambulance Services M: Manual Street Brisbane QLD 4000

Government This email, together with any attachments, is intended for the named recipient(s) only; and may contain privileged and confidential information. If received in error, you are asked to inform the sender as quickly as possible and delete this email and any copies of this from your computer system network.

If not an intended recipient of this email, you must not copy, distribute or take any action(s) that relies on it; any form of disclosure, modification, distribution and /or publication of this email is also prohibited.

Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

Please consider the environment before printing this email.

Fwd: Options Papers - First one and Draft of Second

From: To:	Shaun Drummond <	
Date:	Mon, 06 Jun 2022 09:43:48 +1000	
Attachments:	#184 Review of Microcon Options paper QPS (Final report).pdf (633.18 kB); Assessment of low quant	
	DNA Samples.docm (56.75 kB); Email advice Supt Frieberg on Options Paper_Feb 2018.pdf (1.19 MB)	

Get Outlook for iOS

From: Shaun Drummond <	
Sent: Monday, June 6, 2022 9:41:11 AM	
To: Yvette	<yvette< td=""></yvette<>
Subject: Fwd: Options Papers - First one	e and Dratt of Second

Get Outlook for iOS

From: Lara Keller < Sent: Thursday, Juh Shaun Drummond <shaun drumm<="" td=""><td></td></shaun>	
Matthew Rigby ≤ Cc: FSS Corro ≤ Subject: FW: Op	v.au> Second

Good afternoon All

Papers attached as discussed.

2018 options paper : 1.86% were suitable to be uploaded to the National Criminal Investigation DNA database 2022 review paper: 5.3% " (but note smaller number assessed)

Thanks and Kind Regards



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Cathie Allen < Sent: Thursday, 2 To: Lara Keller < Subject: Options Papers - First one and Draft of Second

Hi Lara

The first options paper is the pdf doc = #184 review of Microcon Options paper QPS. Attached email from Supt Frieberg advising her authorisation to proceed with the 'DNA Insufficient' process (dated Feb 2018).

I'll work on the rest and send as it's done.

Cheers Cathie Cathie Allen Bsc, MSc (Forensic Science) (She/Her*) Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022 Police Services Stream, Forensic & Scientific Services Prevention Division. Queensland Health a 39 Kessels Road, Coopers Plains, QLD 4108 e w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





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

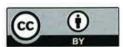
January 2018 Justin Howes and Cathie Allen



Great state. Great opportunity.

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

Published by the State of Queensland (Queensland Health), January 2018



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2018

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

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

Document Details

.

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

 Contact officer:
 Justin Howes

 Title:
 Team Leader – Forensic Reporting and Intelligence Team

 Phone:
 Email:

Contents

Doc	ument Details
1.	Abstract
2.	Definitions
3.	Introduction
4.	Data interrogation
5.	Assessment of 'auto-microcon' results
6. Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
7.	Results and Discussion
7.1 A	Assessment of 'auto-microcon' results 6
7.2 Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
8.	Options for consideration
9.	References

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 postextraction process within Forensic DNA Analysis to reduce the volume of extract from approximately 100uL to $\leq 35\mu$ L 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 0.001 ng/ μ L to 0.0088 ng/ μ L, 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 0.001 ng/µL and less than 0.015 ng/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/ μ L (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

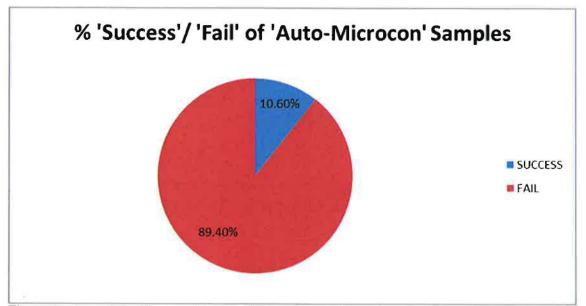


Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

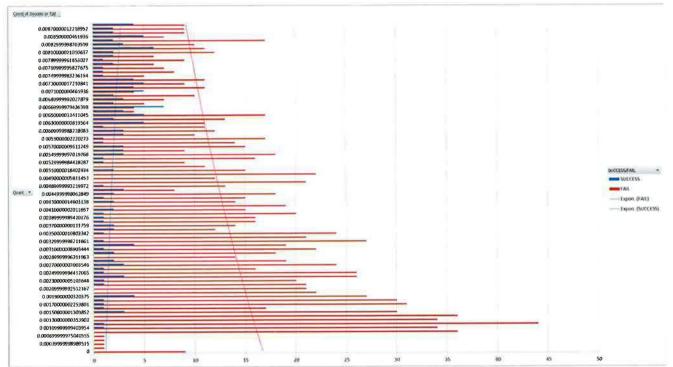


Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

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)..

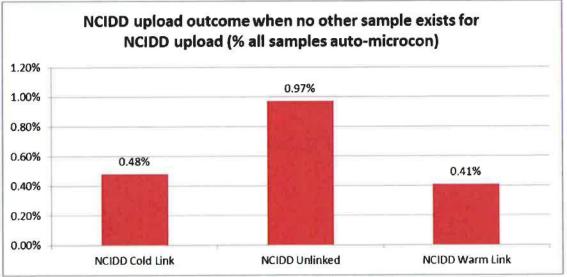


Figure 3: NCIDD outcome for samples that were loaded to NCIDD

A review of the automatic concentration of DNA extracts using Microcon[®] Centrifugal Filter Devices: Options for QPS consideration. 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 0.001 ng/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'.

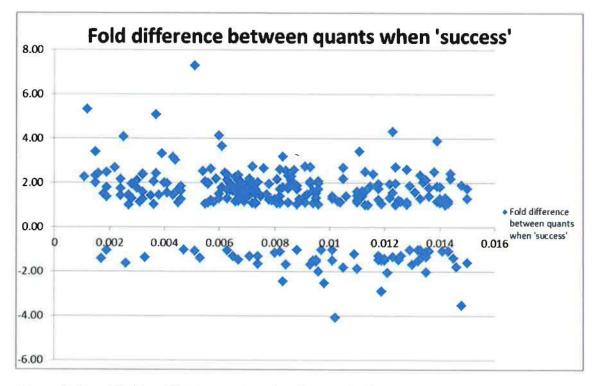


Figure 4: Quantification differences pre and post concentration

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,
- 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);

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

- 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

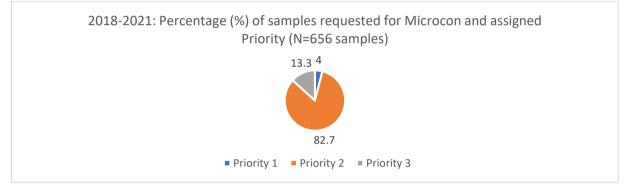
- [1] QIS 19544v11 Concentration of DNA Extracts Using Microcon Centrifugal Filter Devices
- [2] PowerPlex[®] 21– Amplification of Extracted DNA Validation. Megan Mathieson, Thomas Nurthen, Cathie Allen. December 2012. Forensic DNA Analysis.
- [3] QIS 23008v15 Explanation of EXR/EXH Results
- [4] QIS 24012v13 Miscellaneous Analytical Section Tasks

Assessment of Low Quantification Value DNA Samples

Authors: Cathie Allen, Justin Howes and Paula Brisotto

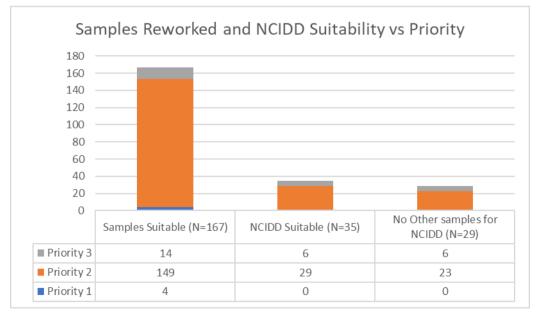
Executive Briefing:

An assessment of all casework DNA samples, with the following criteria was conducted: an initial quantification result of between zero and 0.0088ng/µL, underwent a concentration step and reported results produced between 2018 and 2021. This equated to an assessment of 656 DNA samples. The reported DNA result, which may have been completed after one or more amplifications steps, was categorised into two broad categories - 'suitable for comparison purposes' or 'unsuitable for comparison purposes'.



167 DNA samples (25.5%) were categorised as 'suitable for comparison purposes', with most of these samples being major crime samples. 456 DNA samples (74.5%) were categorised as 'unsuitable for comparison purposes' after concentration and amplification processes.

Of the 167 DNA samples categorised as 'suitable for comparison purposes', 35 DNA samples were able to yield a profile suitable for uploading and searching of the National Criminal Investigation DNA Database (NCIDD). This represents 5.3% of total samples selected for processing.





Please note the current dataset is different to the previous dataset due to, but not limited to: implementation of the statistical interpretation of four-person mixtures, all DNA samples were selected in this dataset (previously the dataset only included DNA samples assigned to Major Crime cases), active selection of samples for processing by either the Queensland Police Service or Forensic DNA Analysis staff members based on the context of the case or scientific knowledge with respect to the associated parameters from the quantification process, and new instrumentation implemented over that period.

Forensic staff are mindful of consuming all DNA extract when requesting a concentration step. Future technologies may be applied to DNA extracts, however if all extract has been exhausted (through concentration and amplifications processes), no extract will be available for these technologies.

Observations:

Review of quantitation parameters, other than quantitation value, did not yield a trend, however further monitoring of these parameters will be conducted.

The value of 0.0088ng/µL is based on assessment of the data (and equates to 132 picograms). The value of 0.0067ng/µL is based on equating to 100 picograms, and not based on assessment of data.

Options for Consideration:

- 1. Continue with the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL (132 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received.
- 2. Amend the current workflow: **RISKS**
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of 0.001 ng/µL to 0.0067ng/µL (100 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received. DNA samples above 0.0067ng/µL will be processed as per routine and will not be subject to a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.
- 3. Amend the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of either 0.001ng/µL to 0.0088ng/µL or 0.001ng/µL to 0.0067ng/µL and processed upon request. Priority 3 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088 ng/µL or 0.001ng/µL to 0.0067ng/µL will be amplified without a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.



Cathie Allen

From:	Frieberg.DaleJ[OSC] <
Sent:	Friday, 2 February 2018 3:38 PM
То:	Cathie Allen; O'Malley.TroyS[OSC]; Taylor.EwenN[OSC]
Cc:	Paul Csoban
Subject:	RE: Options Paper for consideration

Hi Cathie and Paul,

Thank you for your time this afternoon and for discussion around this options paper. Thank you also to both Troy and Ewen with your assistance and expertise/advice around the paper.

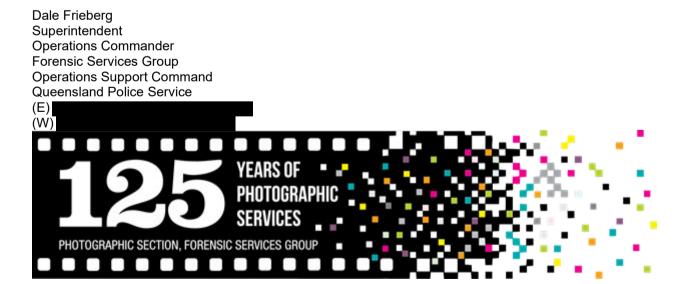
As discussed, I am in agreement that:

- There is clear data that it is not an efficient use of time and resources to continue with the 'auto-microcon' process for Priority 2 (*Major Crime*) samples.
- Option 2. "Cease the 'auto-microcon' process for Priority 2 casework...." Would appear to be a more productive & efficient choice.
- Scientists time and resources would be better spent working samples with a higher DNA yield and more potential.
- It would be beneficial to amend the Forensic Register to provide an automated Q-Prime update advising the Investigators of the option to request further 'Auto-microcon' processing for those samples for unsolved crime, which may prove worthwhile.
- DNA staff can request this additional processing if/when a request is received from the investigators.

I trust this is of assistance.

Kind regards,

Dale.



From: Cathie Allen [mailto:		
Sent: Tuesday, 30 January 2018 4:	56 PM	
To: Frieberg.DaleJ[OSC] <		O'Malley.TroyS[OSC]
<	Taylor.EwenN[OSC] <	
Cc: Paul Csoban <		

Subject: Options Paper for consideration

Hi Dale

Please find attached an Options paper regarding concentration of major crime samples that we have prepared for your consideration. I'd like to discuss this on Friday with you.

Cheers Cathie



Cathie Allen

Managing Scientist – Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health



HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

This email, including any attachments sent with it, is confidential and for the sole use of the intended recipient(s). This confidentiality is not waived or lost, if you receive it and you are not the intended recipient(s), or if it is transmitted/received in error.

Any unauthorised use, alteration, disclosure, distribution or review of this email is strictly prohibited. The information contained in this email, including any attachment sent with it, may be subject to a statutory duty of confidentiality if it relates to health service matters.

If you are not the intended recipient(s), or if you have received this email in error, you are asked to immediately notify the sender by telephone collect on Australia +61 1800 198 175 or by return email. You should also delete this email, and any copies, from your computer system network and destroy any hard copies produced.

If not an intended recipient of this email, you must not copy, distribute or take any action(s) that relies on it; any form of disclosure, modification, distribution and/or publication of this email is also prohibited.

Although Queensland Health takes all reasonable steps to ensure this email does not contain malicious software, Queensland Health does not accept responsibility for the consequences if any person's computer inadvertently suffers any disruption to services, loss of information, harm or is infected with a virus, other malicious computer programme or code that may occur as a consequence of receiving this email.

Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest

immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error, please inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

Fwd: Options Papers - First one and Draft of Second

From: To:	Shaun Drummond <	
Date:	Mon, 06 Jun 2022 09:45:31 +1000	
Attachments:	#184 Review of Microcon Options paper QPS (Final report).pdf (633.18 kB); Assessment of low quant DNA Samples.docm (56.75 kB); Email advice Supt Frieberg on Options Paper_Feb 2018.pdf (1.19 MB)	

Get Outlook for iOS

From: Shaun Drummond <	
Sen Monday June 6 202 To: au	
Subject: Fwa: Options Papers - First one and Dratt of Second	

Get Outlook for iOS

From: Shaun Drummond < Sen_Monday. June 6. 2022 9:41:11 AM	
To: Subject: Fwd: Options Papers - First one and Dratt of Second	

Get Outlook for iOS

From: Lara Keller < Sent: Thursday, Juhe 2, 2022 2:33 pm m	
Cc: FSS Corro < Subject: FW: Op	v.au> Second

Good afternoon All

Papers attached as discussed.

2018 options paper : 1.86% were suitable to be uploaded to the National Criminal Investigation DNA database 2022 review paper: 5.3% " " (but note smaller number assessed)



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

To: Lara Keller < Subject: Options Papers - First one and Dratt of Second

Hi Lara

The first options paper is the pdf doc = #184 review of Microcon Options paper QPS. Attached email from Supt Frieberg advising her authorisation to proceed with the 'DNA Insufficient' process (dated Feb 2018).

I'll work on the rest and send as it's done.







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

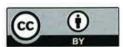
January 2018 Justin Howes and Cathie Allen



Great state. Great opportunity.

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

Published by the State of Queensland (Queensland Health), January 2018



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2018

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

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

Document Details

.

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

 Contact officer:
 Justin Howes

 Title:
 Team Leader – Forensic Reporting and Intelligence Team

 Phone:
 Email:

Contents

Doc	ument Details
1.	Abstract
2.	Definitions
3.	Introduction
4.	Data interrogation
5.	Assessment of 'auto-microcon' results
6. Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
7.	Results and Discussion
7.1 A	Assessment of 'auto-microcon' results 6
7.2 Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
8.	Options for consideration
9.	References

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 postextraction process within Forensic DNA Analysis to reduce the volume of extract from approximately 100uL to $\leq 35\mu$ L 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 0.001 ng/ μ L to 0.0088 ng/ μ L, 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 0.001 ng/µL and less than 0.015 ng/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/ μ L (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

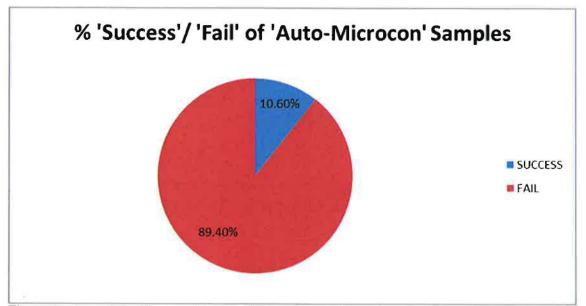


Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

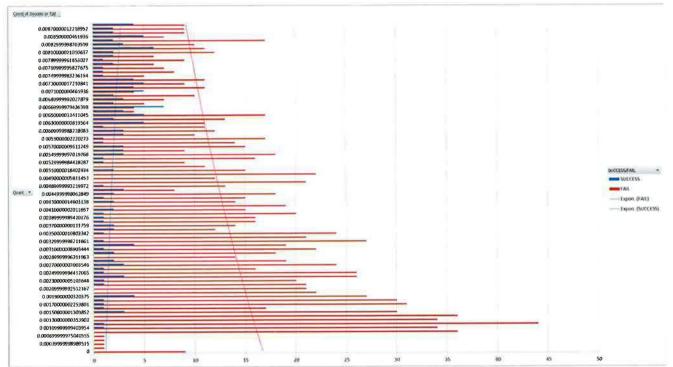


Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

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)..

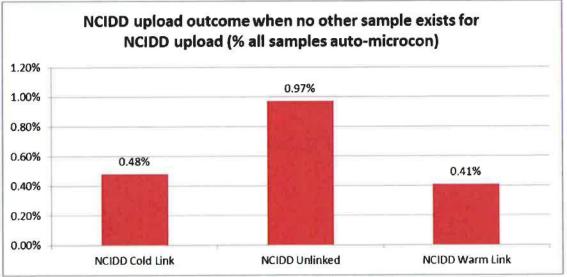


Figure 3: NCIDD outcome for samples that were loaded to NCIDD

A review of the automatic concentration of DNA extracts using Microcon[®] Centrifugal Filter Devices: Options for QPS consideration. 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 0.001 ng/µL where the final result was 'success'.

As the Microcon[®] process concentrates the DNA extract from approximately 100uL to approximately 35 μ L, 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'.

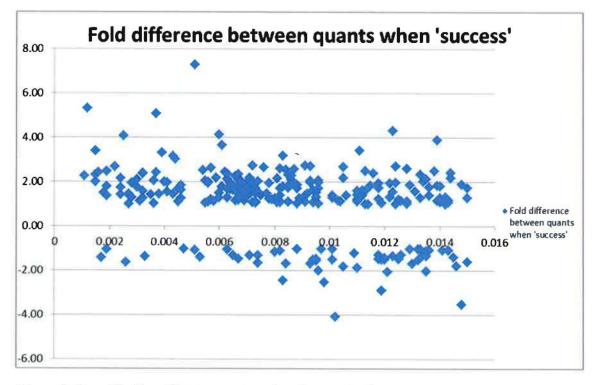


Figure 4: Quantification differences pre and post concentration

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,
- 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);

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

- 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

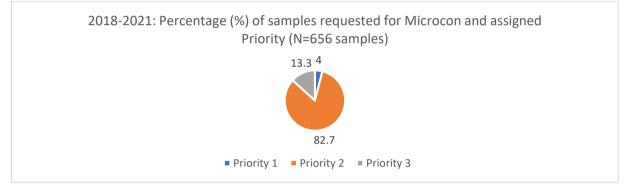
- [1] QIS 19544v11 Concentration of DNA Extracts Using Microcon Centrifugal Filter Devices
- [2] PowerPlex[®] 21– Amplification of Extracted DNA Validation. Megan Mathieson, Thomas Nurthen, Cathie Allen. December 2012. Forensic DNA Analysis.
- [3] QIS 23008v15 Explanation of EXR/EXH Results
- [4] QIS 24012v13 Miscellaneous Analytical Section Tasks

Assessment of Low Quantification Value DNA Samples

Authors: Cathie Allen, Justin Howes and Paula Brisotto

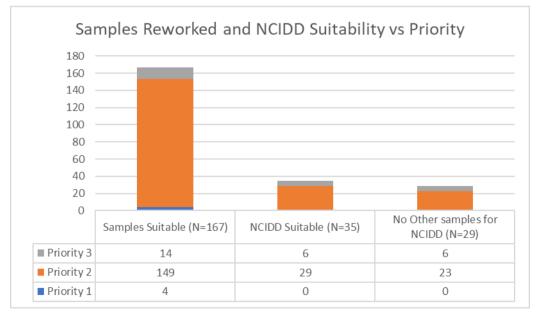
Executive Briefing:

An assessment of all casework DNA samples, with the following criteria was conducted: an initial quantification result of between zero and 0.0088ng/µL, underwent a concentration step and reported results produced between 2018 and 2021. This equated to an assessment of 656 DNA samples. The reported DNA result, which may have been completed after one or more amplifications steps, was categorised into two broad categories - 'suitable for comparison purposes' or 'unsuitable for comparison purposes'.



167 DNA samples (25.5%) were categorised as 'suitable for comparison purposes', with most of these samples being major crime samples. 456 DNA samples (74.5%) were categorised as 'unsuitable for comparison purposes' after concentration and amplification processes.

Of the 167 DNA samples categorised as 'suitable for comparison purposes', 35 DNA samples were able to yield a profile suitable for uploading and searching of the National Criminal Investigation DNA Database (NCIDD). This represents 5.3% of total samples selected for processing.





Please note the current dataset is different to the previous dataset due to, but not limited to: implementation of the statistical interpretation of four-person mixtures, all DNA samples were selected in this dataset (previously the dataset only included DNA samples assigned to Major Crime cases), active selection of samples for processing by either the Queensland Police Service or Forensic DNA Analysis staff members based on the context of the case or scientific knowledge with respect to the associated parameters from the quantification process, and new instrumentation implemented over that period.

Forensic staff are mindful of consuming all DNA extract when requesting a concentration step. Future technologies may be applied to DNA extracts, however if all extract has been exhausted (through concentration and amplifications processes), no extract will be available for these technologies.

Observations:

Review of quantitation parameters, other than quantitation value, did not yield a trend, however further monitoring of these parameters will be conducted.

The value of 0.0088ng/ μ L is based on assessment of the data (and equates to 132 picograms). The value of 0.0067ng/ μ L is based on equating to 100 picograms, and not based on assessment of data.

Options for Consideration:

- 1. Continue with the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL (132 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received.
- 2. Amend the current workflow: **RISKS**
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of 0.001 ng/µL to 0.0067ng/µL (100 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received. DNA samples above 0.0067ng/µL will be processed as per routine and will not be subject to a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.
- 3. Amend the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL
 - b) Priority 2 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of either 0.001ng/µL to 0.0088ng/µL or 0.001ng/µL to 0.0067ng/µL and processed upon request. Priority 3 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088 ng/µL or 0.001ng/µL to 0.0067ng/µL will be amplified without a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.



Cathie Allen

From:	Frieberg.DaleJ[OSC] <
Sent:	Friday, 2 February 2018 3:38 PM
То:	Cathie Allen; O'Malley.TroyS[OSC]; Taylor.EwenN[OSC]
Cc:	Paul Csoban
Subject:	RE: Options Paper for consideration

Hi Cathie and Paul,

Thank you for your time this afternoon and for discussion around this options paper. Thank you also to both Troy and Ewen with your assistance and expertise/advice around the paper.

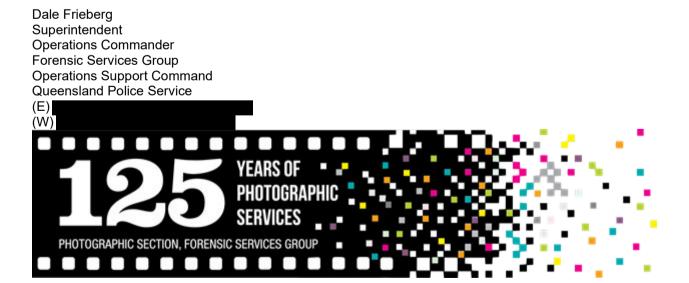
As discussed, I am in agreement that:

- There is clear data that it is not an efficient use of time and resources to continue with the 'auto-microcon' process for Priority 2 (*Major Crime*) samples.
- Option 2. "Cease the 'auto-microcon' process for Priority 2 casework...." Would appear to be a more productive & efficient choice.
- Scientists time and resources would be better spent working samples with a higher DNA yield and more potential.
- It would be beneficial to amend the Forensic Register to provide an automated Q-Prime update advising the Investigators of the option to request further 'Auto-microcon' processing for those samples for unsolved crime, which may prove worthwhile.
- DNA staff can request this additional processing if/when a request is received from the investigators.

I trust this is of assistance.

Kind regards,

Dale.



From: Cathie Allen [mailto:		
Sent: Tuesday, 30 January 2018 4:5	56 PM	
To: Frieberg.DaleJ[OSC] <		O'Malley.TroyS[OSC]
<	Taylor.EwenN[OSC] <	
Cc: Paul Csoban <		

Subject: Options Paper for consideration

Hi Dale

Please find attached an Options paper regarding concentration of major crime samples that we have prepared for your consideration. I'd like to discuss this on Friday with you.

Cheers Cathie



Cathie Allen

Managing Scientist – Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health



HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

This email, including any attachments sent with it, is confidential and for the sole use of the intended recipient(s). This confidentiality is not waived or lost, if you receive it and you are not the intended recipient(s), or if it is transmitted/received in error.

Any unauthorised use, alteration, disclosure, distribution or review of this email is strictly prohibited. The information contained in this email, including any attachment sent with it, may be subject to a statutory duty of confidentiality if it relates to health service matters.

If you are not the intended recipient(s), or if you have received this email in error, you are asked to immediately notify the sender by telephone collect on Australia +61 1800 198 175 or by return email. You should also delete this email, and any copies, from your computer system network and destroy any hard copies produced.

If not an intended recipient of this email, you must not copy, distribute or take any action(s) that relies on it; any form of disclosure, modification, distribution and/or publication of this email is also prohibited.

Although Queensland Health takes all reasonable steps to ensure this email does not contain malicious software, Queensland Health does not accept responsibility for the consequences if any person's computer inadvertently suffers any disruption to services, loss of information, harm or is infected with a virus, other malicious computer programme or code that may occur as a consequence of receiving this email.

Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest

immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error, please inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

Fwd: Options Papers - First one and Draft of Second

From: To:	Shaun Drummond < Jasmina Joldic <
Date:	Mon, 06 Jun 2022 09:47:59 +1000
Attachments:	#184 Review of Microcon Options paper QPS (Final report).pdf (633.18 kB); Assessment of low quant DNA Samples.docm (56.75 kB); Email advice Supt Frieberg on Options Paper_Feb 2018.pdf (1.19 MB)

Get Outlook for iOS

From: Lara Keller < Sent: Thursday, Juh			_		-
au	m				
Ŵ				4	
Cc: FSS Corro <		v.au>			
Subject: FW: Op		Second			
Gubject. 1 W. Op		oecona			

Good afternoon All

Papers attached as discussed.

2018 options paper : 1.86% were suitable to be uploaded to the National Criminal Investigation DNA database 2022 review paper: 5.3% " " (but note smaller number assessed)

Thanks and Kind Regards
Lara
Lara Keller B App Sc (MLS), Grad Cert Health Mgt, MAIMS, CMgr FIML
A/Executive Director
Forensic and Scientific Services
Prevention Division, Queensland Health
p (07)
a Administration, Level 1, 39 Kessels Road, Coopers Plains, QLD, 4108
e www.health.qld.gov.au/fss
www.nearth.qd.gov.au/iss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Cathie Allen < Sent: Thursday, 2 To: Lara Keller < dot for the second d.gov.au> Subject: Options Papers - First one and Draft of Second

Hi Lara

The first options paper is the pdf doc = #184 review of Microcon Options paper QPS. Attached email from Supt Frieberg advising her authorisation to proceed with the 'DNA Insufficient' process (dated Feb 2018).

I'll work on the rest and send as it's done.

Cheers Cathie

Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the

Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022 **Police Services Stream, Forensic & Scientific Services** Prevention Division. Queensland Health

p 07

e

a 39 Kessels Road, Coopers Plains, QLD 4108

w <u>www.health.qld.gov.au/fss</u>

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





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

January 2018 Justin Howes and Cathie Allen



Great state. Great opportunity.

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

Published by the State of Queensland (Queensland Health), January 2018



This document is licensed under a Creative Commons Attribution 3.0 Australia licence. To view a copy of this licence, visit creativecommons.org/licenses/by/3.0/au

© State of Queensland (Queensland Health) 2018

You are free to copy, communicate and adapt the work, as long as you attribute the State of Queensland (Queensland Health).

For more information contact:

Forensic DNA Analysis, Forensic and Scientific Services, Department of Health, GPO Box 48, Brisbane QLD 4001.

Disclaimer:

The content presented in this publication is distributed by the Queensland Government as an information source only. The State of Queensland makes no statements, representations or warranties about the accuracy, completeness or reliability of any information contained in this publication. The State of Queensland disclaims all responsibility and all liability (including without limitation for liability in negligence) for all expenses, losses, damages and costs you might incur as a result of the information being inaccurate or incomplete in any way, and for any reason reliance was placed on such information.

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

Document Details

.

Contact for enquiries and proposed changes

If you have any questions regarding this document or if you have a suggestion for improvements, please contact:

 Contact officer:
 Justin Howes

 Title:
 Team Leader – Forensic Reporting and Intelligence Team

 Phone:
 Email:

Contents

Doc	ument Details
1.	Abstract
2.	Definitions
3.	Introduction
4.	Data interrogation
5.	Assessment of 'auto-microcon' results
6. Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
7.	Results and Discussion
7.1 A	Assessment of 'auto-microcon' results 6
7.2 Qua	Datamine of the difference in pre- and post- Microcon [®] ntification values
8.	Options for consideration
9.	References

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 postextraction process within Forensic DNA Analysis to reduce the volume of extract from approximately 100uL to $\leq 35\mu$ L 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 0.001 ng/ μ L to 0.0088 ng/ μ L, 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 0.001 ng/µL and less than 0.015 ng/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/ μ L (Fig 2). This was considered to be due to there being less DNA detected in the extract, and therefore less DNA to concentrate.

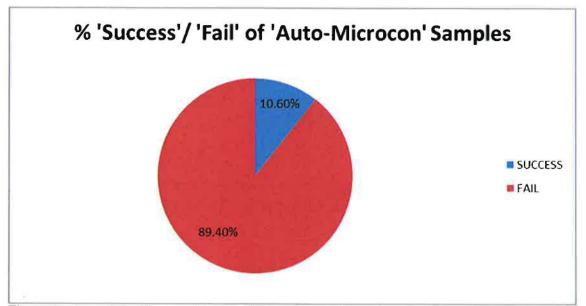


Figure 1: Percentage 'Success'/ 'Fail' of 'Auto-Microcon' samples.

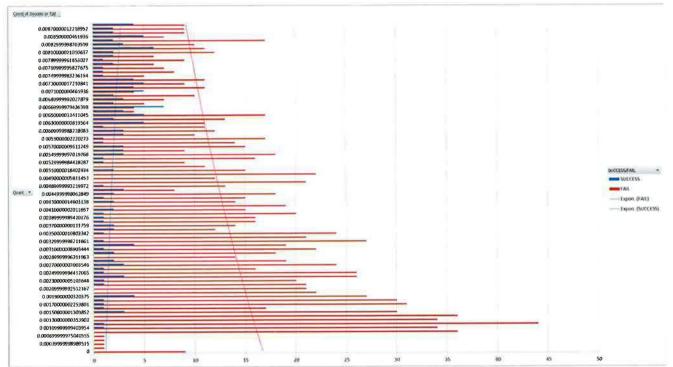


Figure 2: Spread of data and categorised as 'Success'/ 'Fail' for 'Auto-Microcon' samples.

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)..

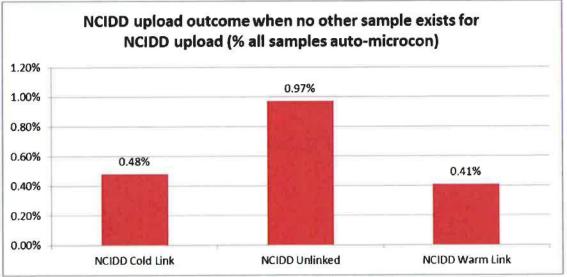


Figure 3: NCIDD outcome for samples that were loaded to NCIDD

A review of the automatic concentration of DNA extracts using Microcon[®] Centrifugal Filter Devices: Options for QPS consideration. 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 0.001 ng/µL where the final result was 'success'.

As the Microcon[®] process concentrates the DNA extract from approximately 100uL to approximately 35 μ L, 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'.

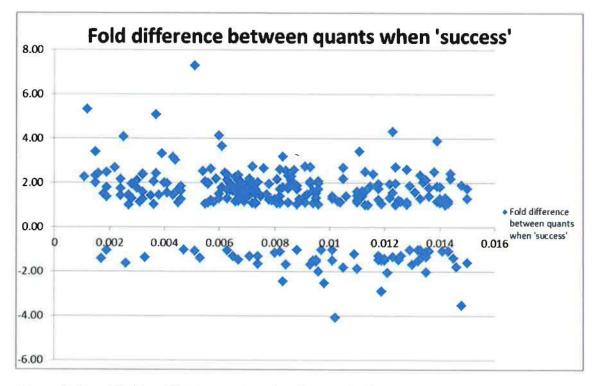


Figure 4: Quantification differences pre and post concentration

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,
- 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);

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

- 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

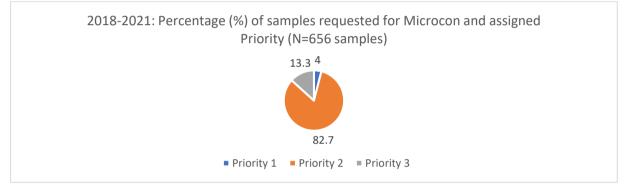
- [1] QIS 19544v11 Concentration of DNA Extracts Using Microcon Centrifugal Filter Devices
- [2] PowerPlex[®] 21– Amplification of Extracted DNA Validation. Megan Mathieson, Thomas Nurthen, Cathie Allen. December 2012. Forensic DNA Analysis.
- [3] QIS 23008v15 Explanation of EXR/EXH Results
- [4] QIS 24012v13 Miscellaneous Analytical Section Tasks

Assessment of Low Quantification Value DNA Samples

Authors: Cathie Allen, Justin Howes and Paula Brisotto

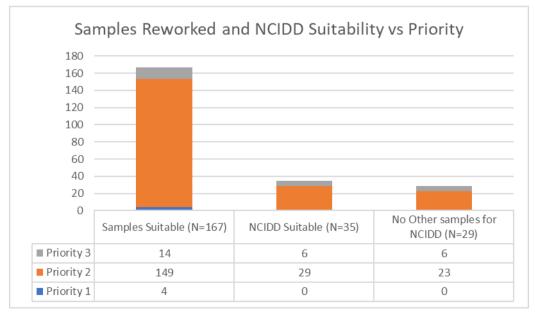
Executive Briefing:

An assessment of all casework DNA samples, with the following criteria was conducted: an initial quantification result of between zero and 0.0088ng/µL, underwent a concentration step and reported results produced between 2018 and 2021. This equated to an assessment of 656 DNA samples. The reported DNA result, which may have been completed after one or more amplifications steps, was categorised into two broad categories - 'suitable for comparison purposes' or 'unsuitable for comparison purposes'.



167 DNA samples (25.5%) were categorised as 'suitable for comparison purposes', with most of these samples being major crime samples. 456 DNA samples (74.5%) were categorised as 'unsuitable for comparison purposes' after concentration and amplification processes.

Of the 167 DNA samples categorised as 'suitable for comparison purposes', 35 DNA samples were able to yield a profile suitable for uploading and searching of the National Criminal Investigation DNA Database (NCIDD). This represents 5.3% of total samples selected for processing.





Please note the current dataset is different to the previous dataset due to, but not limited to: implementation of the statistical interpretation of four-person mixtures, all DNA samples were selected in this dataset (previously the dataset only included DNA samples assigned to Major Crime cases), active selection of samples for processing by either the Queensland Police Service or Forensic DNA Analysis staff members based on the context of the case or scientific knowledge with respect to the associated parameters from the quantification process, and new instrumentation implemented over that period.

Forensic staff are mindful of consuming all DNA extract when requesting a concentration step. Future technologies may be applied to DNA extracts, however if all extract has been exhausted (through concentration and amplifications processes), no extract will be available for these technologies.

Observations:

Review of quantitation parameters, other than quantitation value, did not yield a trend, however further monitoring of these parameters will be conducted.

The value of 0.0088ng/µL is based on assessment of the data (and equates to 132 picograms). The value of 0.0067ng/µL is based on equating to 100 picograms, and not based on assessment of data.

Options for Consideration:

- 1. Continue with the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the sample falls into the quantitation range of 0.001 ng/µL to 0.0088 ng/µL (132 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received.
- 2. Amend the current workflow: **RISKS**
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 and Priority 3 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of 0.001 ng/µL to 0.0067ng/µL (100 picograms) and process upon request by either the QPS or Forensic DNA Analysis staff members. Retain the DNA extract indefinitely, if no request is received. DNA samples above 0.0067ng/µL will be processed as per routine and will not be subject to a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.
- 3. Amend the current workflow:
 - a) Priority 1 samples continue to be automatically concentrated prior to amplification if the sample falls into the quantitation range of 0.001ng/µL to 0.0088 ng/µL
 - b) Priority 2 samples are reported as 'DNA Insufficient for Further Processing' if the DNA sample falls into the quantitation range of either 0.001ng/µL to 0.0088ng/µL or 0.001ng/µL to 0.0067ng/µL and processed upon request. Priority 3 samples that fall into the quantitation range of either 0.001ng/µL to 0.0088 ng/µL or 0.001ng/µL to 0.0067ng/µL will be amplified without a concentration step.
 - c) This amended workflow will require Forensic Register enhancement prior to use.



Cathie Allen

From:	Frieberg.DaleJ[OSC] <
Sent:	Friday, 2 February 2018 3:38 PM
То:	Cathie Allen; O'Malley.TroyS[OSC]; Taylor.EwenN[OSC]
Cc:	Paul Csoban
Subject:	RE: Options Paper for consideration

Hi Cathie and Paul,

Thank you for your time this afternoon and for discussion around this options paper. Thank you also to both Troy and Ewen with your assistance and expertise/advice around the paper.

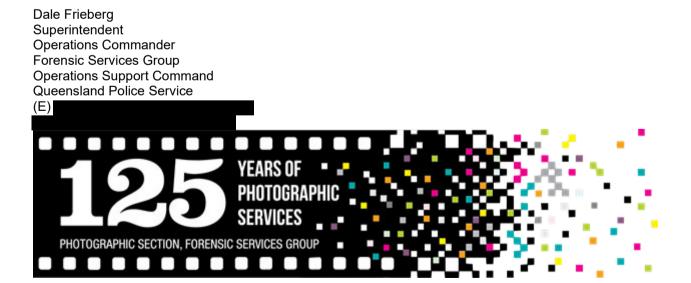
As discussed, I am in agreement that:

- There is clear data that it is not an efficient use of time and resources to continue with the 'auto-microcon' process for Priority 2 (*Major Crime*) samples.
- Option 2. "Cease the 'auto-microcon' process for Priority 2 casework...." Would appear to be a more productive & efficient choice.
- Scientists time and resources would be better spent working samples with a higher DNA yield and more potential.
- It would be beneficial to amend the Forensic Register to provide an automated Q-Prime update advising the Investigators of the option to request further 'Auto-microcon' processing for those samples for unsolved crime, which may prove worthwhile.
- DNA staff can request this additional processing if/when a request is received from the investigators.

I trust this is of assistance.

Kind regards,

Dale.



From: Cathie Allen [mailto:		
Sent: Tuesday, 30 January 2018 4	:56 PM	
To: Frieberg.DaleJ[OSC] <		O'Malley.TroyS[OSC]
<	Taylor.EwenN[OSC] <	
Cc: Paul Csoban <		

Subject: Options Paper for consideration

Hi Dale

Please find attached an Options paper regarding concentration of major crime samples that we have prepared for your consideration. I'd like to discuss this on Friday with you.

Cheers Cathie



Cathie Allen

Managing Scientist – Police Services Stream

Forensic & Scientific Services, Health Support Queensland, Department of Health



HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

This email, including any attachments sent with it, is confidential and for the sole use of the intended recipient(s). This confidentiality is not waived or lost, if you receive it and you are not the intended recipient(s), or if it is transmitted/received in error.

Any unauthorised use, alteration, disclosure, distribution or review of this email is strictly prohibited. The information contained in this email, including any attachment sent with it, may be subject to a statutory duty of confidentiality if it relates to health service matters.

If you are not the intended recipient(s), or if you have received this email in error, you are asked to immediately notify the sender by telephone collect on Australia +61 1800 198 175 or by return email. You should also delete this email, and any copies, from your computer system network and destroy any hard copies produced.

If not an intended recipient of this email, you must not copy, distribute or take any action(s) that relies on it; any form of disclosure, modification, distribution and/or publication of this email is also prohibited.

Although Queensland Health takes all reasonable steps to ensure this email does not contain malicious software, Queensland Health does not accept responsibility for the consequences if any person's computer inadvertently suffers any disruption to services, loss of information, harm or is infected with a virus, other malicious computer programme or code that may occur as a consequence of receiving this email.

Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest

immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error, please inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

Cathie Allen

From:	Lara Keller		
Sent:	Monday, 6 June 2022 12:11 PM		
То:	Cathie Allen		
Subject:	RE: Lead time to change process?		

Thanks for the prompt reply, Cathie Not sure what the meeting is about, but want to be ready. Kind regards Lara

From: Cathie Allen < Sent: Monday, 6 June 2022 12:10 PM To: Lara Keller < Subject: Re: Lead time to change process?

Hi Lara

We will need to contact bdna to request the change in the FR and I'm unsure how long it will take them to make that change.

We have a manual workaround (to hold those DNA Insufficient samples so that they can be profiled) so it can be implemented today. This would be for both options.

Cheers Cathie

Sent from mobile device Cathie Allen Managing Scientist - Police Services Stream

From: Lara Keller < Sent: Monday, June 6, 2022 12:06:00 PM To: Cathie Allen < Subject: Lead time to change process?

Hello Cathie

I hope you had a restful weekend.

If I'm asked, how long would it take to change processes as per Friday's email? Both options please.

I have a mtg with DG at 12.30.

Thanks and kind regards

Lara

Lara Keller, B App Sc (MLS), Grad Cert Health Mgt, MAIMS, CMgr FIML A/Executive Director

Forensic and Scientific Services

Prevention Division, Queensland Health

m a Administration, 39 Kessels Road, Coopers Plains e w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

A. T. Man Date: Page: For DNA Analysis R.D. Meeting 27 05 0000 - New Group for Research 5 helps with priority of task & not impacted by notine. - tomewort for next Ref Tean Workflows in Reporting. neeting Admin Catch-up with wHAT 31/05/2022 - Higher Duties - for July - Man Soi - HPG - Chief Chemis Spy of Date's Approval entar ara Keller objoblood - Phone Call 12. 57pm - Can. of Enguiry - Walter Sofronoff (Reter Davis > 2× SME's - not announced yet hasted by ppc Tok later this neek ~3 nths is to be implemented boday Dotion FTE has been approved - confidentia - there conversation - Expt Menab = not apologetic about it said he wrote some of it

Paula Brisotto

From: Sent:	Luke Ryan Monday, 6 June 2022 1:20 PM A dem Kaiten Alexen Demonsion Anno Change Balinda Anderson Biliana Mising
To:	Adam Kaity; Alanna Darmanin; Amy Cheng; Belinda Andersen; Biljana Micic; Generosa Lundie; Lai-Wan Le; Lisa Farrelly; Maria Aguilera; Melissa Cipollone; Nicole Roselt; Pierre Acedo; Sharelle Nydam; Tara Prowse
Cc:	Paula Brisotto; Cathie Allen
Subject:	DNA Insufficient - Quant transition to Amp
Importance:	High

Afternoon All

The premier has requested we test (amp) all samples in the current DNA Insufficient Range (i.e. above 0.001 - 0.088 ng/µL).

When transitioning Quant batches, please ensure all samples in the DNA Insufficient range are transitioned to the Amp WL. We are not reporting DNA Insufficient result lines as of now.

Please also ensure when reviewing No DNA Detected samples, look for samples with the DNA Insufficient result which have not been transitioned to the Amp WL. Please reallocate these to the Amp WL. I will go through the No DNA review list now and allocate these to the Amp WL.

There is no change to rules for No DNA Detected samples.

FR will be modified so that these rules are incorporated into the Quant transition page, but this will be a manual process until these changes are made.

Thanks Luke



Luke Ryan Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Paula Brisotto

From:	Paula Brisotto
Sent:	Monday, 6 June 2022 1:21 PM
То:	Cathie Allen
Subject:	FW: DNA Insufficient - Quant transition to Amp
Importance:	High

HI Cathie,

Do you want Luke to submit the enhancement, or are you looking after that bit?

Thanks,

Paula

From: Luke Ryan <			
Sent: Monday, 6 June 2022	1:20 PM		
To: Adam Kaity <	Alanna Darmanin <		Amy
Cheng <	Belinda Andersen <		Biljana Micic
<	Generosa Lundie <	La	i-Wan Le <lai-< td=""></lai-<>
	Lisa Farrelly <	Maria Aguilera	
<	Melissa Cipollone <		Nicole Roselt
<	Pierre Acedo <	Sharelle N	lydam
<	Tara Prowse <		
Cc: Paula Brisotto <	Cathie Allen <		

Subject: DNA Insufficient - Quant transition to Amp Importance: High

Afternoon All

The premier has requested we test (amp) all samples in the current DNA Insufficient Range (i.e. above 0.001 - 0.088 ng/µL).

When transitioning Quant batches, please ensure all samples in the DNA Insufficient range are transitioned to the Amp WL. We are not reporting DNA Insufficient result lines as of now.

Please also ensure when reviewing No DNA Detected samples, look for samples with the DNA Insufficient result which have not been transitioned to the Amp WL. Please reallocate these to the Amp WL. I will go through the No DNA review list now and allocate these to the Amp WL.

There is no change to rules for No DNA Detected samples.

FR will be modified so that these rules are incorporated into the Quant transition page, but this will be a manual process until these changes are made.

Thanks Luke



Luke Ryan Senior Scientist – Analytical Team

Forensic DNA Analysis, Forensic and Scientific Services

Prevention Division, Queensland Health

a 39 Kessels Rd, Coopers Plains, QLD 4108

w www.health.qld.gov.au/healthsupport/businesses/forensic-and-scientific-services

Integrity

e

omers and patients first

Accountability

Respect

Engagement

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

From:	Sharon Johnstone
То:	<u>Adrian Pippia: Alicia Quartermain; Angela Adamson; Anne Finch; Cassandra James; Emma Caunt; Jacqui</u> <u>Wilson; Josie Entwistle: Kerry-Anne Lancaster; Rhys Parry: Allan McNevin; Angelina Keller; Claire Gallagher;</u> Deborah Nicoletti; Ingrid Moeller; Matthew Hunt; Penelope Taylor; Tegan Dwyer; Thomas Nurthen
Cc:	<u>Kylie Rika; Allison Lloyd; Luke Ryan</u>
Subject:	FW: DNA Insufficient - Quant transition to Amp
Date:	Monday, 6 June 2022 3:13:00 PM
Attachments:	image003.png image004.png
Importance:	High

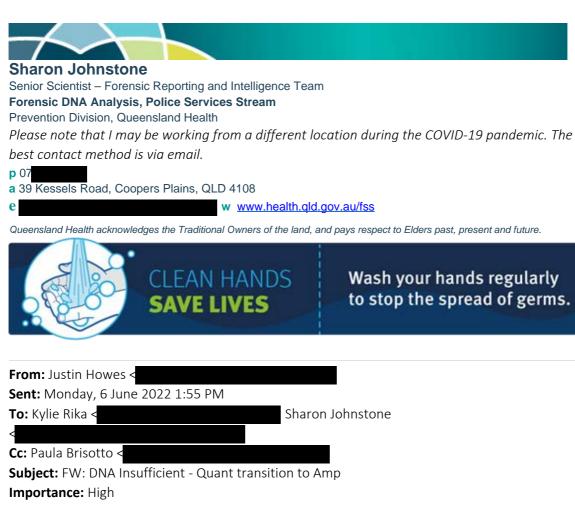
Hi all,

Please see below instructions stemming from today's announcements. These have been agreed to by QPS.

Please also note that any sample that has already been DNA insufficient is to be continued to be reported as such at statement stage. These results are known to the QPS. If it is their wish to have them restarted they will let us know.

Regards,

Sharon



Hi

Please note the DIFP process is currently suspended (the range correction to below is 0.001-0.0088ng/uL). Any new samples in this range will go directly for amp.

Previously reported DIFP that are requested for a restart, will go to microcon as per current process.

P3 samples will continue to be case managed in the same way as always – without rework unless not amped at max (of which the samples in the pertinent range will be amped at max).

Regards Justin



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Paula Brisotto <

Sent: Monday, 6 June 2022 1:23 PM

To: Justin Howes < Subject: FW: DNA Insufficient - Quant transition to Amp Importance: High

FYI

From: Luke Ryan <		
Sent: Monday, 6 June 2022	1:20 PM	
To: Adam Kaity <	Alanna Darmanin	
<	Amy Cheng <	Belinda
Andersen <	Biljana Micic	
<	Generosa Lundie <	Lai-
Wan Le <	Lisa Farrelly <	Maria
Aguilera <	Melissa Cipollone	
<	Nicole Roselt <	Pierre
Acedo <	Sharelle Nydam <	
Tara Prowse <		
Cc: Paula Brisotto <	Cathie Allen	
<		

Subject: DNA Insufficient - Quant transition to Amp

Importance: High

Afternoon All

The premier has requested we test (amp) all samples in the current DNA Insufficient Range (i.e. above $0.001 - 0.088 \text{ ng/}\mu\text{L}$).

When transitioning Quant batches, please ensure all samples in the DNA Insufficient range are transitioned to the Amp WL. We are not reporting DNA Insufficient result lines as of now.

Please also ensure when reviewing No DNA Detected samples, look for samples with the DNA Insufficient result which have not been transitioned to the Amp WL. Please reallocate these to the Amp WL. I will go through the No DNA review list now and allocate these to the Amp WL.

There is no change to rules for No DNA Detected samples.

FR will be modified so that these rules are incorporated into the Quant transition page, but this will be a manual process until these changes are made.



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Paula Brisotto

From: Sent:	Luke Ryan Monday, 6 June 2022 4:21 PM
To:	Adam Kaity; Alanna Darmanin; Amy Cheng; Belinda Andersen; Biljana Micic;
	Generosa Lundie; Lai-Wan Le; Lisa Farrelly; Maria Aguilera; Melissa Cipollone; Nicole
	Roselt; Pierre Acedo; Sharelle Nydam; Tara Prowse
Cc:	Paula Brisotto
Subject:	No DNA Detected
Importance:	High

Afternoon All

I have reviewed the No DNA list and reallocated all DNA Insufficient samples (ordered before the FR changes to quant transition) to the Amp WL.

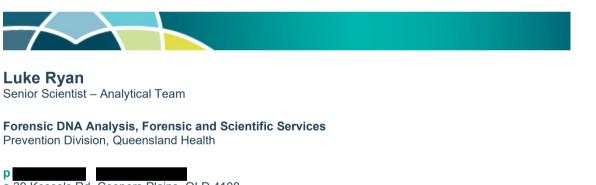
I have tested the changed quant transition, and this is now transitioning samples in the DNA Insufficient range to Amp.

Please return to transitioning quants normally, and reviewing No DNAs normally (by everyone).

When doing so please let me know if you see any DNA Insufficient result lines.

Thanks Luke

Integrity





Accountability

Respect

Engagement

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Customers and patients first

Cathie Allen

From: Sent: To: Subject: Cathie Allen Monday, 6 June 2022 4:50 PM Paula Brisotto RE: No DNA Detected

Thanks Paula

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

p 07 **m**

a 39 Kessels Road, Coopers Plains, QLD 4108

e Cathie.Allen@health.qld.gov.au w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



 From: Paula Brisotto

 Sent: Monday, 6 June 2022 4:29 PM

 To: Justin Howes

 Subject: Fwd: No DNA Detected

Fyi

Get Outlook for Android

From: Luke Ryan <<u>Luke.Ryan@health.qld.gov.au</u>>

Sent: Monday, June 6, 2022 4:20:33 PM

To: Adam Kaity <<u>Adam.Kaity@health.qld.gov.au</u>>; Alanna Darmanin <<u>Alanna.Darmanin@health.qld.gov.au</u>>; Amy Cheng <<u>Amy.Cheng@health.qld.gov.au</u>>; Belinda Andersen <<u>Belinda.Andersen@health.qld.gov.au</u>>; Biljana Micic <<u>Biljana.Micic@health.qld.gov.au</u>>; Generosa Lundie <<u>Generosa.Lundie@health.qld.gov.au</u>>; Lia-Wan Le <<u>Lai-Wan.Le@health.qld.gov.au</u>>; Lisa Farrelly <<u>Lisa.Farrelly@health.qld.gov.au</u>>; Maria Aguilera

<<u>Maria.Aguilera@health.qld.gov.au</u>>; Melissa Cipollone <<u>Melissa.Cipollone@health.qld.gov.au</u>>; Nicole Roselt <<u>Nicole.Roselt@health.qld.gov.au</u>>; Pierre Acedo <<u>Pierre.Acedo@health.qld.gov.au</u>>; Sharelle Nydam <<u>Sharelle.Nydam@health.qld.gov.au</u>>; Tara Prowse <<u>Tara.Prowse@health.qld.gov.au</u>>; Cc: Paula Brisotto <<u>Paula.Brisotto@health.qld.gov.au</u>> Subject: No DNA Detected

Afternoon All

I have reviewed the No DNA list and reallocated all DNA Insufficient samples (ordered before the FR changes to quant transition) to the Amp WL.

I have tested the changed quant transition, and this is now transitioning samples in the DNA Insufficient range to Amp.

Please return to transitioning quants normally, and reviewing No DNAs normally (by everyone).

When doing so please let me know if you see any DNA Insufficient result lines.

Thanks					
Luke					
Luke Ryai	า				
Senior Scienti	st – Analytical Team				
Forensic DNA	A Analysis, Forensic and Scientific S	ervices			
Prevention Div	vision, Queensland Health				
p 07	m				
a 39 Kessels I	Rd, Coopers Plains, QLD 4108				
e	w www.health.qld.go	ov.au/healthsupport/b	usinesses/fore	ensic-and-scientific	-services
Integrity	Customers and patients first	Accountability	Respect	Engagement	

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Paula Brisotto

From:	Justin Howes
Sent:	Tuesday, 7 June 2022 8:17 AM
То:	Cathie Allen; Paula Brisotto
Subject:	RE: Comment against SOPs

Hi, this is against the PDA SOP and will add the same to CM and Release of Results:

Workflow arrangements for samples as of 6 June, 2022:

- samples will not have DIFP added to results in the quant range 0.001-0.0088ng/uL. These will be amplified after Quant. This applies to P2 and P3 samples.

- case managers can assess samples for rework which could include a microcon after the first amplification. This does not apply to P3 samples which are processed without rework as per standard workflow arrangements.

- samples currently reported as DIFP that are requested to be restarted by QPS will undergo a microcon procedure.

- no change to the P1 workflow where samples in the quant range 0.001-0.0088ng/uL will undergo a microcon prior to amplification.

I think that captures what we will be doing.

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p a 39 Kessels Road, Coopers Plains, QLD 4108 e www.health.gld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Cathie Allen < Sent: Monday, 6 June 2022 5:10 PM To: Paula Brisotto < Subject: Comment against SOPs

Justin Howes <

Hi Paula & Justin

I'm assuming that you both remembered before I did, that we need to add comments in QIS against SOPs we've changed today.

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

p 07
a 39 Kessels Road, Coopers Plains, QLD 4108
e www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Paula Brisotto

From: Sent: To: Subject: Paula Brisotto Tuesday, 7 June 2022 9:10 AM Justin Howes; Cathie Allen RE: Comment against SOPs

Hi,

I'll follow up with Luke to ensure this is added to the relevant Analytical SOPs.

Thanks, Paula

From: Justin Howes <	
Sent: Tuesday, 7 June 2022 8:17 AM	
To: Cathie Allen <	Paula Brisotto <
Subject: RE: Comment against SOPs	

Hi, this is against the PDA SOP and will add the same to CM and Release of Results:

Workflow arrangements for samples as of 6 June, 2022:

- samples will not have DIFP added to results in the quant range 0.001-0.0088ng/uL. These will be amplified after Quant. This applies to P2 and P3 samples.

- case managers can assess samples for rework which could include a microcon after the first amplification. This does not apply to P3 samples which are processed without rework as per standard workflow arrangements.

- samples currently reported as DIFP that are requested to be restarted by QPS will undergo a microcon procedure.

- no change to the P1 workflow where samples in the quant range 0.001-0.0088ng/uL will undergo a microcon prior to amplification.

I think that captures what we will be doing.

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p (07) a 39 Kessels Road, Coopers Plains, QLD 4108 e w www.health.gld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

From: Cathie Allen <	
Sent: Monday, 6 June 2022 5:10 PM	
To: Paula Brisotto <	Justin Howes <
Subject: Comment against SOPs	

Hi Paula & Justin

I'm assuming that you both remembered before I did, that we need to add comments in QIS against SOPs we've changed today.

Cheers

Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Paula Brisotto

From: Sent: To: Subject: Paula Brisotto Tuesday, 7 June 2022 9:11 AM Luke Ryan FW: Comment against SOPs

Hi Luke,

Wording used for CM SOPs.

Thanks, Paula

From: Justin Howes <	
Sent: Tuesday, 7 June 2022 8:17 AM	
To: Cathie Allen <	Paula Brisotto <
Subject: RE: Comment against SOPs	

Hi, this is against the PDA SOP and will add the same to CM and Release of Results:

Workflow arrangements for samples as of 6 June, 2022:

- samples will not have DIFP added to results in the quant range 0.001-0.0088ng/uL. These will be amplified after Quant. This applies to P2 and P3 samples.

- case managers can assess samples for rework which could include a microcon after the first amplification. This does not apply to P3 samples which are processed without rework as per standard workflow arrangements.

- samples currently reported as DIFP that are requested to be restarted by QPS will undergo a microcon procedure.

- no change to the P1 workflow where samples in the quant range 0.001-0.0088ng/uL will undergo a microcon prior to amplification.

I think that captures what we will be doing.

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p (07)
a 39 Kessels Road, Coopers Plains, QLD 4108
e w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

From: Cathie Allen <	
Sent: Monday, 6 June 2022 5:10 PM	
To: Paula Brisotto <	Justin Howes <
Subject: Comment against SOPs	

Hi Paula & Justin

I'm assuming that you both remembered before I did, that we need to add comments in QIS against SOPs we've changed today.

Cheers

Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Cathie Allen

From:	Cathie Allen
Sent:	Friday, 10 June 2022 12:47 PM
То:	Angela Adamson
Subject:	RE: Thank you

You're so welcome Angela. Thanks for giving me some of your time, it was great to check-in. I try to be mindful of how busy you are and that I'm disturbing you from getting results out the door, so try not to bother you guys too often.

Cheers Cathie

e



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

p 07 a 39 Kessels Road, Coopers Plains, QLD 4108

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Angela Adamson Sent: Friday, 10 June 2022 12:13 PM To: Cathie Allen < Subject: Thank you

Hi Cathie,

I just wanted to say thank you for taking the time to pop over to reporting to speak with everyone. It really showed your support and that means a lot.

Thanks



Angela Adamson

Reporting Scientist - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

Please note that I may be working from a different location during the COVID-19 pandemic. The best contact method is via email.

 p |
 07

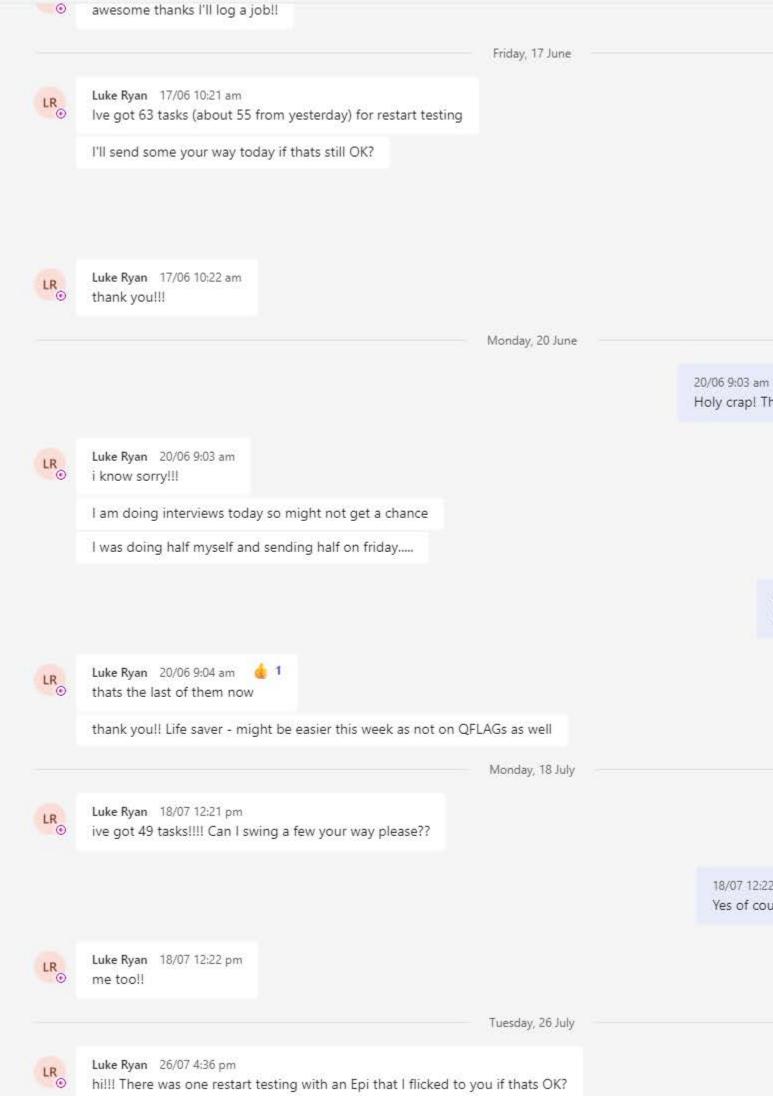
 a |
 39 Kessels Road, Coopres Plains, QLD, 4108

 w |
 www.health.qld.gov.au/healthsupport

 e |

HSQ's vision | Delivering the best health support services and solutions for a safer and healthier Queensland.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.





-

18/07 12:22 pm Yes of course. I'd thought they'd finished...

20/06 9:03 am It's all good. I'll get them sorted.

20/06 9:03 am Holy crap! Those requests just keep coming...

17/06 10:22 am sure thing

Cathie Allen

From: Sent: To: Subject:

Cathie Allen Monday, 20 June 2022 8:57 AM Justin Howes FW:

Hi Justin

I'll let you have a chat with Ingrid regarding the Premier's decision on this.

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 - 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

p 07 a 39 Kessels Road, Coopers Plains, QLD 4108 e

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Ingrid Moeller < Sent: Monday, 20 June 2022 7:45 AM To: Cathie Allen < Justin Howes < Subject:

Hi Cathie and Justin,

I have been off sick for about two weeks and have missed a lot of the discussions surrounding the recent change where DIFP samples are now going through straight to a 15ul amplification and not being concentrated first with a microcon.

I'm a little confused and concerned about this new approach (am I missing something). I'm confused because: -if QPS request work on a DIFP sample, it goes for a microcon first

-P1 samples in the DIFP range go for a microcon

-Automicrocon was the process we used prior to the DIFP process

-P3 samples (which we are not allowed to microcon) could be lost immediately with a potentially suboptimal amplification at 15uls

I have been picking up, from the P2 worklist, the DIFP samples which have been amped at 15uls and putting them through to a microcon. This is obviously not ideal since 15uls of our precious samples have been lost from the get go, not to mention we are doing extra steps in the processing of a sample. (On a hopeful side, I am seeing promising profiles in nearly all of the samples I have looked at so a microcon should help.)

I'm sure I have missed something here and hoping you may be able to enlighten.

Thank you and regards

Ingrid



Ingrid Moeller Scientist

Forensic & Scientific Services Prevention Division, Queensland Health

9

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Cathie Allen

From: Sent: To: Subject:

Cathie Allen Monday, 20 June 2022 9:16 AM Ingrid Moeller; Justin Howes RF:

Hi Ingrid

Welcome back to work. Sorry to hear that you've been absent for sometime feeling unwell. I hope that you're feeling better, and improving.

I'll let Justin have a chat with you regarding this, so that he can bring you up to speed.

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 - 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division. Queensland Health

р a 39 Kessels Road, Coopers Plains, QLD 4108 w www.health.gld.gov.au/fss e

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Ingrid Moeller <	
Sent: Monday, 20 June 2022 7:45 AM	
To: Cathie Allen <	Justin Howes <
Subject:	

Hi Cathie and Justin,

I have been off sick for about two weeks and have missed a lot of the discussions surrounding the recent change where DIFP samples are now going through straight to a 15ul amplification and not being concentrated first with a microcon.

I'm a little confused and concerned about this new approach (am I missing something). I'm confused because: -if QPS request work on a DIFP sample, it goes for a microcon first

-P1 samples in the DIFP range go for a microcon

-Automicrocon was the process we used prior to the DIFP process

-P3 samples (which we are not allowed to microcon) could be lost immediately with a potentially suboptimal amplification at 15uls

I have been picking up, from the P2 worklist, the DIFP samples which have been amped at 15uls and putting them through to a microcon. This is obviously not ideal since 15uls of our precious samples have been lost from the get go, not to mention we are doing extra steps in the processing of a sample. (On a hopeful side, I am seeing promising profiles in nearly all of the samples I have looked at so a microcon should help.)

I'm sure I have missed something here and hoping you may be able to enlighten.

Thank you and regards

Ingrid

е



Ingrid Moeller Scientist

Forensic & Scientific Services Prevention Division, Queensland Health

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

FSS.0001.0052.1243

June 2022 20 Monday Week 25 171-194
7:30
site & Eprort how Cathre, gooding to emost for my
8:30 renempours
9:00
9:30 Spoter to tol to set & parted on icho of
10:00 Ind satematic process
10:30 - KpR by on coronid age renew
() KOK said will that I light.
11:30
12:00
12:30
1:00
1:30
2:00
2:30
3:00
3:30
4:00
4:30
5:00
5:30
6:00
5:30
/:00
If you don't set goals, you can't regret not reaching them. – Yogi Berra January : 22 March : 22 Ancil : 22
M T W T F S S M T

Paula Brisotto

From: Sent: To: Subject: Paula Brisotto Tuesday, 21 June 2022 10:09 AM Cathie Allen FW: Microcons

Hi Cathie,

FYI. This is an indication of the resources involved, should this be useful for any discussions.

Thanks, Paula

From: Allison Lloyd <	
Sent: Monday, 20 June 2022 3:01 PM	
To: Paula Brisotto <	Justin Howes <
Subject: Microcons	

Hi,

FYI, I've ordered 108 m'cons from insufficient requests today and Friday. It has taken me approx. 9 hours. 😕

AL

p



Allison Lloyd

Senior Scientist - Evidence Recovery and Intelligence Teams

DNA Analysis Prevention Division, Queensland Health

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Paula Brisotto

From: Sent: To: Subject: Paula Brisotto Tuesday, 7 June 2022 9:10 AM Justin Howes; Cathie Allen RE: Comment against SOPs

Hi,

I'll follow up with Luke to ensure this is added to the relevant Analytical SOPs.

Thanks, Paula

From: Justin Howes <	
Sent: Tuesday, 7 June 2022 8:17 AM	
To: Cathie Allen <	Paula Brisotto <
Subject: RE: Comment against SOPs	

Hi, this is against the PDA SOP and will add the same to CM and Release of Results:

Workflow arrangements for samples as of 6 June, 2022:

- samples will not have DIFP added to results in the quant range 0.001-0.0088ng/uL. These will be amplified after Quant. This applies to P2 and P3 samples.

- case managers can assess samples for rework which could include a microcon after the first amplification. This does not apply to P3 samples which are processed without rework as per standard workflow arrangements.

- samples currently reported as DIFP that are requested to be restarted by QPS will undergo a microcon procedure.

- no change to the P1 workflow where samples in the quant range 0.001-0.0088ng/uL will undergo a microcon prior to amplification.

I think that captures what we will be doing.

Justin



Justin Howes Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p (07) a 39 Kessels Road, Coopers Plains, QLD 4108 e w www.health.gld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

From: Cathie Allen <	
Sent: Monday, 6 June 2022 5:10 PM	
To: Paula Brisotto <	Justin Howes <
Subject: Comment against SOPs	

Hi Paula & Justin

I'm assuming that you both remembered before I did, that we need to add comments in QIS against SOPs we've changed today.

Cheers

Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Cathie Allen

From: Sent: To: Subject: Cathie Allen Tuesday, 21 June 2022 11:35 AM Lara Keller RE: Advice to the QPS

Hi Lara

I think it would be great if you were able to send that to Supt McNab.

Cheers Cathie

e



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

p 07 a 39 Kessels Road, Coopers Plains, QLD 4108

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Lara Keller < Sent: Tuesday, 21 June 2022 11:24 AM To: Cathie Allen < Subject: RE: Advice to the QPS

Thanks Cathie Will you send that to Supt McNab, or would you like me to? Kind regards Lara

From: Cathie Allen < Sent: Tuesday, 21 June 2022 11:23 AM To: Lara Keller < Subject: Advice to the QPS

Hi Lara

Here's a draft to advise the QPS of the changes made in line with the Premier and Cabinet's announcement:

On Monday, 6th of June, the Premier announced a Commission of Inquiry into Forensic DNA Testing in Queensland. The Premier also announced that, moving forward, samples that fall into the category of 'DNA insufficient for further processing samples' would be profiled. On the 6th of June, the Forensic Register was amended to ensure that all crime scene samples with a quantitation value above 0.001ng/uL are amplified and results provided electronically to the QPS.

Cheers Cathie

e



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

a 39 Kessels Road, Coopers Plains, QLD 4108

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



Kylie Rika

From: Sent: To: Subject: Kylie Rika Wednesday, 27 July 2022 5:00 PM Claire Gallagher RE: Wording for reworks in case

I think that is fine Claire

From: Claire Gallagher < Sent: Wednesday, 27 July 2022 3:15 PM To: Kylie Rika < Subject: RE: Wording for reworks in case

Thanks Kylie.

This better?

Hi John. I have had a look at samples 790715346 and 790715373. These samples were processed in 2018, and therefore they were run on older technology, and we had an older statistical interpretation programme. This meant that we were unable to interpret those results as they were, given these limitations. We now have newer, more sensitive technology, as well as an updated statistical package. I think these samples are good candidates to be processed further with our current profiling system and as such, I have initiated further work on them. These samples have already been concentrated, so that does limit what reworks are available to us. Once the results are finalised, I will get them reviewed and made available to you.

I wanted to clarify the results of these samples in relation to the initial to request further processing. It was not that there was insufficient DNA for further testing with regards to these two samples. The complex result was due to low level DNA that indicated more than one contributor. This made it difficult to determine the number of contributors in this DNA profile, and that's what gives this profile its complexity.

From: Kylie Rika <
Sent: Wednesday, 27 July 2022 3:07 PM
To: Claire Gallagher <
Subject: RE: Wording for reworks in case

Yes OK to clarify that

From: Claire Gallagher < Sent: Wednesday, 27 July 2022 2:10 PM To: Kylie Rika < Subject: RE: Wording for reworks in case

Thanks Kylie. Brilliant wording.

Do you think its ok to clarify that there was not insufficient DNA for testing, but that the DNA profile was low level and showed indications of more than one contributor, and as a result, I am unable to determine the number of contributors. And that is what gives it the complexity.

Thanks Kylie

To: Claire Gallagher < Subject: RE: Wording for reworks in case

See red text below

From: Claire Gallagher < Sent: Wednesday, 27 July 2022 1:59 PM To: Kylie Rika < Subject: Wording for reworks in case

Hi Kylie

Would you mind having a read and letting me know if this wording is OK please?

Hi John. I have had a look at samples 790715346 and 790715373. These samples were processed in 2018, and therefore they were run on older technology and at the time, we had an older statistical interpretation programme. This meant that we were unable to interpret those two results as they were, given these limitations. We now have newer, more sensitive technology, as well as an updated statistical package. I think these samples are good candidates to be processed further with our current profiling system and as such, I have initiated further work on them. Once the results are finalised, I will get them reviewed and made available to you.

Thanks, Claire



Please note that I will be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

Cathie Allen

From: Sent:	Helen Gregg Wednesday, 20 July 2022 10:34 AM
То:	Cathie Allen
Cc:	Peter Culshaw
Subject:	RE: Further clarification previous email: Assessment of low quant DNA samples report

Many thanks!

From: Cathie Allen <	
Sent: Wednesday, 20 July 2022 10:32 AM	-
To: Helen Gregg <	
Cc: Peter Culshaw <	
Subject: RE: Further clarification previous email: As	sessment of low quant DNA samples report
Importance: High	

Hi Helen

In 2018, an Options Paper was provided to the QPS with options regarding processing. The QPS reviewed the options and approved for the implementation of the Option where samples with a quant value between 0.0001 and 0.0088ng/ul would be advised as 'DNA Insufficient for processing' and QPS officers could request testing of these samples, which would involve a concentration step prior to amplification.

A Follow-up paper was provided to the QPS last month or so ago, regarding samples that had been concentrated prior to amplification and the outcome of those samples.

Prior to the announcement of the commission of inquiry, the DG requested options for processing that did not include the 'DNA insufficient' process. Options were provided and the Premier announced that Cabinet had decided the DNA insufficient process was no longer being used, and all samples were being processed. From this, we take it that the Premier and Cabinet did not appear to choose the option that included concentration of samples within a particular range, given potential workplace health and safety issues.

Lara advised Supt McNab of the decision and process in the attached email, given the announcement by the Premier of the Cabinet's decision.

Samples are processing through DNA profiling and upon review of the profile obtained, staff will assess if concentration of the sample would be of benefit, within the context of the case. The option of concentration is available, as it has always been since it's implementation in the late 1990's.

From a Forensic DNA Analysis perspective, the most conservative option has been chosen – in that all samples are being profiled, concentration can be done once an appropriate evaluation of the resulting profile has been reviewed, and allows the work unit to gather data on the effectiveness of the concentration step when applied to samples with low quantitation values.

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

a 39 Kessels Road, Coopers Plains, QLD 4108 e www.health.gld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Helen Gregg <

Sent: Wednesday, 20 July 2022 9:54 AM

To: Cathie Allen <

Subject: FW: Further clarification previous email: Assessment of low quant DNA samples report **Importance:** High

Hi Cathie

Could you please advise me asap?

I understood that we were concentrating everything now. Is that correct?

Н

From: Pobar.DarrenJ[OSC] <

Sent: Wednesday, 20 July 2022 9:51 AM

To: Helen Gregg <

Subject: Further clarification previous email: Assessment of low quant DNA samples report

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Good morning Helen

Further to the below query, I am seeking further clarification of the current testing process by QHFSS announced by the Minister. With the 0.0088ng/ul threshold removed, are some samples now being processed without any microconcentration step in place. Ie those between .001 and .0088 which would potentially benefit from concentration.

Regards Darren



Darren Pobar | Acting Superintendent Forensic Services Group Operations Support Command Queensland Police Service

200 Roma Street Brisbane



From: Pobar.DarrenJ[OSC] Sent: Friday, 15 July 2022 12:00 To: Subject: Assessment of low quant DNA samples report

Good morning Helen

I am currently relieving for a short term in Superintendent Bruce Mcnab's role in Forensic Services Group.

I refer to attached report provided by Acting Executive Director Lara Keller to Supt Mcnab on 24 June 2022 regarding a review assessment of low quant DNA samples and I thank QHFSS for compiling and providing this new report. I note that the success rate in this new review of the micro-concentration process is approximately 25%. This is considerably higher than predicted in the 2018 Options Paper that recommended the removal of the process as a matter of routine. We are still considering the material provided and hope to discuss the options with QHFSS in the near future.

I understand the Health Minister announced on 30 May 2022 the .0088ng/uL processing threshold has been removed and that all samples are now processed as a matter of routine. I am seeking clarification on the current process on testing low quant value samples. If correct that all samples from priority 1 to 3 are being processed despite low quant values, the QPS has concerns how this change will impact anticipated backlogs and turn around times of results. Should this present as a risk, could you also please advise what strategies are in place to mitigate this issue.

Thank you again for providing the report and I look forward to receiving your advice on these queries.

Regards

Darren Pobar | Acting Superintendent Forensic Services Group Operations Support Command Queensland Police Service



CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error, please inform the sender or contact

inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

FW: Updated memo for consideration

From:	Helen Gregg <	
	Paula Brisotto < Cathie Allen <	Justin Howes <
	Fri, 19 Aug 2022 11:23:17 +1000	
Attachments:	DG Memo - Required amendment to FSS SOP 1	7117V19 - 19 August 2022 updated DR.docx (57.81 kB)

From: Matthew Rigby < Sent: Friday, 19 August To: Megan Fairweather <Megan.Fairwea Subject: Updated memo for consideration

Helen Gregg <

Hi Megan and Helen,

Can I please seek your feedback on this updated memo. Once you are comfortable with the content, I will seek David's final approval and arrange for this to be issued from DG Corro.

Thanks Matt

	ealth.qld.gov.au evel 14, 33 Charlotte Street, Brisbane QLD 200
--	---

From:	Helen Gregg	
Sent:	Friday, 19 August 2022 4:20 PM	
То:	Paula Brisotto; Justin Howes; Cathie Allen	
Subject:	FW: URGENT: request to change workflow ub FR	

High

Importance:

FYI

From: Helen Gregg Sent: Friday, 19 August 2022 4:19 PM To: Troy O'Malley < Cc: FSS Corro < Subject: URGENT: request to change workflow ub FR Importance: High

Good afternoon Troy,

We have been requested by the Director-General that for all Priority 1 and Priority 2 samples with a quantitation result between 0.001ng/uL (LOD) and 0.0088ng/uL, that these be concentrated and undergo amplification- what I term 'concentration of samples in the range'

I have been instructed to undertake a review of the laboratory information system to identify any sample results within this quantitation range from 6 June 2022 to today's date inclusive – hence my urgent email to you.

For the period: 6 June 2022 to 19 August 2022:

- Could you please prepare a report to identify Priority 1, 2 and 3 samples that have a quantitation value between 0.001ng/uL and 0.0088ng/uL that have been processed up to and including 19 August 2022.
- Could you please also prepare a report to identify the Priority 1, 2 and 3 samples with a quantitation value between 0.001ng/uL and 0.0088ng/uL that have had a Microcon PowerPlex 21 Method applied to them
- Could this information please be provided in a Spreadsheet with the parameters listed below
- For Priority 2 samples with quantitation value between 0.001ng/ul and 0.0088ng/uL, please request the line 'Sample undergone further testing', then add Microcon PowerPlex 21 Method to those that do not currently have this applied
- Please amend in FR Production the workflow for Priority 2 samples with a quantitation value between 0.001ng/ul and 0.0088ng/uL to automatically have the Microcon PowerPlex21 Method added to them (as per Priority 1 samples)

Parameters for Spreadsheet:

- Forensic Number
- Exhibit Number
- SRP Date
- Analytical Priority
- BatchID
- Well
- TSAQty
- TSAIPCCT
- TLAQty
- TYQty
- TASDegIndex

- TSALOWQT
- Results
- Well
- TSAQty
- TSAIPCCT
- TLAQty
- TYQty
- TASDegIndex
- TSALOWQT
- MicroconDate
- BatchID
- Well
- TSAQty
- TSAIPCCT
- TLAQty
- TYQty
- TASDegIndex
- TSALOWQT
- Results

I would appreciate this being done as a matter or urgency.



Helen Gregg A/Executive Director

p (07)

е

Forensic and Scientific Services Prevention Division, Queensland Health

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

RE: written approval steps and wording

From: To: Date:	Cathie Allen < Justin Howes < Alison Slade < Fri, 19 Aug 2022 16:48:10 +1000	Helen Gregg <	Paula Brisotto <
Hi Justir	1		

I support all below (with minor amendment that I've done in red below).

Cheers Cathie

From: Justin Howes < Sent: Friday, 19 August 2022 4:28 Piv			
To: Hele Slade <	qld.gov.au>; Cathie Allen <	Paula Brisotto <	Alison
Subject: RE: written approval steps ar	nd wording		

Hi, I like Cathie's addions and I ha ve an extra edit knowing NATA need a 'fina'I a. er reporng 'pr elim/inial'. Wording below removes 'inial' and 'final'.

Suggested Template for wording: 'Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for addional w ork to be undertaken on the sample, in an all empt to obtain a suitable DNA profile for interpretaon. Please be advised if this addional w ork is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternavie technologies are under consideraon. We understand that consultaon with the In vesgi ang Officer mainly be necessary and will await the outcome of those discussions. Once finalised, please advise via returned Request/Task if the addional w ork is approved. If approval is not provided, the DNA profile obtained will be reported.

I think we can use the 'SOHAA - Sample on hold, awaing advice' e xhibit result as the expanded comment is sll r elevant. This is used when sending the task. This result will require a validaon fr om a second operator.

SOHA	A Sample on hold, awai	This item/sample has been placed on hold and is awaiting additional information from QPS before processing can recommence. This information may relate to, but
		is not limited to; examination priority, screening requirements.

When QPS request a further process, staff would add below, and then the final interp.

TRQ Testing restarted on advice from QPS QPS have provided advice that testing is now re been restarted.	quired for this item/sample. Testing has
--	--

Jusn

Justin Howes Team Leader - Forensic Reporting and Intelligence Team	
Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services	
Prevention Division, Queensland Health p (07)	
a 39 Kessels Road, Coopers Plains, QLD 4108	
e www.health.qld.gov.au/fss Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is	via email.
Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging	



	CLEAN HANDS Save Lives	Wash your hands regularly to stop the spread of germs.		
From: Helen Gregg < Sent: Friday, 19 Augu To: Just Slade < Subject: RE: written ap		; Cathie Allen <	Paula Brisotto <	Alison
Excellent – than I have	e no problems with the wording	3		
From: Justin Howes < Sent: Friday, 19 Augus To: Hele Slade < Subject: RE: written ag	S	Cathie Allen <	Paula Brisotto <	Alison

Hi, yes, it is sll a DNA pr ofile that is deemed too complex and unsuitable for interpretaon.

Jusn				
Justin Howes Team Leader - Forensic Reporting a Forensic DNA Analysis, Police Se Prevention Division, Queensland He p (07) a 39 Kessers Road, Coopers Plans, Prease note that I may be working for	alth QLD 4108 w <u>www.health.qld.go</u> y	v.au/fss	e best contact method is via email.	
Queensland Health acknowledges the Trad	tional Owners of the land, ar	nd pays respect to Elders past, present and	l emerging.	
	n hands Lives	Wash your hands reg to stop the spread o		
From: Helen Gregg < Sent: Friday, 19 Augu To: Justin Howes < Justin Howes Slade < Subject: RE: written approval ste		Cathie Allen <	Paula Brisotto <	Alison
Thanks – so complex/unsuitable	is sll r eferred to as a	DNA profile?		
From: Justin Howes < Sent: Friday, 19 Augus To: Helen Greaa <helen greaa@<br="">Slade < Subject: RE: written approval ste</helen>		Cathie Allen <	Paula Brisotto <	Alison
Hi, in many situaons the pr ofile every me – ther e may be some			S don't approve, then the interp will be that bu	t can't say that will be the case
Jusn				
Justin Howes Team Leader - Forensic Reporting a Forensic DNA Analysis, Police Se Prevention Division, Queensland He p (07) a 39 Kessels Road, Coopers Plains, e Prease note that I may be working for	rvices Stream, Forensi alth QLD 4108 w www.health.qld.gov	v.au/fss	e best contact method is via email.	
Queensland Health acknowledges the Trad	tional Owners of the land, ar	nd pays respect to Elders past, present and	l emerging.	
	n hands Lives	Wash your hands reg to stop the spread o		
From: Helen Gregg < Sent: Friday, 19 August 2022 4:0 To: Just Slade < Subject: RE: written approval ste	<u>h.qld.gov.au</u> >;	Cathie Allen <	Paula Brisotto <	Alison
Thanks Jusn – will ther e be an i	nial pr ofile that can	be reported? Or could it be a 'c	omplex/uninterpretable'?	
From: Justin Howes < Sent: Friday, 19 Augus To: Cath	<u>ld.gov.au</u> >; P	aula Brisotto <	Helen Gregg <	Alison

Hi
Please check all on the same page and let me know if any edits are required.
Thease check an on the same page and let the know hany calls are required.

Subject: written approval steps and wording

Slade

* When seeking wri en approval from QPS for a second amplificaon if c onsidered beneficial, send a Request/Task via the Forensic Register to the relevant Forensic Officer found by the field below. Add the Forensic Officer's ID number to the Acon Officer field, and link the r elevant crime scene barcode to the Request/Task.

Template for wording: '*Hello, an initial DNA profile has been obtained from the linked crime scene sample. I am seeking approval for a second DNA amplification process in an attempt to obtain a suitable DNA profile for interpretation. Please note if this process is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternative technologies are under consideration Please advise via returned Request/Task if a second amplification is approved, o if the initial DNA profile can be reported.'*

Location / Owner					
From the front driver se	eat adjustment levers				
Exem Source				1	
Vehicle:	Van				
Exhibit Notes & Analysis A	dvice				
Parent Barcode	Property Tag	Current Location PSD	Investorator Perenaiz Officer		
Ownership / Relationship /	PriorRisation	Examination Section			
Suspect	Entry / Exit	Analytical Services	Photographic Section		
Justin How					1
Forensic DNA / Prevention Divis	Forensic Reporting and In Analysis, Police Servic sion, Queensland Health	ntelligence Team es Stream, Forensic	& Scientific Services		
a 39 Kessels Ro	Dad, Coopers Plains, QLI W W T may be working from a		au/fss ing the COVID-19 Pande	emic. The best cont	act method is via email
Queensland Health	acknowledges the Traditiona	l Owners of the land, and	pays respect to Eklers past, p	oresent and emerging.	



RE: Volume for DNA analysis

From: To:	Helen Gregg < Cathie Allen <	Paula Brisotto <	Justin
Cc: Date:	Howes < Alison Slade < Fri, 19 Aug 2022 17:06:32 +1000		
Yay!			
Sent: F T <cc: Subjec</cc: 	Cathie Allen < riday, 19 Augu t: RE: Volume for DNA analysis iuL would be ok for either Mini or Y's. Thar	Helen Gregg <	Justin Howes
Sent: F T Hel Cc: Alls Subjec	Paula Brisotto < riday, 19 August 2022 5:03 PM en Gregg <he son Stade < t: RE: Volume for DNA analysis his info, yes it appears 15uL is sufficient.</he 	Cathie Allen <	Justin Howes
Thanks Paula			
Sent: F T CC: Subjec	Helen Gregg < riday, 19 Augu t: RE: Volume for DNA analysis	Cathie Allen <	Justin Howes
Hi Even Minifile	yone, er: max amp volume is 10ul		
Y-Filer I	Plus: same as ID+ which is 5ul		
Full em So 15ul	ail trail attached		
Agree?			
Sent:F T ⊂c:	Justin Howes < riday, 19 Augu t: RE: Volume for DNA analysis	Paula Brisotto <	Cathie Allen

Hi, I think we need to ask about Minifiler and Y-Filer Plus amplifications as well.

They are the two processes that QPS seek assistance from ESR with.

Thanks Justin



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Turlough Thomas-Stone < Sent: Friday, 19 August 2022 1:3		
To: Helen Gregg < Cc: Cathie Allen <	>	
Subject: RE: Volume for DNA analysis		

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Hello,

We use the Identifiler + kit for casework.

We amp a maximum of 5ul per amp. This is normally for samples that have low levels of DNA.

Do you have quant values for the samples in question? If so then if there is a decent amount of DNA present then we may amp less than 5ul per amp.

Our optimal amp vol is 1.0ng/ul.

So to answer you question if we amp at max then we could do 3 amps out of the 15ul you have remaining from your extracted DNA

Thanks

Regards Turlough Turlough Thomas-Stone BSc (Hons) Team Leader / Senior Scientist (Forensic Biology) Institute of Environmental Science and Research Limited (ESR) Mt Albert Science Centre, 120 Mt Albert Road, Auckland 1025



From: Helen Gregg < Sent: Friday, 19 Augu To: Turlough Thomas-Stone <Turlough Cc: Cathie Allen < Subject: FW: Volume for DNA analysis

Hi Turlough,

I got a out of office response from Sarah Cockerton, which referred me to you.

I am acting Executive Director at FSS for a few weeks, and am interested in the minimum volume your lab would require for DNA analysis.

We concentrate to a volume of 35ul, and use about 20ul in our amplification and CE. We have about 15uL left over if we want to go back and do another amp.

If we were to want to have the amp and CE done by ESR, would 15uL be sufficient?

Thanks in advance Helen



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Disclaimer: This email and any attachments may contain legally privileged or confidential information and may be protected by copyright. You must not use or disclose them other than for the purposes for which they were supplied. The privilege or confidentiality attached to this message and attachments is not waived by reason of mistaken delivery to you.

If you are not the intended recipient, you must not use, disclose, retain, forward or reproduce this message or any attachments. If you receive this message in error, please notify the sender by return email or telephone and destroy and delete all copies. Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

Queensland Health carries out monitoring, scanning and blocking of emails and attachments sent from or to addresses within Queensland Health for the purposes of operating, protecting, maintaining and ensuring appropriate use of its computer network.

The information contained in this message and/or attachments from ESR is intended solely for the addressee and may contain confidential and/or privileged material. If you are not the intended recipient, any review, disclosure, copying,

distribution or any action taken or omitted to be taken in reliance on it is prohibited by ESR. If you have received this message in error, please notify the sender immediately.

This email has been filtered by SMX. For more information visit smxemail.com

RE: Process following A/DG memo

From:	Justin Howes <	
To:	Sharon Johnstone <	Kylie Rika <
Date:	Thu, 01 Sep 2022 12:46:47 +1000	

Hi, responses below. Essenally, these are quesons that QPS have requested be added as a stnd to the task in order for them to advise approval or not.

Hopefully, I have answered your points. Just really a. er your assistance of template/suggested wording that could help your staff when sending these requests.



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Sharon Johnstone < Sent: Thursday, 1 S To: Justin Howes < Subject: RE: Process following A/DG memo

Hi,

Just trying to clarify what it is you are looking for:

So are these quesons that the QPS have asked us give info for as a standard in the wording of the request? Yes Are we trying to provide the standard wording examples for our sciens to use? Yes – some assistance to guide their comments in the Req/Tasks

Do we know if FLU are going to answer these requests or are they really going to send to an I/O? That will be up to the FLU. My understanding is that FLU will liase with the Investigators. Asking because the wording we use might need to be different for an I/O with no DNA knowledge versus FLU that should have some idea.

For the 2nd point re mic – a yes / no should be sufficient because regardless the volume le is the ne xt point. Yes, the QPS are interested if it has already been concentrated here.

I would also be hesitant to use the term microcon and replace with concentraon of the sample. Can seek removal of the word Microcon, but to retain Microcon to full (which is what a process is called).

Do we really want to be talking about external services as an alternav e like it is something that's the done thing? Realisc ally this sort of tesng and the c ost of it is reserved for samples that are high profile cases with very lile leads. I don't like having to menon this sort of thing f or every sample. Especially when these samples are ones that were NDNA or Insuff and were asked rightly or wrongly to be tested again by QPS already. Yes, I take your point. This is a QPS request so that they can decide to approve exhaustion or not. The QPS are clear that on their samples, they wish to be consulted if likely to exhaust moving forward.

Cheers, Sharon



From: Justin Howes < Sent: Thursday

To: Kylie Rika <

>; Sharon Johnstone <

. . . .

Subject: RE: Process following A/DG memo

Hi Kylie and Sharon, QPS have requested further informaon to be provided to assist them in the approval process where the sample is likely to be exhausted.

Please see the points below that are to be completed when sending the Request/Task. Given the informaon that Request/Tasks can be sent to a group at QPS in the FR, Request/Tasks of this type can be sent to 'FLU'.

Please note the Quant value below relates to the TSAQty.

Hello a DNA profile has been obtained from the linked crime scene sample I am seekina approval for additional work to be undertaken on the sample in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

Additional information to assist:

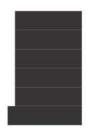
- * Quant value:
- * Undergone Microcon: No/Yes (if yes, please provide approx. volume remaining after Microcon concentration):
- * Current Volume Remaining: uL
- * Further Processing Requested eg. Microcon to full, additional amplification
- * Will further processing exhaust the sample: No/Yes
- * Description of DNA profile obtained to date:
- * Scientific Opinion on the likelihood that further internal testing may provide additional probative information:
- * Recommendation as to whether the sample may be better tested by an external service provider:

Could you both please work on generic wording for the last three points?

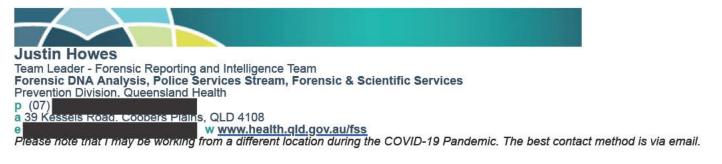
Inial though ts from me:

• * The descripon of the DNA pr ofile – this will be in the absence of STRmix, so please think on generic words with 'inial in tuiv e assessment' declared. * Likelihood of providing further information – this is unknown without having done the rework, but the request in seeking approval is all about an attempt to obtain more from the sample.

Could you please work on this today as there could be samples in these categories now, or at least very soon? The QPS have asked that the following barcodes be provided with the further information above. When you have advised wording, I will send to staff and add to the SOP, and then could you please work with the staff involved with the samples below.



Thanks Justin



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Subject: Process following A/DG memo

Hi all

Following this memo, the information below will be added to 17117 which will be sent to review early next week:

When seeking written approval from QPS for additional work if considered beneficial, send a Request/Task via the Forensic Register to the relevant Forensic Officer found by the field below. Add the Forensic Officer's ID number to the Action Officer field, and link the relevant crime scene barcode to the Request/Task.

ocation / Owner				
From the front dri	ver seat adjustment levers			
Exam Source				
Vehicle:	Van			
Exhibit Notes & Anal	lvsis Advice			
Parent Barcode	Property Tag	Current Location	Investigator	Forensic Officer
Parent Barcode	Property Tag	Current Location PSD	Investigator	Forensic Officer
		PSD	Investigator	Forensic Officer
		CONTRACTOR AND A CONTRACTOR	Investigator	Forensic Officer
Parent Barcode Ownership / Relation		PSD	C Finger	Forensic Officer rprint Bureau prechic Section

Suggested Template for wording:

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for additional work to be undertaken on the sample, in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

When sending the Request/Task, the exhibit result line **SOHAA** – **Sample on hold, awaiting advice** should be added, and validated by a second operator.

When QPS respond, the exhibit result line **TRQ – Testing restarted on advice from QPS** hould be added irrespective of whether approval for further processing has been granted or not. The result will either be reported based on the one amplification result, or will be reported after the further processing.

Regards Justin Justin Justin Howes Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health p (07) a 39 Kessels Road, Coopers Plans, QLD 4108 W www.health.qld.gov.au/fss Prevase note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

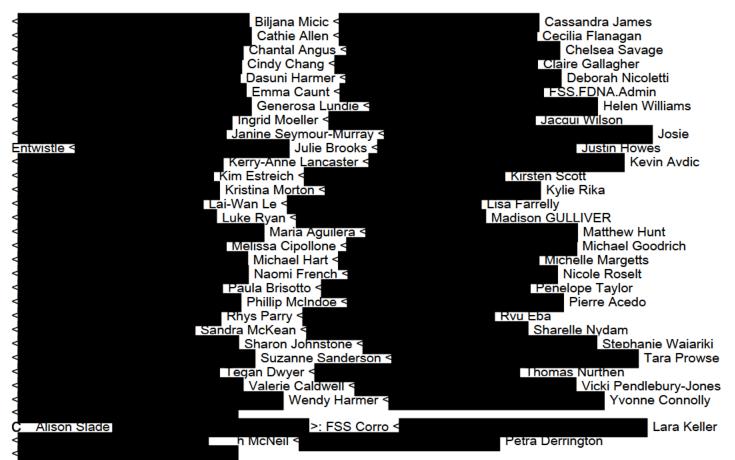
Anne Finch <



Wash your hands regularly to stop the spread of germs.

From: Helen Gregg <
Sent: Friday, 19 Augu
Abigail Rvan <Abigail.Rvan@health.qld.gov.au>; Adam Kaitv <
Alanna Darmanin <
Allan McNevin <
Allan McNevin <
Amy Cheng <
Angela Adamson <

Adrian Pippia Alicia Quartermain Alison Lloyd Amy Morgan Angelina Keller Belinda Andersen



Subject: FW: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health -Subject of memorandum

Good afternoon everyone,

Please see attached memo. I have asked for an enhancement to FR to assist with this change.

Please hold all quants effective immediately, until the FR enhancement is complete. Paula has specific details for the analytical team.

For batches that have already progressed beyond quant, proceed as per this morning's processes.

Could you please update SOPs asap.

Contact me if you have any queries.

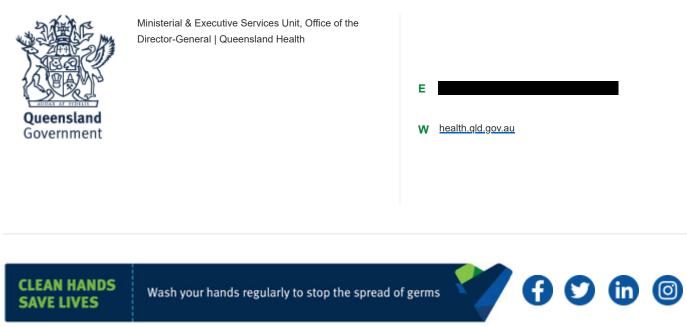
Regards Helen



Please see attached the Memorandum from Dr David Rosengren, Acting Director-General, Queensland Health, for your attention.

Should you have any questions in relation to this advice, please contact Professor Keith McNeil, Acting Deputy Director-General on telephone 07

Kind Regards



Kylie Rika

From: Sent: To: Cc: Subject: Justin Howes Monday, 22 August 2022 10:53 AM Kylie Rika Angelina Keller RE: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi

The memo mentions the range, so if bone/teeth are in the range, then they would be microconned in the way the memo describes. If at examination, an analytical note of a different approach is made, then that could be made. This would be similar to cold case Q&H processes where the note is made to hold and consult after quant.

Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p (07)
a 39 Kessels Road, Coopers Plains, QLD 4108
e w www.health.qld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

From: Kylie Rika < Sent: Monday, 22 August 2022 9:59 AM

To: Justin Howes <

Cc: Angelina Keller <

Subject: FW: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi Justin

Please see query below from Angelina. Are we able to get some clarification on this please?

Thanks Kylie From: Angelina Keller < Sent: Monday, 22 August 2022 9:51 AM To: Kylie Rika <

Subject: FW: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Hi Kylie,

Is it possible to clarify the sample categories affected by this latest direction. For example I would assume bone / teeth aliquots are exempt as well as No DNA samples.

Kind regards, Angelina

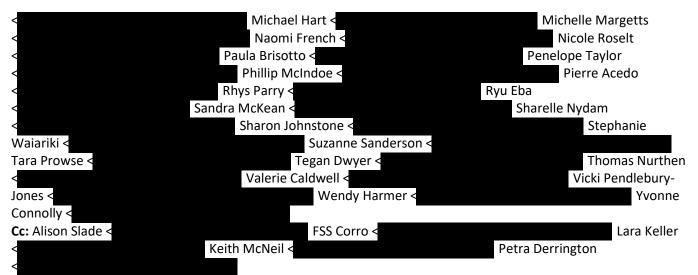


Angelina Keller Reporting Scientist

Forensic DNA Analysis, Forensic & Scientific Services Prevention Division, Queensland Health

p (07)
a 39 Kessels Road, Coopers Plains, Qld, 4108
e www.health.qld.gov.au/fss

From: Helen Gregg < Sent: Friday, 19 August 20	022 3:33 PM	
To: Abigail Ryan <	Adam Kaity <	Adrian Pippia
<	Alanna Darmanin <	Alicia Quartermain
<	Allan McNevin <	Allison Lloyd
<	Amy Cheng <	Amy Morgan
<	Angela Adamson <	Angelina Keller
<	Anne Finch <	Belinda Andersen
<	Biljana Micic <	Cassandra James
<	Cathie Allen <	Cecilia Flanagan
<	Chantal Angus <	Chelsea Savage
<	Cindy Chang <	Claire Gallagher
<	Dasuni Harmer <	Deborah Nicoletti
<	Emma Caunt <	FSS.FDNA.Admin
<	Generosa Lundie <	Helen Williams
<	Ingrid Moeller <	Jacqui Wilson
<	Janine Seymour-Murray <	Josie
Entwistle <	Julie Brooks <	Justin Howes
<	Kerry-Anne Lancaster <	Kevin Avdic
<	Kim Estreich <	Kirsten Scott
<	Kristina Morton <	Kylie Rika
<	Lai-Wan Le <	Lisa Farrelly
	Luke Ryan <	Madison GULLIVER
<	Maria Aguilera <	Matthew Hunt
<	Melissa Cipollone <	Michael Goodrich



Subject: FW: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health - Subject of memorandum

Good afternoon everyone,

Please see attached memo. I have asked for an enhancement to FR to assist with this change.

Please hold all quants effective immediately, until the FR enhancement is complete. Paula has specific details for the analytical team.

For batches that have already progressed beyond quant, proceed as per this morning's processes.

Could you please update SOPs asap.

Contact me if you have any queries.

Regards Helen



Helen Gregg A/Executive Director

Forensic and Scientific Services Prevention Division, Queensland Health

w www.health.qld.gov.au/fss

Please see attached the Memorandum from Dr David Rosengren, Acting Director-General, Queensland Health, for your attention.

Should you have any questions in relation to this advice, please contact Professor Keith McNeil, Acting Deputy Director-General on telephone



RE: clarification

From:	Cathie Allen <		
To:	Helen Gregg < Brisotto <	Justin Howes <	Paula
Cc:	Alison Slade <		
Date:	Mon, 22 Aug 2022 12:45:59 +1000		

I agree – any process that we undertake that could consume the DNA extract, can only be done with QPS approval.

From: Helen Gregg < Sent: Monday, 22 Aug T < CC: Subject: RE: clarification	; Cathie Allen <	Paula Brisotto
l agree		
From: Justin Howes < Sent: Monday, 22 Aud T < CC: Subject: RE: clarification	; Cathie Allen <	Paula Brisotto

Hi all

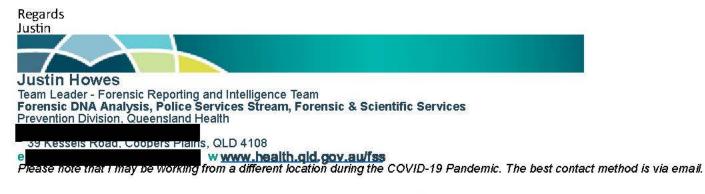
I have had some questions from staff, so one point to ensure we all understand the same thing:

The staff question was: 'samples that are 0.010 and have bene amped on their initial run, and we would like to M'con it to 35uL and then amp it again after that, potentially using up all of the extract? Do we need to ask permission from QPS for those too? If we do, does this mean m'conning to full can only happen if we request permission from QPS to use up all of the extract?

My answer was

 * My understanding from the seeking of approval for a second amp is that QPS want extract volume retained, and only with approval, are QPS fine to accept the consumption of the extract. So with the scenario below, any work that would totally consume the extract (ie. full microcon, or second amp after microcon) would need prior approval from QPS.

Do we all agree that the general point is that any decision which could use up all the extract would need QPS approval? This would be second amp after microcon, or if microconned to full.



CLEAN HANDS SAVE LIVES	Wash your hands regularly to stop the spread of germs.	
From: Helen Gregg < Sent: Monday, 22 Aud T Cc: Subject: RE: clarification Hi,	Paula Brisotto <	Justin Howes
Regards Helen		
From: Cathie Allen < Sent: Monday, 22 Au T < Cc: Subject: RE: clarification	Justin Howes <	Helen Gregg
Good questions Paula! I've responded in blue below for ease.		
From: Paula Brisotto < Sent: Friday, 19 August 2022 5:18 PM T Justin Howes < Cc: Allson Slade < Subject: clarification	Cathie Allen <	Helen Gregg

HI all,

A couple of things have popped into my head, which may be questions from staff come Monday (or may be my tired Friday afternoon thoughts):

For any samples processed prior to 5 June 202218 that were reported as *DNA Insufficient for Further Processing* and **are requested by QPS to proceed to testing** (which as per previous process, involves microconning), QPS have already approved additional processing, so further approval is not required? From my perspective, I would say the 2rd amp would require approval, however if 2rd amp has already proceeded then it has occurred before the QPS direction of 19th Aug 2022. Agree - 2nd amp approval required if we are doing post 19 August. If 2^d amp started pre-19 August we cannot get approval.

For any samples processed after 5 June 202218 until today, where FSS staff requested a microcon, before proceeding to a second amplification, approval from QPS is required? **Yes, written approval requiredagree – written approval required**

For any samples after 5 June where a microcon by FSS staff was requested to full, or a second amplification has already occurred and all sample is consumed, as this was previous process, no further advice is required...? In these instances, do we need to formally advise the QPS -Helen, what's your thoughts? Wed need bdna to search the FR to find these ones (any in the quant range, that have Microcon and have 2 amps after Microcon As above, the request for 2nd amp was prior to 19th August, so QPS approval is not possible, and the sample has been exhausted. I donthink QPS can do anything with the additional information we could provide except to know that the sample has been exhausted. Also - who would we give that message to so that it would get through (would we put it in FR/on the statement)? Given TAT are going up, and the information is unactionable, I think we do not need to do anything.

Thanks, Paula

Paula Brisotto

Team Leader – Evidence Recovery & Quality Team Forensic DNA Analysis, Police Services Stream Forensic & Scientific Services. Prevention Division, Queensland Health

Iains OLD 4108

p 07 a <u>39</u>

е

w www.health.qld.gov.au/fss

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

RE: clarification

From: To:	Justin Howes < Paula Brisotto < <	Helen Gregg <	Cathie Allen
Cc:	Alison Slade <		
Date:	Mon, 22 Aug 2022 16:38:24 +1000		

Hi, closing this off:

The staff member confirmed they meant the second amp after the microcon to 35uL. This would be where the extract would be consumed. I replied to them that QPS approval would be needed for that second amp after concentration to 35uL.

Thanks Justin



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Subject: RE: clarification

Hi,

I may have not understood the question from the staff, so apologies.

For the first part of the question – amping at 15μ L (at max) would have ~80 μ L left. Micrcon to 35μ L, then quant and amp (at max) - would have~~18 μ L. This would fall into what is currently allowable prior to seeking QPS approval....?

For the second part of the question – yes agree – my understanding is that any microcon to full being requested from today (for samples processed pre 19 August) will require approval. And any **second amp after microcon to 35µL** will require approval.

Thanks, Paula

TJustin Howes < Cc: Allson Slade < Subject: RE: clarification	Cathie Allen <	Paula Brisotto
l agree		
From: Justin Howes < Sent: Monday, 22 Aud T < C C:	Cathie Allen <	Paula Brisotto

Subject: RE: clarification

Hi all

I have had some questions from staff, so one point to ensure we all understand the same thing:

The staff question was: 'samples that are 0.010 and have bene amped on their initial run, and we would like to M'con it to 35uL and then amp it again after that, potentially using up all of the extract? Do we need to ask permission from QPS for those too? If we do, does this mean m'conning to full can only happen if we request permission from QPS to use up all of the extract?

My answer was

• * My understanding from the seeking of approval for a second amp is that QPS want extract volume retained, and only with approval, are QPS fine to accept the consumption of the extract. So with the scenario below, any work that would totally consume the extract (ie. full microcon, or second amp after microcon) would need prior approval from QPS.

Do we all agree that the general point is that any decision which could use up all the extract would need QPS approval? This would be second amp after microcon, or if microconned to full.



	CLEAN HANDS SAVE LIVES	Wash your hands regularly to stop the spread of germs.	
From: Helen Gregg < Sent: Monday, 22 Auc T CC: Subject: RE: clarificati	P	aula Brisotto <	Justin Howes
Hĭ,			
See below in green			
Regards Helen			

From: Cathie Allen < Sent: Monday, 22 Au T Cc: Subject: RE: clarification	Justin Howes <	Helen Gregg
Good questions Paula!		
I've responded in blue below for ease.		
From: Paula Brisotto < Sent: Friday, 19 Augus T < C c:	Cathie Allen <	lelen Gregg

Subject: clarification

Hi all,

A couple of things have popped into my head, which may be questions from staff come Monday (or may be my tired Friday afternoon thoughts):

For any samples processed prior to 5 June 202218 that were reported as *DNA Insufficient for Further Processing* and are requested by QPS to proceed to testing which as per previous process, involves microconning), QPS have already approved additional processing, so further approval is not required? From my perspective, I would say the 2nd amp would require approval, however if 2nd amp has already proceeded then it has occurred before the QPS direction of 19th Aug 2022. Agree – 2nd amp approval required if we are doing post 19 August. If 2^d amp started pre-19 August we cannot get approval.

For any samples processed after 5 June 202248 until today, where FSS staff requested a microcon, before proceeding to a second amplification, approval from QPS is required? **Yes, written approval requiredagree – written approval required**

For any samples after 5 June where a microcon by FSS staff was requested to full, or a second amplification has already occurred and all sample is consumed, as this was previous process, no further advice is required...? In these Instances, do we need to formally advise the QPS -Helen, what's your thoughts? Wed need bdna to search the FR to find these ones (any in the quant range, that have Microcon and have 2 amps after Microcon) above, the request for 2nd amp was prior to 19th August, so QPS approval is not possible, and the sample has been exhausted. I donthink QPS can do anything with the additional information we could provide except to know that the sample has been exhausted. Also - who would we give that message to so that it would get through (would we put it in FR/on the statement)? Given TAT are going up, and the information is unactionable, I think we do not need to do anything.

Thanks, Paula



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

CLEAN HANDS

SAVE LIVES

Wash your hands regularly to stop the spread of germs.

From:	Justin Howes
To:	Emma Caunt; Sharon Johnstone; Kylie Rika
Subject:	RE: recent A/DG memo
Date:	Tuesday, 23 August 2022 3:35:49 PM
Attachments:	image003.png
	image004.png

Hi

Yes QPS are aware that the options for further testing elsewhere are limited. FASS and ESR use half-volumes for Y-STRs, and ESR at least (being generally the preferred external) use max vol of 10uL for minifiler and 5uL for ID+ and YSTRs.

Analytical staff have been working with 35uL for a long time (since full-vol PP21) and I am sure they will continue to do their best in the manual process to achieve this which is a difficult assignment - all staff were shared the direction from the A/DG. I am not sure how often QPS will not approve a second amp post-mic, but will be interesting to monitor over time. We do have a number of external transfers per year but less often for current/active casework.

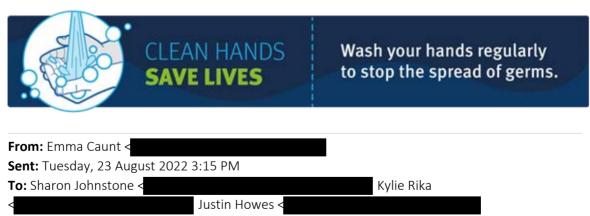
Will be interesting.

Justin



Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Subject: RE: recent A/DG memo

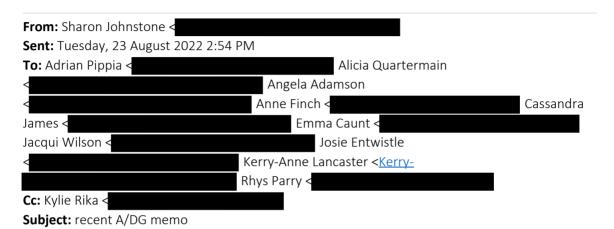
Hi

I have a couple of questions. I don't know who to address them to so I thought I'd include you all

- 1. Do we know how much volume other labs require for further testing whether it be Y-STRs, LCN, minifiler, etc?
- 2. Have analytical staff been advised of the requirement to conserve sample? The reason I ask is that often a m'con to 35uL will result in a volume <35uL. This will affect our ability to conserve sample and therefore it is important that analytical staff don't over spin the sample.

Thanks

Emma



Hi there.

Attached is a workflow that has been added to the 17117 SOP as a comment. The recent changes may be a bit hard to remember key dates so hopefully this will help. Please note that that the overarching principle from the DG memo is that the DNA extract cannot be exhausted without QPS approval. (eg. whether second amp post mic, or consideration of mic to full) Regards,

Sharon





From:	Sharon Johnstone
To:	Adrian Pippia; Alicia Quartermain; Angela Adamson; Anne Finch; Cassandra James; Emma Caunt; Jacqui
	Wilson; Josie Entwistle; Kerry-Anne Lancaster; Rhys Parry
Subject:	A/DG memo
Date:	Wednesday, 24 August 2022 3:33:00 PM
Attachments:	image002.png

Hi all,

There have been a few questions asked as a result of this change. Please see the information below that will likely answer any questions you may have. If you have any others please send them through

- Microcon to full was a common (though not default) strategy in use for many years and recently became quite a common strategy for reporters after DIFP samples started to appear on the worklists again. It was widely seen as more effective with very low template samples than the usual microcon to 35 to give the best chance of obtaining useable profile info (I note this is based on conversations with other reporters and therefore maybe somewhat subjective).
- Is the mic to full rework strategy no longer available under any circumstances, due to the risk of DNA extract exhaustion?
- Could there be any negative consequences for our having previously used this strategy fairly liberally without informing QPS of the fact?
- If so, then do we need to inform QPS now of the microcon to full samples which have previously been processed (providing a list of the potentially exhausted extracts they are currently unaware of)? Is there any feasible way to collect this data if necessary?
- How much extract is it necessary to preserve before it is classified as 'exhausted'? Can we presume 15uL is required, to allow for a potential future amp to max?
- With a view to preserving extract and maximising DNA concentration and profile peak heights, could we consider altering the microcon to 35uL workflow, so that a second quantification step is <u>not</u> performed after microcon, but the concentrated extract is immediately amplified at 15uL?
- In my view the number of samples within the low template category which would be
 overamplified by a straight amp at max is extremely low. For multi-contributor mixtures the
 quant may indicate total DNA as requiring a reduction in the extract added to SV1 in
 reality if we are trying to interpret these profiles, it is only the 'major' peaks which are
 potentially going to be meaningful, if at all. We should give them the best possible chance
 by amping at max, without wasting resources on another quant and potentially lowering
 the amp vol added. This may help offset some of the impact of the absence of the 'mic to
 full' rework option.
- The workflow note about P3 sample states 'Reworks are limited and only performed in exceptional circumstances'. Does the prior policy of not allowing microcons (of any type) as an option for this priority type, or could this be considered as a possible option now (for the occasional profile where this may yield an upload, where another amp to max would be unlikely to).

The answers to questions really all come back to the Memo. The background/context isn't known but I think we are able to work with the memo directive as it is. The A/DG mentions in the memo that consultation with QPS has occurred and I do know that they are not keen on material exhaustion unless with their approval. With this overall principle in not exhausting extract without prior approval from QPS, Helen Gregg gave words to me on this today to ensure it is in the SOP (now back in QIS as 17117v21.5 for your review). I don't know when or what circumstances QPS would not approve a second amp post mic, but the hope is that there is approx. 15uL for this effort, which could also be used externally.

As per the memo, we need to find the samples that have not been microconned that had initial quants in this range. Bdna are working on finding these samples at the moment, and then these samples will have the mic process as per the memo and SUFP line added unless a final interp has not been added (in which case the end result would be reported). Data is not available on this yet – Paula will let me know when more is known.

There isn't any change to the P3 process to my knowledge - it is as per the SOP. These will not have the mic process, and will be amped straight after quant. I am not aware of any further information.

Paula asked Helen about the second quant and she mentioned that the A/DG wanted the process to be the same as what we have had before, and there is no change to this process with the memo release ie a second quant is performed. In thinking further on it too, the quant post mic will also help the client in external consultation if required.

- 1. Do we know how much volume other labs require for further testing whether it be Y-STRs, LCN, minifiler, etc?
- 2. Have analytical staff been advised of the requirement to conserve sample? The reason I ask is that often a m'con to 35uL will result in a volume <35uL. This will affect our ability to conserve sample and therefore it is important that analytical staff don't over spin the sample.

Yes QPS are aware that the options for further testing elsewhere are limited. FASS and ESR use half-volumes for Y-STRs, and ESR at least (being generally the preferred external) use max vol of 10uL for minifiler and 5uL for ID+ and YSTRs.

Analytical staff have been working with 35uL for a long time (since full-vol PP21) and I am sure they will continue to do their best in the manual process to achieve this which is a difficult assignment - all staff were shared the direction from the A/DG. I am not sure how often QPS will not approve a second amp post-mic, but will be interesting to monitor over time. We do have a number of external transfers per year but less often for current/active casework.

So just to clarify:

The new workflow only applies to P1 and P2 samples within the 0.001-0.0088ng/uL quant range

All other P1, P2 and P3 samples outside of this quant range (0.0089 and above) are case managed as usual

Cheers, Sharon



Sharon Johnstone Senior Scientist – Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream Prevention Division, Queensland Health

Please note that I may be working from a different location during the COVID-19 pandemic. The best contact method is via email.

p 07

e

a 39 Kessels Road, Coopers Plains, QLD 4108

w www.health.qld.gov.au/fss

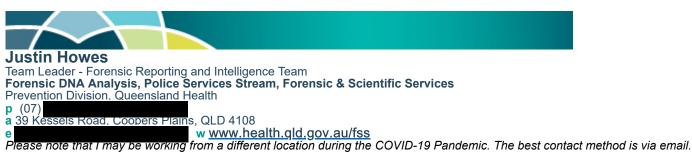


RE: Workflow - Exhaustion of extract

From:	Justin Howes <
То:	Helen Gregg <
Date:	Thu, 25 Aug 2022 12:04:09 +1000

Thanks, please let me know a good met o come over to discuss key points.

Jusn



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Helen Gregg < Sent: Thursday, 25 To: Justin Howes < Idd.gov.au> Subject: RE: Workflow - Exhaustion of extract

Hi Jusn – ab solutely – I would love to speak to you and your team. I am getting emails direct

Н

Hi Helen

Would you please have met o speak with me a. er 12pm today on this thread? There are a few threads, but below has most informaon t o assist our chat.

Pls let me know – whether today, or tomorrow.

Thanks Jusn



Team Leader - Forensic Reporting and Intelligence Team **Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services** Prevention Division, Queensland Health



Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Justin Howes Sent: Thursday To: Kylie Rika <

<u>.au</u>>; Sharon Johnstone <

Subject: RE: Workflow - Exhaustion of extract

Hi

My understanding is the overriding principle is to not exhaust any DNA extract without QPS wri en approval.

I will seek word from Helen Gregg whose office the memo can through. Hopefully, she will get back today on this.

Jusn



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Kylie Rika < Sent: Wednesday, 2 To: Justin Howes < ______<u>.gov.au</u>>; Sharon Johnstone < ______ Subject: RE: Workflow - Exhaustion of extract

Thanks Jusn

So for samples that are not P1/2 in the range 0.001-0.0088ng/ul – can we exhaust them? I am really confused – and it is very difficult to give the correct guidance to staff when we don't have all the informaon.

Thanks Kylie

From: Justin Howes < Sent: Wednesda	
To: Kylie Rika < Subject: RE: Workflow - Exhaustion of extra	u>; Sharon Johnstone <

Hi, yes the A/DG bolded that part in the memo. The new workflow is only for P1/2 in the range 0.001-0.0088ng/uL.

Justin Howes Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division. Queensland Health (07)D 39 Kessels Road, Coopers Plains, QLD 4108 а w www.health.gld.gov.au/fss e

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Wash your hands regularly to stop the spread of germs.

From: Kylie Rika < Sent: Wednesday <u>qov.au</u>>; Sharon Johnstone < To: Justin Howes < Subject: RE: Workflow - Exhaustion of extract

Thanks Jusn

So just to clarify:

The new workflow only applies to P1 and P2 samples within the 0.001-0.0088ng/uL quant range?

All other P1, P2 and P3 samples outside of this quant range (0.0089 and above) are case managed as usual?

Thanks Kylie

From: Justin Howes < Sent: Wednesday, 24

To: Sharon Johnstone <<u>Sharon.Johnstone@health.qld.gov.au</u>>; Kylie Rika < Subject: FW: Workflow - Exhaustion of extract

Hi

The answers to below really all come back to the Memo. The background/context isn't known but I think we are able to work with the memo direcve as it is. The A/DG menons in the memo that consultaon with QPS has occurred and I do know that they are not keen on material exhauson unless with their appr oval. With this overall principle in not exhausing e xtract without prior approval from QPS, Helen Gregg gave words to me on this today to ensure it is in the SOP (now back in QIS as 17117v21.5 for your review). I don't know when or what circumstances QPS would not approve a second amp post mic, but the hope is that there is approx. 15uL for this effort, which could also be used externally.

As per the memo, we need to find the samples that have not been microconned that had inial quan ts in this range. Bdna are working on finding these samples at the moment, and then these samples will have the mic process as per the memo and SUFP line added unless a final interp has not been added (in which case the end result would be reported). Data is not available on this yet – Paula will let me know when more is known.

There isn't any change to the P3 process to my knowledge - it is as per the SOP. These will not have the mic process, and will be amped straight after quant. I am not aware of any further informaon.

Paula asked Helen about the second quant and she menoned that the A/DG wanted the process to be the same as what we have had before, and there is no change to this process with the memo release ie a second quant is performed. In thinking further on it too, the quant post mic will also help the client in external consultaon if r equired.

Hope that helps!

Angelina Keller



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Penelope Tavlor <

Subject: RE: Workflow - Exhaustion of extract

Hi,

Moeller <

Thanks for forwarding the new workflow, it generally makes sense, but I have a few initial questions about it:

- * Microcon to full was a common (though not default) strategy in use for many years and recently became quite a common strategy for reporters after DIFP samples started to appear on the worklists again. It was widely seen as more effective with very low template samples than the usual microcon to 35 to give the best chance of obtaining useable profile info (I note this is based on conversations with other reporters and therefore maybe somewhat subjective).
- * Is the mic to full rework strategy no longer available under any circumstances, due to the risk of DNA extract exhaustion?
- * Could there be any negative consequences for our having previously used this strategy fairly liberally without informing QPS of the fact?
- * If so, then do we need to inform QPS now of the microcon to full samples which have previously been processed (providing a list of the potentially exhausted extracts they are currently unaware of)? Is there any feasible way to collect this data if necessary?
- * How much extract is it necessary to preserve before it is classified as 'exhausted'? Can we presume 15uL is required, to allow for a potential future amp to max?
- * With a view to preserving extract and maximising DNA concentration and profile peak heights, could we
 consider altering the microcon to 35uL workflow, so that a second quantification step is <u>not</u> performed after
 microcon, but the concentrated extract is immediately amplified at 15uL?
- * In my view the number of samples within the low template category which would be overamplified by a straight amp at max is extremely low. For multi-contributor mixtures the quant may indicate total DNA as requiring a reduction in the extract added to SV1 – in reality if we are trying to interpret these profiles, it is only the 'major' peaks which are potentially going to be meaningful, if at all. We should give them the best possible chance by amping at max, without wasting resources on another quant and potentially lowering the amp vol added. This may help offset some of the impact of the absence of the 'mic to full' rework option.
- * The workflow note about P3 sample states 'Reworks are limited and only performed in exceptional circumstances'. Does the prior policy of not allowing microcons (of any type) as an option for this priority type, or could this be considered as a possible option now (for the occasional profile where this may yield an upload, where another amp to max would be unlikely to).

Appreciate your thoughts on these points.

Regards,



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

From: Kylie Rika < Sent: Tuesday, 23 To: Allan McNevin < <u>Allan.McNevin@hea</u> Matthew Hunt < Nicoletti < Subject: FW: Exhaustion of extract	alth.ald.gov.au>; Thomas Nurthen < Claire Gallag <u>her <</u> Ingrid <u>Moeller <</u> Angelina Keller <	Deborah Penelope Taylor Tegan Dwyer
FYI		
From: Justin Howes <		

Sent: Tuesday, To: Kylie Rika <	n Johnstone <
Cc: Paula Brisot Subject: RE: Exhaustion of extract	

Hi

Please try this workflow first Kylie which has been made available to Helen Gregg. I did this to get my head around it and am hoping that this is clear on what samples go where, and the overriding principle. This is in 17117 as an Appendix which is currently in review.

Justin

Justin Howes	
Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services	
Prevention Division. Queensland Health	
p (07)	
a 39 Kessels Road, Coopers Plains, QLD 4108	
e w <u>www.health.qld.gov.au/fss</u>	
Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact m	ethod is via email.

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



From: Kylie Rika < Sent: Tuesday, 23		
To: Justin Howes < J	Sharon Johnstone <	
Cc: Paula Brisotto <		
Subject: RE: Exhaustion of extract		

Thanks Justin

I am wondering if a meeting with staff would be a good way for staff to better understand the changes? and allow all questions to be answered in one go.

Thanks Kylie

From: Justin Howes < Sent: Tuesday, To: Kylie Rika < Cc: Paula Brisot Subject: Exhaustion of extract	v au>: Sharon Johnstone <	
--	---------------------------	--

Hi

I know there have been some questions regarding the A/DG Memo and extract volumes. I just spoke to Helen Gregg who asked if I thought the message on extract availability is clear with staff.

I said there have been some questions to me, and perhaps more with seniors but that I would reiterate the message that the overarching principle in any situation (eg. whether second amp post mic, or consideration of mic to full) from the DG memo is that the DNA extract cannot be exhausted without QPS approval. She was happy with this and I mentioned it is already in the draft SOP for further review (17117v21.4).

Could you pls ensure that staff understand the key principle?

Thanks Justin





From: Sent: To: Cc: Subject: Attachments: Justin Howes Monday, 29 August 2022 2:11 PM Helen Gregg Alison Slade; Paula Brisotto Thread on MS Teams_DG memo workflow_29082022 Thread on MS Teams_DG memo workflow_29082022.docx

Hi

Here is a thread that was on MS Teams on Fri, that I just copied and thought may or may not be useful to you.

I think there are many practical issues with this proposal here – remember the 'low level' samples are only about 10-15% of the total samples we process. Approx 4500 per year.

Thanks Justin



Justin Howes

Team Leader - Forensic Reporting and Intelligence Team

Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health

p (07)
a 39 Kessels Road, Coopers Plains, QLD 4108
e w www.health.gld.gov.au/fss

Please note that I may be working from a different location during the COVID-19 Pandemic. The best contact method is via email.



	noughta age to be s	after extrac tored for fu	iture testin		ke the suggestion (to whomever it needs to be suggested to) to remove 15uL of ? That way we could m'con to full if necessary, without needing to ask permission in
ose all					
	rtermain 2	5/08 5:02 pr	n		
FRIT					
	int 26/08 9 artermain):07 am that's an e	xcellent id	еа	🔞 1 🖤 1
		/08 10:29 am			
				he impact c	in the sample that will be process - at 15uL approximately 18% of sample is lost by
splitting	the sample	5,005	70		Ĩ
-	85	15	70	1048	-
Conc	Total DNA	Total DNA	Total DNA	Amp input	
ng/uL	ng	ng	ng	ng	
0.001	0.085	0.015	0.070	0.02	
0.002	0.17	0.03	0.140	0.03	
0.003	0.255	0.045	0.210	0.05	
0.004	0.34	0.06	0.280	0.06	
0.005	0.425	0.075	0.350	0.08	
0.006	0.51	0.09	0.420	0.09	
0.007	0.595	0.105	0.490	0.11	
0.008	0.68	0.12	0.560	0.12	
0.009	0.765	0.135	0.630	0.14	
0.01	0.85	0.15	0,700	0,15	
0.011	0.935	0.165	0.770	0.17	
0.012	1.02	0.18	0.840	0.18	
0.013	1.105	0.195	0.910	0.20	
0.014	1.19	0.21	0.980	0.21	
0.015	1.275	0.225	1.050	0.23	
0.016	1.36	0.24	1.120	0.24	
0.017	1.445	0.255	1.190	0.26	
0.018	1.53	0.27	1.260	0.27	

4 1

0.019	1.615	0.285	1.330	0.29
0.02	1.7	0.3	1.400	0.30
0.021	1.785	0.315	1.470	0.32
0.022	1.87	0.33	1.540	0.33
0.023	1.955	0.345	1.610	0.35
0.024	2.04	0.36	1.680	0.36
0.025	2.125	0.375	1.750	0.38
0.026	2.21	0.39	1.820	0.39
0.027	2.295	0.405	1.890	0.41
0.028	2.38	0.42	1.960	0.42
0.029	2.465	0.435	2.030	0.44
0.03	2.55	0.45	2,100	0.45
0.031	2.635	0,465	2.170	0,47
0.032	2.72	0.48	2.240	0.48
0.033	2.805	0.495	2.310	0.5

See less

Alicia Quartermain 26/08 11:16 am

Thomas Nurthen Very true. I still think it's better than the current process though. Either way, if QPS want sample available for future testing, either they get it put aside from the start or we have to put it aside at the end.

Emma Caunt 26/08 11:19 am

If we start with 90ul and m'con to 35uL we have concentrated by 2.6x. 15uL of this will be saved for further testing. If we start with 90uL and remove 15uL for further testing and then m'con the remaining 75uL to 15uL (full) that is a 5x concentration. So Alicia's idea is probably better for obtaining an optimum result.

Reply

0

.

From:	Kylie Rika
То:	Sharon Johnstone; Josie Entwistle; Justin Howes; Paula Brisotto
Subject:	RE: Further processing of DNA insufficient
Date:	Tuesday, 30 August 2022 11:02:29 AM
Attachments:	image002.png

Hi all

I understand where Sharon is coming from with regards to QPS role and responsibilities, however, at this time we can only control what happens once samples are here with us and so I do think there are some improvements that could be made to this process.

I understand Josie's perspective on having another scientist make decisions on her behalf given that she is the reporter for this case. Whilst all rework requests of this type are sent to Luke, it would be prudent to forward these requests to the case scientist, if one exists, to enable them to reprocess the sample with the case context in mind. I appreciate that this is extra work for Luke, however it would be possible for another scientist to access his requests and forward on any that relate to allocated cases.

Ideally, I would like ALL (internal and QPS) initiated further processing requests to go onto a list that CMers can assess and address.

We all have a lot of extra work to do at the moment, but I think we should continue to keep the best interests of the sample in mind.

Thanks Kylie

From: Sharon Johnstone <			
Sent: Tuesday, 30 August 2			
To: Josie Entwistle <		Kylie Rika	
<	Justin Howes <		Paula Brisotto
<			
Subject: RE: Further proce	ssing of DNA insufficient		

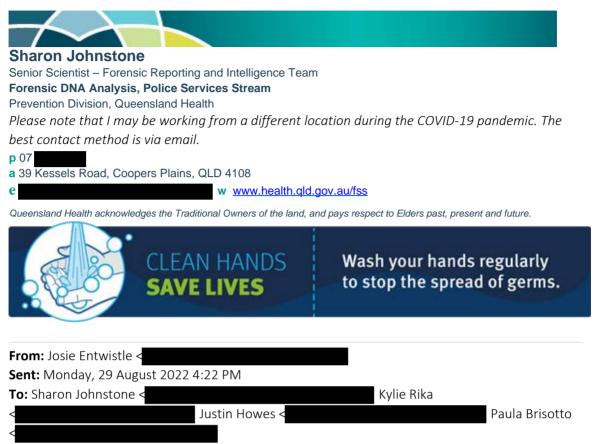
.

Hi Josie,

These samples are one of hundreds that were re-activated by the QPS with no consultation with us and no prior warning. The list that was populated was managed in the same way for all samples due to the large numbers. Luke will not be reporting this nor other cases that he was involved with to process this list.

Ideally, the QPS would not have sent through a request without reviewing all of the results obtained as the rework has not added any value to the case. Nor did it make any sense to rework samples after a trial date had been set. Perhaps it would be a good idea to send a message through SSLU to the I/O to see if an addendum statement will be required given the events. The last one that I encountered like this asked us not to include in an addendum statement. Alternatively, you could send a message to Olivia McIntyre who made the request and has said that "she had reviewed the investigation and was supportive of the reworking of these samples" to see why the request was received to rework them.

Regards, Sharon



Subject: Further processing of DNA insufficient

Hi all,

I have been made aware that two samples from a case that I have previously reported

were requested to undergo further processing by QPS. This request was made on the case summary page in the FR, where there are entries relating to a subpoena I was previously issued, as well as the statement allocation. The samples were submitted to a microcon to 35uL by Luke without any communication or consultation of myself as the reporting scientist of the case.

A microcon to 35uL is not the option I would have chosen for further processing of these samples. I would have recommended a microcon to full for these samples, and for any samples with a quant in the range of what has been reported as NDNA. I am now unsure of the most appropriate pathway for reporting of these samples as results and in a statement of witness. I'm wondering if perhaps this case should now be adopted by Luke?

I understand that these re-activated samples may populate to a worklist, however a response was entered in a case file notation in the FR, where information regarding allocation is readily available. Samples that have already been reported have been allocated to a case manager either to report a result or the entire case, and it's certainly my preference that I am in involved in any further interpretation or processing of samples I've allocated, or that the case is adopted by someone else.

Is it possible to request for the QPS to forward reprocessing enquiries directly to the CMer/reporter, and/or for Analytical to consult the CMer/reporter prior to submitting a processing request, or for Reporting staff to access and monitor the worklist (if relevant, perhaps

an FR enhancement), or for NDNA rework requests to proceed to a microcon to full?

Kind regards

Josie



RE: URGENT: New reporting process involving low quant samples

From:	Cathie Allen <	
To:	Paula Brisotto <	Justin Howes <
Date:	Thu, 01 Sep 2022 15:24:32 +1000	

Thanks Paula & Jusn – I'll send off no w with the below.



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Paula Brisotto < Sent: Thursday, 1 Sept To: Cathie Allen < Subject: RE: URGENT: New reporting process involving low quant samples

Hi all,

Sorry if I'm too late in my response.

See highlighted below.

Thanks, Paula

From: Cathie Allen < Sent: Thursday, 1 S To: Justin Howes < <u>ov.au</u>>; Paula Brisotto < Subject: RE: URGENT: New reporting process involving low quant samples

Hi there – can you please peer review prior to me sending? Thanks.

Hi Steve

The workflow has been reverted to one that was used immediately prior to February 2018 as per DG advice. P1 workflow had not changed – and were automac ally concentrated. Can you please confirm that authorisaon is required prior to exhausng a sample f or P1s.

P2 samples within the specified low quant range are being automatically concentrated to approx. 35uL, quantified and then amplified. This leaves approx. 15 – 18uL of available sample remaining.

Should further additional processing be required, QPS have requested that their authorisation is provided prior to exhausting all remaining sample volume, so contact will be made via Request / Task to 'FLU'. If written approval is provided, the sample may be amplified again (rework) which will likely exhaust the sample.

Samples processed between 6/6/22 and approx. 19/8/22 have been amplified without concentration. Between 6/6/22 and approx. 19/8/22, Forensic DNA Analysis scientists were able to request concentration of a P2 sample to occur, as a rework option. If the scientists have requested a 'concentration to approx 35uL', there may or may not be sample volume available (this would be dependent on how many further reworks were requested). If scientists have requested a 'concentration to full' during this period, the sample will be exhausted.

All samples which had initial quant values within the specified range that were processed during this period and have not yet been subjected to a concentration process will undertake that process – being a microcon concentration to 35µL. These samples will then go through the authorisation process prior to any sample volume being exhausted.



*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Foxover.StephanP[OSC] < Sent: Thursday, 1 To: Cathie Allen < Subject: FW: URGENT: New reporting process involving low quant samples

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Hi Cathie

Can you confirm the accuracy of my understanding of the process as :

- 1. 1. P1 and P2 samples in the low quant range will be microconcentrated to a volume of about 35uL.
- 2. 2. 15uL of the concentrate will then be analysed and the result reported.
- 3. 3. If the result is suboptimal or analysis is unsuccessful, QHFSS will consult with QPS (DNA Management FLU) in relation to possible rework. If the sample is reworked the remainder of the extract will be consumed.

I understand that some samples (those tested between 6/6/22 and 19/8/22) in the low quant range may have already been fully tested without micro concentration. As a result there remains only one opportunity to test which will exhaust the extract.

Can you please let me know if is this is correct.

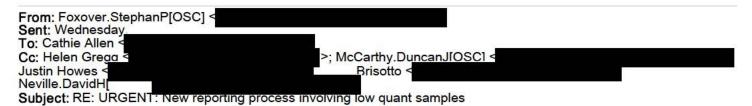
Regards

Steve



Stephan Foxover Acting Inspector Biometrics Forensic Services Group Operations Support Command

GPO Box 1440, Brisbane QLD 4001, Australia



Hi Cathie,

Thanks, I really appreciate the quick response and solution, it will help us adjust to the recent changes in methodology.

Please send the request/task to FLU, staff there will advise on further testing as required. FLU staff will be guided by the information you provide and liaise with QPS stakeholders.

Regards

Steve



Stephan Foxover Acting Inspector Biometrics Forensic Services Group Operations Support Command

GPO Box 1440, Brisbane QLD 4001, Australia

CAUTION: This email originated from outside of Queensland Police Service. Do not click links or open attachments unless you recognise the sender and know the content is safe.

Hi Stephan

Thanks for your email with feedback on a new process.

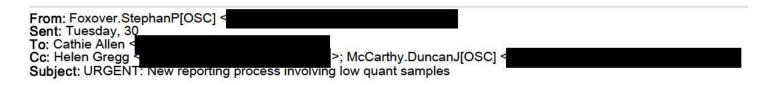
I've worked with Helen Gregg, Paula Brisotto and Justin Howes and we've devised a workflow that will include the below dot points. We will implement this new workflow from here forward, and will add the additional information for the barcodes listed below.

Could you please confirm that the Request / Task should be directed to Results Management Team (RMT) of QPS DNA Unit? We just want to ensure that it goes to the right team.



*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Hi Cathie,

I am aware that recent changes have been made in relation to testing of samples in the concentration range of .001-.0088ng/uL. Advice was received from the Director General of Queensland Health that samples in this range would automatically undergo micro-concentration to 35uL before being further processed in an attempt to obtain a profile. The advice also included that if the testing was unsuccessful and further testing was required, the scientist would liaise with QPS to determine if there was a need to preserve the sample before another attempt was made.

We are now receiving tasks on the FR that include the following wording:

Hello a DNA profile has been obtained from the linked crime scene sample I am seekina approval for additional work to be undertaken on the sample in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory. or elsewhere if alternative technologies are under consideration We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Reauest/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

The relevant barcodes include:



Could vou please advise if each of the above samples subject to this task have been through the process as described by the Director General (i.e. have been micro-concentrated and analysed).

We don't have sufficient information to make an informed decision on further testing. Could these (and future tasks) tasks please be amended to include the following information:

- * The actual QuantTrio results
- * Please indicate if the sample has already undergone micro-concentration and the volume produced
- * The approximate volume remaining.
- * A full description of the the actual profile already obtained.
- * An indication (expert opinion) on the likelihood that further internal testing may provide additional probative information.
- * A recommendation as to whether the sample may be better tested by an external service provider.

Finally. could you please ensure that these tasks are forwarded to the DNA Management Section in the first instance rather than investigators, forensic, or scientific officers.

Your urgent advice is sought on this matter please.

Regards



Stephan Foxover Acting Inspector Biometrics Forensic Services Group Operations Support Command

GPO Box 1440, Brisbane QLD 4001, Australia

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthonsed. If you

have received this electronic inform the sender or contact This footnote also confirms that this email message has been checked for the presence of computer viruses.

Disclaimer: This email and any attachments may contain legally privileged or confidential information and may be protected by copyright. You must not use or disclose them other than for the purposes for which they were supplied. The privilege or confidentiality attached to this message and attachments is not waived by reason of mistaken delivery to you. If you are not the intended recipient, you must not use, disclose, retain, forward or reproduce this message or any attachments. If you receive this message in error, please notify the sender by return email or telephone and destroy and delete all copies. Unless stated otherwise, this email represents only the views of the sender and not the views of the Queensland Government.

Queensland Health carries out monitoring, scanning and blocking of emails and attachments sent from or to addresses within Queensland Health for the purposes of operating, protecting, maintaining and ensuring appropriate use of its computer network.

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic inform the sender or contact This footnote also confirms the been checked for the presence of computer viruses.

RE: URGENT: New reporting process involving low quant samples

10	Cathie Allen <		tilland in
To:	Paula Brisotto < Gregg <	Justin Howes <	Helen
Date:	Wed, 31 Aug 2022 17:29:07 +1000		

Hi Everyone

How about this?

Hello a DNA profile has been obtained from the linked crime scene sample I am seeking approval for additional work to be undertaken on the sample, in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported Additional information to assist: Ouant value: Undergone Microcon: No / Yes (if yes, please provide approx. volume remaining after microcon concentration) Current Volume Remainina: Further processing requested: e.g. Microcon to full, additional amplification Will further processing exhaust the sample: No / Yes Scientific Opinion on the likelihood that further internal testing may provide additional probative information: Recommendation as to whether the sample may be better tested by an external service provider: Cheers Cathie Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022 Police Services Stream, Forensic & Scientific Services

Prevention Division, Queensland Health

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Paula Brisotto <
Sent: Wednesday. 31 A
T
T
A
Helen Gregg

Subject: RE: URGENT: New reporting process involving low quant samples

Hi all,

Some thoughts again (sorry) in red below. After reading the updated version and Justin's further comment, I put the following as suggestions.

Hello a DNA profile has been obtained from the linked crime scene sample I am seeking approval for additional work to be undertaken on the sample, in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported

Additional information to assist:

Ouant value:

Undergone Microcon: No/Yes (if yes, please provide approx. volume remaining after microcon concentration)

Current Volume Remainina:

Current testing performed: Microcon/Amplification

Further processing requested: e.g. Microcon to full, additional amplification Scientific Opinion on the likelihood that further internal testing may provide additional probative information:

Recommendation as to whether the sample may be better tested by an external service provider:



Subject: RE: URGENT: New reporting process involving low quant samples

Hi Paula

Great addition. So it now reads:

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for additional work to be undertaken on the sample. in an attempt to obtain a suitable DNA profile for interpretation Please be advised if this additional work is approved the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Reauest/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported

Additional information to assist:

Ouant value:

Undergone Microcon: No/Yes (if yes, please provide approx. volume remaining after microcon concentration):

aining after Microcon concentration: uL (this is likely Appro to be 35mL delete as not required

Approx Volume remaining after any further testing: uL (**this is likely to be 18uL (for example – after quant and** one amp) still discussion about what this means, as we are reading this differently. My interp is that, as we are asking to consume the whole sample by further testing, this would be what is remainin<u>before</u> the testing was progressed. I am unsure why the final volume after microcon is required and is different to approx. volume remaining after any further testing. I think this still creates confusion.

Description of DNA profile obtained to date:

Scientific Opinion on the likelihood that further internal testing may provide additional probative information:

Recommendation as to whether the sample may be better tested by an external service provider:

Cheers Cathie Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022 Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Paula Brisotto < Sent: Wednesday, <u>31 A</u>		
T	Cathie Allen <	Helen Gregg

Subject: RE: URGENT: New reporting process involving low quant samples

Hi all,

I copied over the info again so I could read in full.

My only additional thought is in red below.

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seekina approval for additional work to be undertaken on the sample, in an attempt to obtain a suitable DNA profile for interpretation Please be advised if this additional work is approved the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported

Additional information to assist:

Ouant value:

Underaone Microcon: Yes/No – could this be combined with the one below? To read "Underaone Microcon: No/Yes (if ves please provide approx. volume remaining after microcon concentration). Just thinking if no, the following line is not required.

Approx Final volume remaining after Microcon concentration: uL This would be 35mL (for example) Approx Volume remaining after any further testing: uL This would be 18uL (for example – after quant and one amp)

Description of DNA profile obtained to date:

Scientific Opinion on the likelihood that further internal testing may provide additional probative information:

Recommendation as to whether the sample may be better tested by an external service provider:

Thanks, Paula



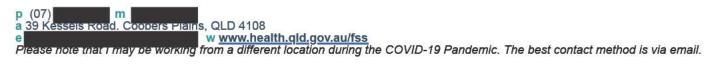
Subject: RE: URGENT: New reporting process involving low quant samples

Hi, in directing back to QPS DNa Mgt Unit, please confirm that it will be group 'RMT' who we direct it to.

Thanks Justin

Justin Howes

Team Leader - Forensic Reporting and Intelligence Team Forensic DNA Analysis, Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.





From: Cathie Allen Sent: Tuesday, 30 August 2022 4:48 PM		
T	Justin Howes <	Paula Brisotto
Subject: RE: URGENT: New reporting process invol-	ving low quant samples	

Hi Everyone

A/Insp Foxover advised the following clarification for these 2 dot points:

- * Please indicate if the sample has already undergone micro-concentration and the volume produced
- * The approximate volume remaining.

For Dot point 1 in lieu of 'the volume produced' insert 'the volume remaining after micro-concentration',

For Dot Point 2 Please change that to 'the approximate volume remaining after any further testing'.





From: Helen Gregg < Sent: Tuesday, 30 Au		
T	Justin Howes <	Paula Brisotto

Subject: RE: URGENT: New reporting process involving low quant samples

Undergone Microcon: Yes/No Approx Volume remaining after Microcon: uL Approx Volume remaining now: uL

- * Please indicate if the sample has already undergone micro-concentration and the volume produced
- * The approximate volume remaining.

Hi Cathie,

I meant that you had already asked about the undergone microcon (first line above – unhighlighted)), and that the two highlighted lines were a duplicate for Stephans second dot point

Н

From: Cathie Allen < Sent: Tuesday, 30 August 2022 3:03 PM		
T Helen Gread <	Justin Howes <	Paula Brisotto

Subject: RE: URGENT: New reporting process involving low quant samples

Hi Everyone

I've asked A/Insp Foxover to clarify what's meant by the 2 dot points – duplication or different things. I'll let you know his reply.

Cheers Cathie





From: Helen Gregg < Sent: Tuesday, 3 <u>0 Au</u>		
T	Justin Howes <	Paula Brisotto
<		

Subject; RE: URGENT: New reporting process involving low quant samples

Thanks Cathie,

Is it a duplication? It seems to be asking the same thing to me.

Approx Volume remaining after Microcon: uL Approx Volume remaining now: uL

From: Cathie Allen < Sent: Tuesday, 30 <u>Au</u>		
T	Paula Brisotto <	Helen Gregg

Subject: RE: URGENT: New reporting process involving low quant samples

It's taken from A/Insp Foxover's email:

- * The actual QuantTrio results
- * Please indicate if the sample has already undergone micro-concentration and the volume produced * The approximate volume remaining.
- * A full description of the the actual profile already obtained.
- * An indication (expert opinion) on the likelihood that further internal testing may provide additional probative information.
- * A recommendation as to whether the sample may be better tested by an external service provider.

So my assumption is that it's Microcon – Yes, Volume after microcon – 35uL and Volume remaining – 15uL since an amp's been done.

Cheers Cathie Cathie Allen BSc, MSc (Forensic Science) (She/Her*)

Managing Scientist Social Chair, Organising Committee for 25th International Symposium of the

Helen Gregg



From: Cathie Allen < Sent: Tuesday, 30 August 2022 2:21 PM T___Paula Brisotto <

Justin Howes <

Subject: RE: URGENT: New reporting process involving low quant samples

Thanks everyone

So the revised suggested wording will be something like this:

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for additional work to be undertaken on the sample in an attempt to obtain a suitable DNA profile for interpretation Please be advised if this additional work is approved the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternative technologies are under consideration We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions Once finalised please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported. Additional information to assist:

Ouant value: Ouant value: Undergone Microcon: Yes/No Approx Volume remaining after Microcon: uL Approx Volume remaining now: uL Description of DNA profile obtained to date: Scientific Opinion on the likelihood that further internal testing may provide additional probative information: Recommendation as to whether the sample may be better tested by an external service provider:

Regarding the suggested wording for the Description of the DNA profile can we ask Kylie & Sharon to develop wording, for review by Justin & Paula?

Is everyone ok if I reply to A/Insp Foxover and advised that we've developed a process which incorporates the additional information requested, this may increase the TAT for results and will provide that from here on in? Plus we'll add the additional information for the barcodes listed below.

Cheers	
Cathie	
Cathie Allen BSc, MSc (Foren	sic Science) (She/Her*)
Managing Scientist	
	e for 25th International Symposium of the
	sic Science Society (ANŹFŚS), Brisbane, 11 – 15 Sept 2022
Police Services Stream, Forensi	
Prevention Division, Queensland H	lealth
p 07 m	
a 39 Kessels Road, Coopers Plain	s, QLD 4108
e	w www.health.qld.gov.au/fss
Queensland Health acknowledges the Tra	ditional Owners of the land, and pays respect to Elders past, present and future.
*If you're wondering about the use of pror	ouns She/Her on this signature block, I encourage you to read some resources available here



From: Paula Brisotto < Sent: Tuesday, 30 Aud		
τ	Justin Howes <	Helen Gregg

Subject: RE: URGENT: New reporting process involving low quant samples

Hi all,

I'm okay with this process.

In thinking about some scenarios, I think standard wording may be difficult.

For example, DNA interpretation may not be appropriate in all cases, hence why additional processing may be requested, which could consume the sample. Could be "mixed DNA profile obtained. Re-amp requested to determine number of contriubotrs" or "Interpretational difficulties within result DNA profile that may resolve through rework, which may include inhibition, degradation" (etc)

For microcon to full requests if these are NDNAD, this may be slightly different wording i.e. "DNA was not detected above the limit of detection at quantitation. Concentration to full may consume the remaining sample"

Or "Partial DNA profile obtained. Concentration to full may provide further DNA profile information, which may consume the remaining sample".

I think some suggestion wording could be provided for use, and I definitely think the review is a good step.

Thanks, Paula

From: Cathie Allen < Sent: Tuesday, 30 Au T	Paula Brisotto <	Helen Gregg
Subject: Importance: High	rting process involving low quant samples	

Hi Everyone

Justin and I have had a quick chat about the below and have come up with the following as a workflow that we'd like your feedback on:

- * Amend the suggested wording for this workflow to include the dot points below
- * Staff members would add a Request / Task and add the detail, including the DNA interpretation for the samples
- * Staff member would then send the Request / Task to a peer reviewer
- * Peer reviewer would add detail next to DNA interpretation something like 'Reviewed by [name and date]'
- * Peer reviewer would then release the Request / Task to QPS DNA Management Unit
- * If there is a difference of scientific opinion about the DNA interpretation, the Peer reviewer would discuss this with the staff member

Additional Exhibit Reporting Lines could be devised which would mean that the reporting of the 'first' DNA profile could be reported as per SOPs. In the interim, we'd need to do the above – but happy for your feedback to see if it could be done better or a different way.

Cheers	
Cathie	
Cathie Allen BSc, MSc (Forensic Science) (She/Her*)	
Managing Scientist	
Social Chair, Organising Committee for 25th International Symposium of the	
Australian and New Zealand Forensic Science Society (ANŹFŚS), Brisbane, 11 – 15 Sept 2022	
Police Services Stream, Forensic & Scientific Services	
Prevention Division, Queensland Health	
p 07 m m	
a 39 Kessels Road, Coopers Plains, QLD 4108	
e w www.health.gld.gov.au/fss	

Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here



From: Cathie Allen Sent: Tuesday, 30 <u>August 2022 11:13 AM</u> To: Justin Howes < Cc: 'Paula Brisotto' < Subject: FW: URGEN Importance: High

>; Helen Gregg < ng low quant sam Could you please see the below email from A/Insp Foxover. The QPS are requesting additional advice be provided with the email as per below.

Could you please amend the suggested communication wording to include the below and advise the team. Once you've been able to do that, could you please respond to A/Insp Foxover to advise that the requested information has been included the barcodes listed below and all further advice to QPS DNA Management Unit.

Cheers
Cathie
Cathie Allen BSc, MSc (Forensic Science) (She/Her*)
Managing Scientist
Social Chair, Organising Committee for 25th International Symposium of the
Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022
Police Services Stream, Forensic & Scientific Services
Prevention Division, Queensland Health
a 39 Kessels Road, Coopers Plains, QLD 4108
e w www.health.qld.gov.au/fss
Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.
*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here.



From: Foxover.StephanP[OSC] < Sent: Tuesday, 30 To: Cathie Allen < Cc: Helen Gregg < Subject: URGENT: New reporting process involving low quant samples

>; McCarthy.DuncanJ[OSC]

This email originated from outside Queensland Health. DO NOT click on any links or open attachments unless you recognise the sender and know the content is safe.

Hi Cathie,

I am aware that recent changes have been made in relation to testing of samples in the concentration range of .001-.0088ng/uL. Advice was received from the Director General of Queensland Health that samples in this range would automatically undergo micro-concentration to 35uL before being further processed in an attempt to obtain a profile. The advice also included that if the testing was unsuccessful and further testing was required, the scientist would liaise with QPS to determine if there was a need to preserve the sample before another attempt was made.

We are now receiving tasks on the FR that include the following wording:

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for additional work to be undertaken on the sample. in an attempt to obtain a suitable DNA profile for interpretation Please be advised if this additional work is approved the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised. please advise via return Reduest/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

The relevant barcodes include:





Could vou please advise if each of the above samples subject to this task have been through the process as described by the Director General (i.e. have been micro-concentrated and analysed).

We don't have sufficient information to make an informed decision on further testing. Could these (and future tasks) tasks please be amended to include the following information:

- * The actual QuantTrio results
- * Please indicate if the sample has already undergone micro-concentration and the volume produced
- * The approximate volume remaining.
- * A full description of the the actual profile already obtained.
- * An indication (expert opinion) on the likelihood that further internal testing may provide additional probative information.
- * A recommendation as to whether the sample may be better tested by an external service provider.

Finally. could you please ensure that these tasks are forwarded to the DNA Management Section in the first instance rather than investigators, forensic, or scientific officers.

Your urgent advice is sought on this matter please.

Regards

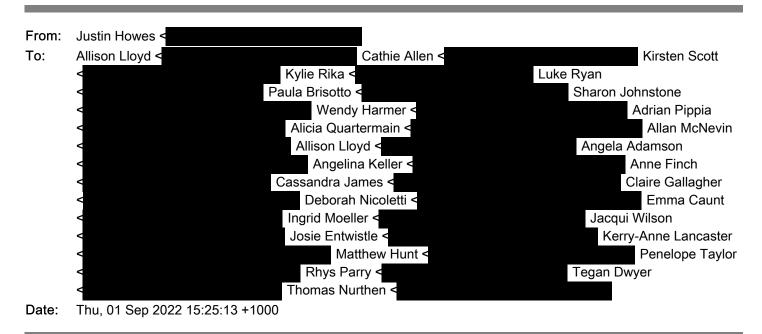


Stephan Foxover Acting Inspector Biometrics Forensic Services Group Operations Support Command Mobile Ph: 07 Fax: 07 GPO Box 1440, Brisbane QLD 4001, Australia

CONFIDENTIALITY: The information contained in this electronic mail message and any electronic files attached to it may be confidential information, and may also be the subject of legal professional privilege and/or public interest immunity. If you are not the intended recipient you are required to delete it. Any use, disclosure or copying of this message and any attachments is unauthorised. If you have received this electronic message in error please inform the sender or contact This footnote also confirms th

been checked for the presence of computer viruses.

RE: Process following A/DG memo



Hi all

QPS have requested addional in formaon t o be provided to Request/Tasks when seeking approval for tesng that might exhaust the DNA extract.

Firstly, the Request/Task is to be directed to Acon Unit: 'FL U'. From there, my understanding is the Invesg ang Officer will be contacted and approval will be considered.

The addional in formaon r equired is below. Also provided is suggested wording that took on Kylie and Sharon's feedback.

Hello. a DNA profile has been obtained from the linked crime scene sample. I am seekina approval for additional work to be undertaken on the sample in an attempt to obtain a suitable DNA profile for interpretation Please be advised if this additional work is approved the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory. or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions Once finalised please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

Additional information to assist:

- * Quant value:
- * Undergone concentration (Microcon): No/Yes
- * Current Volume Remaining: uL
- * Further Processing Requested eg. Microcon to full, additional amplification
- * Will further processing exhaust the sample: No/Yes
- * Description of DNA profile obtained to date: eq. Low level DNA profile difficult to interpret, complex DNA profile, Low level profile that may not be suitable for interpretation
- * Scientific Opinion on the likelihood that further internal testing may provide additional probative information: eq. further work is likely to/ may assist in the confirmation of information currently obtained. Further work may also confirm that the profile is too complex to interpret.
- * Recommendation as to whether the sample may be better tested by an external service provider: If this item is critical to the outcomes of the case then a discussion is requested to explore all possible options.

This has been added to 17117 SOP which is in review.

Please add the extra informaon t o all Request/Tasks when seeking approval that might exhaust the DNA extract.

Regards Jusn



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



Subject: Process following A/DG memo

Hi all

Following this memo, the information below will be added to 17117 which will be sent to review early next week:

When seeking written approval from QPS for additional work if considered beneficial, send a Request/Task via the Forensic Register to the relevant Forensic Officer found by the field below. Add the Forensic Officer's ID number to the Action Officer field, and link the relevant crime scene barcode to the Request/Task.

rom the front dri	ver seat adjustment levers			
Exam Source				
Vehicle:	Van			
whibit Notes & Ana	lvan Advira			
Parent Barcode	Property Ten	Current Location	Investigator	Forensic Officer
arent Barcode	Property Ten	Current Location PSD	Investigator	Forensic Officer
Parent Barcode	Property Ten	Contraction and a second	Investigator	Forenaic Officer
	Property Ten	Contraction and a second	Investigator	Forenais Officer
Parent Barcode Ownership / Relation	S. Co.	PSD		Forensic Officer

Suggested Template for wording:

Hello, a DNA profile has been obtained from the linked crime scene sample. I am seeking approval for additional work to be undertaken on the sample, in an attempt to obtain a suitable DNA profile for interpretation. Please be advised if this additional work is approved, the DNA extract will be consumed. This means there will be no opportunity for further processing in this laboratory, or elsewhere if alternative technologies are under consideration. We understand that consultation with the Investigating Officer may be necessary and will await the outcome of those discussions. Once finalised, please advise via return Request/Task if the additional work is approved. If approval is not provided, the DNA profile obtained will be reported.

When sending the Request/Task, the exhibit result line *SOHAA – Sample on hold, awaiting advice* should be added, and validated by a second operator.

When QPS respond, the exhibit result line *TRQ – Testing restarted on advice from QPS* hould be added irrespective of whether approval for further processing has been granted or not. The result will either be reported based on the one amplification result, or will be reported after the further processing.

Regards Justin



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.



<	Phillip McIndoe <	Pierre Acedo
<	Rhys Parry <	Rvu Eba
<	<u>Sandr</u> a McKean <	<u>Sharelle N</u> ydam
<	Sharon Johnstone <	<u>Steph</u> anie Waiariki
<	Suzanne Sanderson <	Tara Prowse
<	<u>⊺e</u> gan Dwyer <	Thomas Nurthen
<	<u>Valeri</u> e Caldwell <	Vicki Pendlebury-Jones
<	Wendy Harmer <	Yvonne Connolly
<		
C	Alison Slade < FSS Corro <	Lara Keller
<	h McNeil <	Petra Derrington

Subject: FW: C-ECTF-22/13557 - DG MEMO - from Dr David Rosengren, Acting Director-General, Queensland Health -Subject of memorandum

Good afternoon everyone,

Please see attached memo. I have asked for an enhancement to FR to assist with this change.

Please hold all quants effective immediately, until the FR enhancement is complete. Paula has specific details for the analytical team.

For batches that have already progressed beyond quant, proceed as per this morning's processes.

Could you please update SOPs asap.

Contact me if you have any queries.

Regards Helen



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Good Afternoon

Please see attached the Memorandum from Dr David Rosengren, Acting Director-General, Queensland Health, for your attention.

Should you have any questions in relation to this advice, please contact Professor Keith McNeil, Acting Deputy Director-General on telephone 07

Kind Regards



Ministerial & Executive Services Unit, Office of the Director-General | Queensland Health

E	
w	health.qld.gov.au

CLEAN HANDS SAVE LIVES

Wash your hands regularly to stop the spread of germs



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

Cathie Allen

From: Sent: To: Cc: Subject: Cathie Allen Thursday, 8 September 2022 5:42 PM Ingrid Moeller Justin Howes Clarification

Hi Ingrid

I refer to your emails of 8, 11 and 12 August abut reworking a DIFP sample and my email to you of 23 August.

I am trying to support our scientists and provide clear communication and guidance on the scope of our work. Therefore, I thought it would be appropriate to clarify my email to you of 23 August, particularly in light of the Acting Director-General's memorandum dated 19 August 2022.

If a reporting scientist wishes to rework a sample, before they are asked to give evidence in court about a previous statement with a DIFP result, I will be happy to approve the request. However, it would prudent for the scientist to first seek confirmation of the reworking from the QPS investigating officer before seeking my approval as the samples are the property of QPS. This will also ensure, as I have explained, that QPS is aware of potential additional results or addendum statement/s. Also, in view of the A/DG's memorandum of 19 August, the scientist must consult with and obtain QPS's approval if reworking of the sample would exhaust the sample volume.

I hope I have clarified what should occur if a scientist wishes to rework a sample with a DIFP result. If you have any further questions or concerns about this, please contact me for advice.

Cheers Cathie



Cathie Allen BSc, MSc (Forensic Science) (She/Her*) Managing Scientist

Social Chair, Organising Committee for 25th International Symposium of the Australian and New Zealand Forensic Science Society (ANZFSS), Brisbane, 11 – 15 Sept 2022

Police Services Stream, Forensic & Scientific Services Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and future.

*If you're wondering about the use of pronouns She/Her on this signature block, I encourage you to read some resources available here





FSS.0001.0079.2902

Josie Entwistle

From:	Josie Entwistle
Sent:	Monday, 29 August 2022 4:22 PM
То:	Sharon Johnstone; Kylie Rika; Justin Howes; Paula Brisotto
Subject:	Further processing of DNA insufficient

Hi all,

I have been made aware that two samples from a case that I have previously reported (QP1800511236) were requested to undergo further processing by QPS. This request was made on the case summary page in the FR, where there are entries relating to a subpoena I was previously issued, as well as the statement allocation. The samples were submitted to a microcon to 35uL by Luke without any communication or consultation of myself as the reporting scientist of the case.

A microcon to 35uL is not the option I would have chosen for further processing of these samples. I would have recommended a microcon to full for these samples, and for any samples with a quant in the range of what has been reported as NDNA. I am now unsure of the most appropriate pathway for reporting of these samples as results and in a statement of witness. I'm wondering if perhaps this case should now be adopted by Luke?

I understand that these re-activated samples may populate to a worklist, however a response was entered in a case file notation in the FR, where information regarding allocation is readily available. Samples that have already been reported have been allocated to a case manager either to report a result or the entire case, and it's certainly my preference that I am in involved in any further interpretation or processing of samples I've allocated, or that the case is adopted by someone else.

Is it possible to request for the QPS to forward reprocessing enquiries directly to the CMer/reporter, and/or for Analytical to consult the CMer/reporter prior to submitting a processing request, or for Reporting staff to access and monitor the worklist (if relevant, perhaps an FR enhancement), or for NDNA rework requests to proceed to a microcon to full?

Kind regards

Josie



Josie Entwistle Reporting Scientist - Forensic Reporting & Intelligence Team

Forensic DNA Analysis, Forensic & Scientific Services Prevention Division, Queensland Health



Queensland Health acknowledges the Traditional Owners of the land, and pays respect to Elders past, present and emerging.

Project #70 – Maxwell project Phase 1

Phase 1 Report- Verification of Promega DNA IQ™ for the Maxwell[®]16

Megan Mathieson, Belinda Andersen, Cecilia lannuzzi, Allan McNevin

1 Abstract

Implementation of the Promega Maxwell[®]16 MDx instrument will provide an alternative to the current manual and automated (liquid handling units) DNA IQ[™] methods of extracting DNA to supplement the existing high throughput and to improve workflow efficiency. Initially pre-lysis methods were tested to determine which method gave acceptable results and then would be used for the remainder of the verification. It was determined that the Promega recommended procedure with a few modifications was deemed to be the most suitable pre-lysis procedure. For the repeatability and reproducibility studies blood samples were found to have acceptable results, whereas cell samples were initially found to be variable when processed using the Maxwell[®]16 MDx instruments. This was due to a single batch that produced yields below expectation and further testing of cell substrates demonstrated reproducible, repeatable results. The Promega Maxwell[®]16 MDx instrument with a modified Promega procedure was comparable or outperformed the Manual DNA[™] IQ method in the sensitivity studies. There was no evidence suggesting cross contamination occurred between any of the extraction batches performed for each experiment on either of the Maxwell[®]16 MDx instruments. The use of the Promega Maxwell[®]16 MDx instruments has been shown to be an acceptable alternative to manual DNA IQ[™] method and is suitable for routine use in DNA Analysis Unit.

2 Background

The Promega Maxwell[®] 16 MDx instrument is a pre-programmed, automated paramagnetic particle handler that is specifically designed for optimal DNA extraction of forensic casework samples using the Promega DNA IQ[™] chemistry. Samples undergo a pre-processing step prior to DNA extraction and are then added to disposable cartridges containing pre-dispensed, ready to use extraction reagents. The Maxwell[®] 16 MDx instrument can process up to 16 samples taking approximately 30 minutes.

3 Purpose

The aim of this study was to assess the suitability, reproducibility, repeatability, sensitivity and crosscontamination of the Maxwell[®]16 MDx instruments for the purpose of extracting DNA from blood and cell swabs. This study also aimed to verify the Maxwell[®]16 MDx instrument using Promega DNA IQ[™] chemistry to provide a comparable alternative method to current in-use protocols for routine processing of casework and reference samples as per operational requirements of DNA Analysis Unit.

4 Equipment and Materials

- o STORstar instrument (Process Analysis & Automation, Hampshire, UK)
- MultiPROBE[®] II PLUS HT EX with Gripper[™] Integration Platform (PerkinElmer, Downers Grove, IL, USA)
- ABI 7500 Real Time PCR System (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o GeneAmp PCR system 9700 (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o ABI 3130x/ Genetic Analyzer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- Promega Maxwell[®] 16 MDx A and B Instrument (Promega Corp., Madison, WI, USA)
- o 5804 centrifuge (Eppendorf,Germany)
- 5424 centrifuge (Eppendorf,Germany)
- o Vortex
- Minifuge (Tomy)



Project #70 – Maxwell project Phase 1

- Miscellaneous consumables and labware (eg 1.5mL screw-cap tubes, pipettes, pipette tips, 96-well PCR plates, 2.0mL sterile screw-cap tubes)
- Sterile Conductive Filtered Roborack 175µl and 25µl disposable tips (PerkinElmer, Downers Grove, IL, USA)
- o Cytobrush™ Plus Cell Collector devices (Cooper Surgical, Inc.,)
- Baxter 0.9% saline solution
- o Sterile rayon swabs (Copan Italia SPA, Brescia, Italy)
- o DNA IQ[™] System Kit 400 sample kit (Promega Corp., Madison, WI, USA)
- o DNA IQ[™] casework Pro Kit for Maxwell[®]16 (Promega Corp., Madison, WI, USA)
- o Reagents
 - TNE
 - Proteinase K (20mg/mL) (Sigma)
 - DTT (Dithiothreitol) 1M (Sigma)
 - 5% v/v Trigene
 - 70% v/v and 100% v/v Ethanol
 - 5% v/v Bleach
 - 1% v/v Amphyl
 - 0.2% v/v Amphyl
 - 40% w/v Sarcosyl
 - Analytical Positive Control lot#29102010
 - Nuclease Free Water
 - Isopropyl Alcohol
- Quantifiler[™] Human DNA Quantification kits (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o Promega Genomic Male DNA G147A (Promega Corp., Madison, WI, USA)
- AmpF{STR[®] Profiler Plus[®] PCR Amplification kits including 9947A control DNA (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o Hi-Di[™] Formamide (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o 3130 POP-4[™] Polymer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- GeneScan[™] 500 ROX[™] Size Standard(Life Technologies Applied Biosystems, Foster City, CA, USA)
- o Running Buffer (Life Technologies Applied Biosystems, Foster City, CA, USA)
- AmpFlSTR[®] Profiler Plus[®] Allelic Ladder (Life Technologies Applied Biosystems, Foster City, CA, USA)
- o GeneMapper-IDX ver. 1.1.1(Life Technologies Applied Biosystems, Foster City, CA, USA)



Project #70 - Maxwell project Phase 1

5 Methods

5.1 Sample Creation

5.1.1 Collection procedure for buccal cells

Buccal cells were collected from a donor using the Cytobrush[™] method. Two Cytobrush[™] Plus Cell collector devices were used to collect buccal cells from each cheek for 1 minute then collected into 500µL of 0.9% saline solution. The cell solutions were stored at 4°C until they were required for use.

5.1.2 Collection procedure for blood

A donor (different to the buccal donor) was selected and 10mL of blood was collected in EDTA tubes by a qualified phlebotomist and stored at 4°C until it was required for use.

5.1.3 Sample creation for swabs with buccal cells

Four collections of buccal cells were made and combined to ensure a uniform suspension. Pipetting of the buccal suspension and drying of swabs was performed in a Class II biohazard cabinet. Working areas were decontaminated using 10% v/v bleach and 70% v/v ethanol.

49 swabs were prepared for extraction, swab heads were cut away from the stick of the swab using a sterile scalpel and forceps. The swab heads were placed upside (end of swab head pointing up) into 2mL tubes ready for the cells to be spotted on.

The buccal cell suspension was resuspended by vortexing prior to dispensing onto swabs.

30µL of cell suspension was dispensed onto 49 swabs. Swabs were dried in an open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head was pointing down in the bottom of the tube, re-capped and stored at \leq -10°C.

5.1.4 Sample creation for swabs with blood

Pipetting of blood and drying of swabs was performed in a Class II biohazard cabinet. Working areas were decontaminated using 10% v/v bleach and 70% v/v ethanol.

77 swabs were prepared for extraction, swab heads were cut away from the stick of the swab using a sterile scalpel and forceps. The swab heads were placed upside (end of swab head pointing up) into 2mL tubes ready for sample creation.

The blood was resuspended by vortexing prior to dispensing onto swabs.

 30μ L of blood was dispensed onto 56 swabs. Swabs were dried in the open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head is pointing down in the bottom of the tube, recapped and stored at \leq -10°C.

A series of sevene and each with a different amount of blood were created in triplicate (three swabs per volume) as per Table 1. The blood was resuspended by vortexing prior to pipetting onto swabs.



Project #70 – Maxwell project Phase 1

Ta	Table 1 Volume of blood added to swabs				
	Sample	Volume of blood			
	1	60µL			
	2	30µL			
	3	15µL			
	4	5µL			
	5	2µL			
	6	1µL			
	7	0.5µL			

Swabs were dried in an open 2mL tube at 56°C on a dry block heater for 2 hours.

Once dry, the swabs were inverted so the swab head was pointing down in the bottom of the tube recapped and stored at \leq -10°C.

5.2 Extraction

Samples were extracted using the Promega DNA IQ[™] System kit according to either the current in house standard laboratory procedure (QIS 24897 DNA IQ[™] Method of Extracting DNA from Casework and Reference samples) or to Technical Manual DNA IQ[™] Casework Pro Kit for Maxwell[®]16 (Part# TM332 revised 10/10 - recommended procedure from the manufacturer). The latter protocol was revised during the course of this verification to include;

- combining Proteinase K and DTT into the initial extraction buffer before adding to each sample,
- an additional pulse spin after incubation and prior to the addition of lysis buffer and
- an increase in the final elution volume from 50µL to 100µL.

This revised method is referred to as the 'modified Promega method' in this report.

5.3 Quantification

All quantification reaction setups were performed using a MultiPROBE[®] II PLUS HT EX with Gripper[™] Integration Platform and quantified according to the standard laboratory procedure (QIS 19977 'Automated Quantification of Extracted DNA using the Quantifiler Human DNA Quantitation Kit').

5.4 Amplification

All samples were amplified with the Applied Biosystems AmpF{STR[®] Profiler Plus[®] PCR Amplification Kit at the volumes calculated from the quantification result. Approximately 1.2ng of DNA template was added for amplification reaction. The PCR reaction was set up using a MultiPROBE[®] II PLUS HT EX with Gripper[™] Integration Platform and amplified according to the standard laboratory procedures (QIS 19976 "Amplification of Extracted DNA using the AmpFISTR[®] Profiler Plus[®] kit or AmpFISTR[®] COfiler[®] Kit").

5.5 DNA Fragment Analysis and Profile Interpretation

All samples were sent for capillary electrophoresis and processed according to the standard laboratory procedure (QIS 15996 'Procedure for the use and Maintenance of the AB 3130xl Genetic Analyzers). All samples were analysed according to the standard laboratory procedure (QIS 17130 'CE Quality Check of Samples from the ABI Prism 3130xl Genetic Analyzers).

All sample results were interpreted using GeneMapper *ID-X* ver. 1.1.1 according to the standard laboratory processing (17137 "Procedure for the Interpretation & Acceptance of Results using Profiler & COfiler systems').



Project #70 – Maxwell project Phase 1

5.6 Statistical Tests

Microsoft Excel was used to calculate averages, standard deviations, maximum and minimum values. It was also used to perform two-tailed t-tests to assess comparable data sets for significant difference, unless specified total DNA yield was used for this assessment. A *p*-value of <0.05 was considered to be significantly different.

6 Experimental Design

6.1 Experiment 1 - Suitability

Suitability studies were carried out to compare DNA yields (ng) between manual DNA IQ[™] and DNA IQ[™] extraction on the Maxwell[®]16 using both the current in-house pre-lysis method and the Promega pre-lysis method.

6.1.1 Pre-Lysis of samples for lysates to be extracted using DNA IQ[™] Casework Pro Kit for Maxwell[®]16

Seven blood and seven buccal cell swab samples along with one positive and one negative control, were pre-lysed according to the current in-house pre-lysis procedure outlined in section 5.2.

Seven blood and seven buccal cell swab samples along with one positive and one negative control, were pre-lysed according to the Promega recommended pre-lysis procedure outlined in section 5.2.

6.1.2 Lysates to be extracted using DNA IQ[™] Casework Pro Kit for Maxwell[®]16

Lysates obtained from the pre-lysis steps were extracted on both Maxwell[®]16 MDx instruments, using the recommended procedure from the manufacturer.

6.1.3 Samples extracted using Manual DNA IQ™

Seven blood and seven buccal cell swab samples along with a positive and negative control were extracted according to the in house procedure outlined in section 5.2.

6.2 Experiment 2 – Reproducibility and Repeatability

Reproducibility and repeatability studies were carried out to compare run to run variation and instrument to instrument variation. Note, due to an apparent failure of one batch of cell samples (see results and discussion), the entire experiment was repeated for the cell samples.

6.2.1 Reproducibility

The run to run variation was assessed by processing two further batches on each of the Maxwell[®]16 MDx instruments, using the modified Promega method outlined in section 5.2. Each batch consisted of seven buccal cell lysates, seven blood lysates, and a positive and negative control.

6.2.2 Repeatability

The instrument to instrument variation was assessed by comparing batches (using data from the reproducibility study) processed on one Maxwell[®]16 MDx instrument to batches processed on the other Maxwell[®] for the rument.



Project #70 – Maxwell project Phase 1

6.3 Experiment 3 – Sensitivity and DNA Yield

Sensitivity studies were carried out to show the difference in performance of the DNA IQ[™] Casework Pro Kit for Maxwell[®]16 and the DNA IQ[™] manual extraction using different volumes of blood applied to swabs.

6.3.1 Sensitivity testing

A sensitivity series with duplicate blood samples with volumes of 60μ L, 30μ L, 15μ L, 5μ L, 2μ L, 1.0μ L and 0.5μ L and a positive and negative control were extracted using the modified Promega method on instrument Maxwell[®] 16 A.

A further sensitivity series of blood samples with volumes of 60μ L, 30μ L, 15μ L, 5μ L, 2μ L, 1.0μ L and 0.5μ L and a positive and negative control were extracted manually according to the current in house procedures outlined in section 5.2.

6.4 Experiment 4 – Cross-Contamination

Cross-contamination studies were carried out to determine whether any cross contamination occurs during the extraction process and to show no cross contamination occurred between extraction batches on the Maxwell[®]16 MDx instruments.

6.4.1 Cross- Contamination

Eight blank lysates and eight blood lysates containing DNA were placed on the Maxwell[®]16 MDx instrument in an alternating pattern and were extracted using the modified Promega method.



Project #70 - Maxwell project Phase 1

7 Results and Discussion

7.1 Suitability

The average DNA yields produced for blood and cell samples processed through DNA IQ[™] extraction on the Maxwell[®]16 instruments using the current in-house pre-lysis method (DNA Analysis) and the Promega pre-lysis method and the manual DNA IQ[™] process are shown in Figure 1 below.

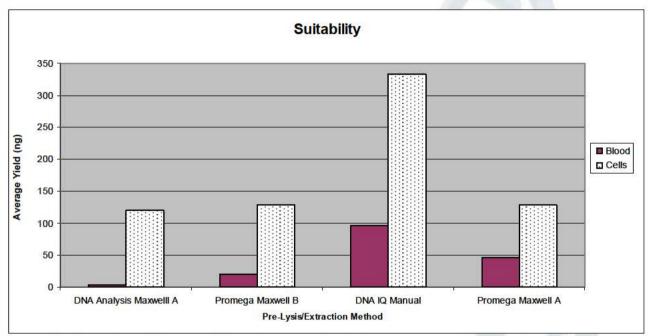


Figure 1 Comparison of in-house and Promega recommended pre-lysis procedure and Manual DNA IQ™.

A summary of the average yield, standard deviation, maximum and minimum yield values obtained for each method and sample type tested is outlined in Table 2 below.

Lysis / extraction Method	Sample type	Instrument	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
DNA Analysis	Blood	A	3.63	1.90	7.10	1.01
DNA Analysis	Cell	A	120.21	42.79	183.00	76.50
Promega	Blood	В	20.63	6.28	26.75	11.65
Promega	Cell	B	128.29	44.67	198.00	80.50
DNA IQ Manual	Blood	N/A	96.56	28.78	136.00	46.80
DNA IQ Manual	Cell	N/A	332.57	87.30	489.00	238.00
Promega	Blood	A	45.76	11.47	59.00	27.20
Promega	Cell	A	128.14	23.81	172.50	104.00

Table 2 Summary of suitability results

The original validation of the manual DNA IQTM chemistry gave an average yield of 317ng for blood swabs with a standard deviation of 102.36; cell swabs produced an average yield of 134.54ng with a standard deviation of 41.30 (Nurthen *et al.*, 2007). The results of the manual DNA IQTM in this verification showed a significantly lower yield with a lower standard deviation for the blood swabs



Page 7 of 17

Project #70 - Maxwell project Phase 1

and a much greater yield for the cell swabs with an increased standard deviation when compared to the original validation of DNA IQ[™] chemistry.

The average DNA yields for blood samples extracted using manual DNA IQTM (refer section 6.1.3 above) were significantly higher than yields obtained using DNA IQTM extraction on the Maxwell[®]16 instruments (refer section 6.1.2 above) using both the current in-house pre-lysis method (p = 0.000136345) and the Promega pre-lysis method (p = 0.000329464). The average DNA yields for cell samples extracted using manual DNA IQTM were significantly higher than yields obtained using DNA IQTM extraction on the Maxwell[®]16 MDx using both the current in-house pre-lysis method (p = 0.000299508) and the Promega pre-lysis method (p = 0.000383315).

The Promega pre-lysis procedure was repeated using the alternate Maxwell[®]16 instrument. The average DNA yields compared to manual DNA IQTM also showed a significant difference for blood samples (p = 0.002593137) and cell samples (p = 0.000589507).

The Promega pre-lysis method outperformed the current in-house pre-lysis method and was subsequently deemed to be the most suitable for DNA Analysis' applications. This is most likely due to lack of DTT present in the buffer used with the current in-house pre-lysis method.

The relatively low yield noted with the Promega pre-lysis method coupled with extraction on the Maxwell[®]16 MDx compared with the routine manual DNA IQ[™] procedure was possibly due to the difference in elution volume (the manual method uses a "double elution" method resulting in 100µL of eluent, the standard Maxwell[®]16 MDx protocol results in a 50µL elution).

To improve yield values and bring this process in line with manual DNA IQ method small modifications were made to the published protocol in the Promega Technical Manual (refer section 5.2 above). This protocol was revised to include;

- combining Proteinase K and DTT into the initial extraction buffer before adding to each sample and,
- an increase in the final elution volume from 50μ L to 100μ L.

Note: This revised method is referred to as the 'modified Promega method'. This was used for all subsequent experiments (refer sections 6.2, 6.3 and 6.4 above)

The average yields produced for blood and cell samples processed through DNA IQ[™] extraction on Maxwell[®]16 A and B with the modified Promega method (refer section 5.2 above) compared to the manual DNA IQ[™] method (refer to section 6.1.3 above) is shown in Figure 2 below.



Project #70 - Maxwell project Phase 1

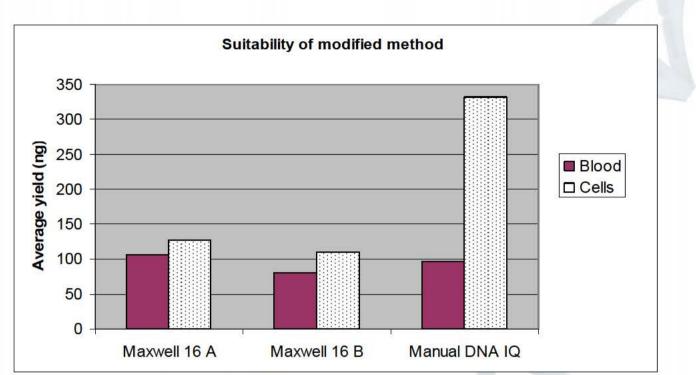


Figure 2 Average yield obtained with 100µL elution volume.

A summary of the average yield, standard deviation, maximum and minimum yield values obtained for the modified Promega method and sample type tested is outlined in Table 3. Also shown are the results from the manual DNA IQ[™] extraction previously shown in Table 2.

Lysis / extraction Method	Sample type	Instrument	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
Promega (modified)	Blood	A	106.36	9.47	116	92.10
Promega (modified)	Cell	A	127.29	7.76	142	119.00
Promega (modified)	Blood	В	92.27	9.53	103	78.60
Promega (modified)	Cell	В	110.04	25.29	136	64.50
DNA IQ Manual	Blood	N/A	<mark>96.5</mark> 6	28.78	136	46.80
DNA IQ Manual	Cell	N/A	332.57	87.30	489	238

Table 2 Cummon	u of regulto for quitabili	ty of modified Drom	aga mathad
Table J Summan	y of results for suitabili	ty of mouned From	ega meulou.

Using the modified Promega method the yield of the blood samples improved showing no significant difference when comparing manual DNA IQTM to Maxwell[®]16 A (p = 0.419380318) and Maxwell[®]16 B (p = 0.719012613). The yields for the cell samples were significantly different when comparing manual DNA IQTM to Maxwell[®]16 A (p = 0.000766146) and Maxwell[®]16 B (p = 0.000341129). This significant difference results from the manual DNA IQTM cell extraction producing much higher than expected yields. Differences in operators, shaking, incubation time, equipment used and preparation of mock samples could contribute to the difference in results. It is also possible that the binding capacity for the pre-dispensed resin had been reached in some cartridges therefore limiting the yields obtained from the extraction on the Maxwell[®]16 MDx instruments.

DNA profiles of all the blood and cell samples gave the expected profile in all suitability studies with no evidence of cross contamination.



Project #70 - Maxwell project Phase 1

As a result of the suitability studies the modified Promega method was employed for the repeatability, reproducibility, sensitivity and cross-contamination testing (refer sections 6.2, 6.3 and 6.4 above).

7.2 Repeatability and Reproducibility

7.2.1 Run to Run Variation- Repeatability

The average yields obtained for blood and cell samples from each of the extraction batches performed on Maxwell[®]16 A and Maxwell[®]16 B are shown in Figure 3.

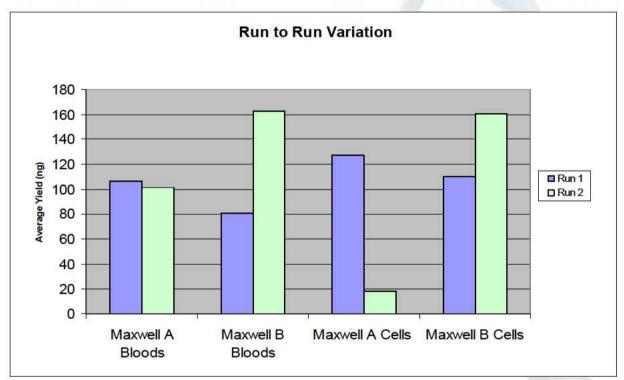


Figure 3 Comparison of run to run for blood and cells swabs on Maxwell A and Maxwell B.

A summary of the data for the repeatability studies, comparing run to run variation for each of the Maxwell[®]16 MDx instruments for blood and cells is outlined in Table 4.

Instrument	sample type	Run number	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
A	Blood	1	106.36	9.47	116	92.10
A	Blood	2	101.31	41.14	134	19.00
В	Blood	1	92.27	9.53	103	78.60
В	Blood	2	163.00	41.41	221	119.00
А	Cells	1	127.29	7.76	142	119.00
A	Cells	2	18.32	46.60	124	0.32
В	Cells		110.04	25.29	136	64.50
В	Cells	2	160.14	34.46	207	117.00

Table 4 Summary of Repeatability Tests



Page 10 of 17

Project #70 – Maxwell project Phase 1

This data shows average yield, the standard deviation and the maximum and minimum yields for each run. The standard deviation increased on batch 2 compared to batch 1 on Maxwell[®]16 A for blood samples due to a wide range of yields as seen in Table 4. This was also evident for blood samples on Maxwell[®]16 B. This range of standard deviation is similar to that observed with the original validation of the DNA IQ chemistry as outlined above and is similar to that observed with manual DNA IQ[™] results obtained in this verification.

The cell data for run 2 on Maxwell[®]16 A was also variable, with one sample showing a yield of 124ng and the other 6 samples giving yields less than 1.1ng which were unexpectedly low. The approximate yield for this extraction was expected to be 100ng. The yield for the positive extraction control for this run (data not shown) was consistent with the yields observed for the positive extraction controls for the other runs shown in Table 4. Therefore, the inconsistency observed in the 2nd run of cells on Maxwell[®]16 A indicates that the instrument itself was not the cause of the low yield values, rather the cause was likely to be related to sample creation or the pre-lysis procedure (possible operator error).

The DNA yields from the first run compared to the second run on Maxwell[®]16 A for blood samples showed no significant difference (p = 0.761677182), indicating acceptable repeatability.

The DNA yields compared from the first run and second run on Maxwell[®]16 B for blood samples showed a significant difference (p = 0.003577971). The second batch outperformed the first batch as can be seen in Table 4. The improvement in yield for blood samples on the second batch from Maxwell[®]16 B may be due to a difference in mixing of the samples and centrifugation of the samples after incubation. This removed the liquid from the lids prior to the addition of lysis buffer allowing the lysis buffer access to all of the liquid containing DNA. These minor changes in technique improved the results and were utilised in later experiments. Additionally, the effect noted may also have been related to the wide standard deviation associated with the method relative to the average yield.

There was a significant difference (p = 0.000720208) in DNA yields from the first batch to the second batch on Maxwell[®]16 A for cell samples. The difference between the cell samples on the second batch on Maxwell[®]16 A gave unexpectedly low yields compared to the yields obtained on the first batch as discussed above.

The DNA yields compared from the first batch and second batch on Maxwell[®]16 B for cell samples also showed a significant difference (p = 0.010074). Cell samples on the second batch on Maxwell[®]16 B gave higher yields than the cell samples extracted on the first batch. The difference in yield between runs for the cell samples on Maxwell[®]16 B may be due to difference in mixing of the samples and centrifugation of the samples after incubation to remove lysate from the lids as discussed above. Additionally, the effect noted may also have been related to the wide standard deviation associated with the method relative to the average yield.

7.2.2 Instrument to Instrument Variation- Reproducibility

The average yields obtained for 14 blood swabs run on Maxwell[®]16 A compared to 14 blood swabs run on Maxwell[®]16 B and 14 cell swabs run on Maxwell[®]16 A and 14 cell swabs on Maxwell[®]16 B are shown in Figure 4.



Project #70 - Maxwell project Phase 1

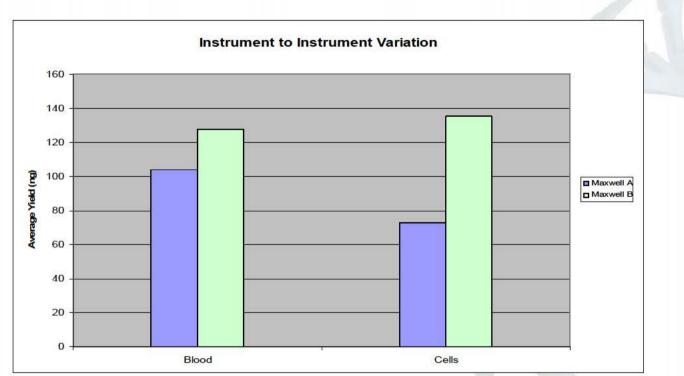


Figure 4 Comparison of Maxwell A to Maxwell B for blood and cell swabs.

The average yields, standard deviation and maximum and minimum for instrument to instrument comparison are displayed in Table 5.

Instrument	sample type	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
A	Blood	103.84	28.80	134	19.00
В	Blood	127.64	46.69	221	78.60
A	Cells	72.81	65.01	142	0.32
В	Cells	135.09	38.97	207	64.50

Table 5 A summary of the Instrument to Instrument Testing

The DNA yields comparing blood samples between Maxwell[®]16 A and Maxwell[®]16 B showed no significant difference (p = 0.119016). The DNA yields comparing 14 cell swabs on Maxwell[®]16 A and 14 cell swabs on Maxwell[®]16 B was significantly different (p = 0.005689). This was due to the unexpectedly low yield results obtained from the second run on Maxwell[®]16 A as previously discussed.

Comparing the DNA yields for cell swabs between Maxwell[®]16 A and Maxwell[®]16 B excluding the low yield second batch results from Maxwell[®] 16 A, a t-test showed no significant difference (p = 0.481774). This suggested, as discussed above, that there had been a problem with the samples or the pre-lysis treatment rather than the instrument itself. Further testing was carried out, and these results are shown in 7.2.3 below.



Project #70 - Maxwell project Phase 1

7.2.3 Further testing

After a preliminary report was presented to the Management Team of the DNA Analysis Unit, it was decided the overall variability of the cell aspect of this verification was unacceptable and further testing was requested.

The reproducibility and repeatability experiments were repeated with new cell substrates created from a new collection of buccal cells from the same donor. Figure 5 shows the average yields from the further testing of cell samples.

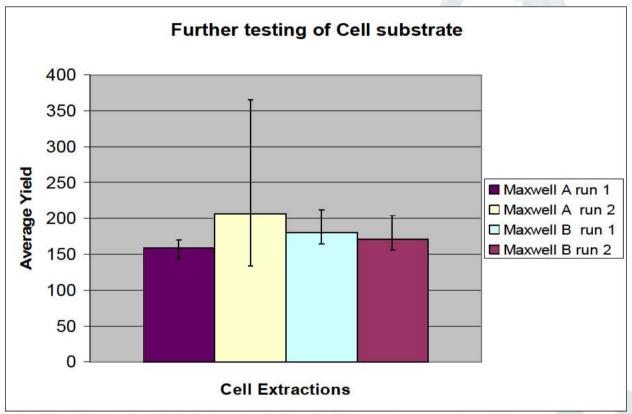


Figure 5 Second set of data for Average yield of cell substrates

Table 6 shows the average yields, the standard deviation and the maximum and minimum of the second set of cell data. The second batch of cells processed on Maxwell[®] A showed a higher standard deviation when compared to the other batches; this was due to a wide range of yield values.

Instrument	Run number	Average Yield (ng)	Standard deviation	Maximum yield (ng)	Minimum yield (ng)
Α	1	158.86	9.14	170	144
A	2	206.14	73.90	365	134
В	1	180.29	19.34	212	164
В	2	.97	16.96	204	156

Table 6 Summary of the second set of cell data



Project #70 - Maxwell project Phase 1

T-tests using the data from the further testing of cell substrates showed no significant difference between each run on Maxwell[®] A (p= 0.142182171) and each run on Maxwell[®] B (p= 0.357212553). There was also no significant difference in the instrument to instrument comparison (p=0.991410996).

The data from the further testing of cell substrate showed repeatability between batches processed on Maxwell[®] A and Maxwell[®] B and reproducibility between the instruments. This supports the premise the variability seen in earlier testing was due to the cell substrates and not the instruments.

DNA profiles obtained from all the blood and cell swabs gave the expected profile in all reproducibility studies with no evidence of cross contamination.

7.3 Sensitivity Testing and DNA Yield

For the 0.5µL and 1.0µL volumes both the manual DNA IQ[™] method and the modified Promega method extraction on the Maxwell[®]16 MDx gave similar DNA yields. For the 2µL to 60µL volumes the extraction on the Maxwell[®]16 MDx gave better yields than the yields obtained with the manual DNA IQ[™] method as shown in Figure 5.

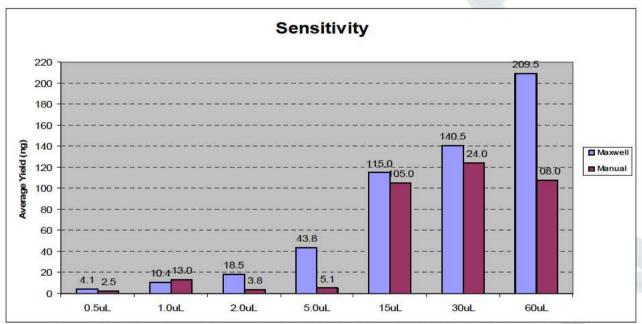


Figure 5 Sensitivity and DNA Yield comparing Maxwell®16 A and Manual DNA IQ™ methods using blood swabs.

7.4 Cross-Contamination

In an alternating pattern, eight blood samples and eight blank/negative controls were extracted using the modified Promega method on each of the Maxwell[®]16 MDx instruments. The quantification values, CT values and IPCCT values obtained for each of the blank controls and blood samples are shown in Table 5 and Table 6 below.



Project #70 - Maxwell project Phase 1

	Sample type	Instrument	Quant (ng/µL)	CT	IPC
1	Negative Control	Maxwell [®] 16 A	undetermined	undetermined	26.7
2	Positive Control	Maxwell [®] 16 A	1.82	26.78	26.57
3	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.91
4	Blood swab	Maxwell [®] 16 A	1.55	27	26.79
5	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.95
6	Blood Swab	Maxwell [®] 16 A	2.49	26.34	26.69
7	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.89
8	Blood Swab	Maxwell [®] 16 A	1.71	26.86	26.83
9	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.91
10	Blood Swab	Maxwell [®] 16 A	1.61	26.95	26.66
11	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.82
12	Blood Swab	Maxwell [®] 16 A	1.51	27.04	26.75
13	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.81
14	Blood Swab	Maxwell [®] 16 A	2	26.65	26.65
15	Blank Control	Maxwell [®] 16 A	undetermined	undetermined	26.84
16	Blood Swab	Maxwell [®] 16 A	2.41	26.39	26.67

Table 6 Cross Contamination Results for Maxwell B

	Sample type	DNA IQ [™] EXT method	Quant (ng/µL)	CT	IPC
1	Negative Control	Maxwell [®] 16 B	undetermined	undetermined	27.63
2	Positive Control	Maxwell [®] 16 B	2.16	26.49	27.5
3	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.56
4	Blood swab	Maxwell [®] 16 B	2.21	26.46	27.44
5	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.56
6	Blood Swab	Maxwell [®] 16 B	1.82	26.73	27.42
7	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.72
8	Blood Swab	Maxwell [®] 16 B	1.96	26.63	27.69
9	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.65
10	Blood Swab	Maxwell [®] 16 B	2.2	26.47	27.48
11	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.6
12	Blood Swab	Maxwell [®] 16 B	2	26.6	27.47
13	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.6
14	Blood Swab	Maxwell [®] 16 B	1.25	27.26	27.53
15	Blank Control	Maxwell [®] 16 B	undetermined	undetermined	27.73
16	Blood Swab	Maxwell [®] 16 B	1.07	27.48	27.71

All blank/negative controls gave undetermined quantification values and all the blood samples gave quantification values consistent with results seen in the previous studies. The C_T (cycle threshold) values for all blank/negative controls gave values of undetermined indicating there was no DNA or not enough DNA to be amplified to reach the set cycle threshold. The blood samples gave C_T values within a range of 20-30 which is expected and is within normal range for samples with the presence of DNA.

The blank/negative states and blood samples gave IPCCT (internal PCR control) values within a range of 20-30 which is within normal range indicating no presence of inhibitors in any of the samples. The blank/negative controls were profiled and analysed at 16RFU, which is the peak



Project #70 – Maxwell project Phase 1

detection threshold used for all negative controls processed in DNA Analysis Unit. The blood samples were profiled and analysed at 50RFU, which is the standard peak detection threshold for casework and reference samples. The blank/negative controls run on Maxwell[®]16 A displayed no DNA (NSD) profiles and the blood samples displayed excess (XS) sized peaks that were consistent with the expected profile. The blank/negative controls run on Maxwell[®]16 B displayed no DNA (NSD) profiles and the blood samples displayed profiles consistent with the expected profile with one sample displaying excess (XS) sized peaks.

There was no evidence suggesting cross contamination occurred between any of the extraction batches performed for each experiment on either of the Maxwell[®]16 MDx instruments. All blood and cell samples for the suitability, reproducibility, sensitivity and cross contamination studies obtained single source profiles with no presence of mixtures.

8 Conclusion and recommendations

This verification has determined the Maxwell[®]16 MDx instruments using the modified Promega method have produced repeatable, reproducible results and are suitable for routine processing of blood and cell swabs in the DNA Analysis Unit. It has also shown that this extraction procedure will give results comparable to the current routine manual DNA IQ[™] method. It has also been shown that there is no evidence to suggest cross contamination between samples (between runs or between samples within a run) is likely to occur.

Therefore, it is recommended that the Maxwell[®]16 MDx instruments using the modified Promega method be introduced for the routine extraction of crime-scene swabs within the DNA Analysis laboratory.

It is also recommended that further investigation into the suitability of this procedure for the processing of other substrates - specifically tape-lifts and cigarette butts be carried out.

This would further enhance the workflow and throughput of DNA Analysis Unit as this technology would reduce the time taken for substrates in small batches to be processed, thereby improving current turn around times. The reduction in the amount of pipetting required compared with the labour intensive current routine manual DNA IQ[™] method would also be of an occupational health and safety benefit to laboratory workers.



Forensic and Scientific Service CaSS

Project #70 - Maxwell project Phase 1

9 References

- 1. DNA IQ^{™™} Casework Pro Kit for Maxwell[®] 16, 2010, Technical Manual, Part#TM332, Promega.
- Maxwell[®] 16 MDx Instrument, 2009, Technical Manual, Part# TM320, Promega
 Validation Guide for the DNA IQ[™] Casework Pro Kit for Maxwell[®] 16, 2010, Reference Manual,
- 4. Part# GE621, Promega.
- QIS 24897 DNA IQ[™] Method of Extracting DNA from Reference and Casework Samples.
 QIS 19977 Automated Quantification of Extracted DNA using the Quantifiler Human DNA
- 7. Quantification Kit.
- QIS 19976 Automated Amplification of Extracted DNA using the AmpFISTR Profiler Plus Kit or
 AmpiFISTR Cofiler Kit.
- 10. QIS 19978 Capillary Electrophoresis Setup
- 11. QIS 17137 Procedure for STR fragment analysis using GeneMapper ID-X software.
- 12. QIS 15996 Procedure for the use and Maintenance of the AB 3130xl Genetic Analyzers.
- 13. QIS 17130 CE Quality Check of Samples from the ABI Prism 3130xl Genetic Analyzers.
- 14. Nurthen, T., Hlinka, V., Muharam, I., Gallagher, B., Lundie, G., Iannuzzi, C. "Project 9. Automation: Report on the Evaluation of DNA Extraction Chemistries." June 2007.

Copyright protects this publication. However, Queensland Health has no objection to this material being reproduced with acknowledgment, except for commercial purposes. Permission to reproduce for commercial purposes should be sought from:

Managing Scientist **DNA** Analysis Forensic and Scientific Services PO Box 594, Archerfield QLD Australia 4108

Or by telephone (07)

