

Extraction Batch Audit

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DNA Analysis FSS (September 2008)

1. Abstract

An internal audit of the extraction process was deemed necessary to address adverse quality events identified through the laboratory quality system and Opportunity for Quality Improvement (OQI) process. The purpose of this audit was to maintain the continuous high quality standards within the DNA Analysis laboratory.

All initial results obtained from casework samples processed with DNA IQ™ and the MultiPROBE® II PLUS HT EX platforms between the 23 October 2007 and the 28 July 2008 were investigated, this included a total of 216 extraction batches. Of these, 202 batches were released (94%), six batches were placed on hold pending the release of results (3%) and five batches were placed on hold due to the involvement in previous OQI's (2%). An additional three batches were quarantined through the identification of new contamination events that resulted in three new OQI's (1%).

The methodology applied in this audit provides scientists with a new tool to reinforce the existing quality system. It may also assist in strengthening the confidence scientists have in reporting results. This quality tool could have applications outside the scope of this audit and be applied to other types of batches.

2. Aim

- To evaluate casework data obtained from extractions processed with DNA IQ™ on the MultiPROBE® II PLUS HT EX platforms for contamination events by comparing initial results for each sample from within an extraction batch.

3. Background

Through the laboratory quality system (OQI process) a number of adverse quality events were identified on the MultiPROBE® II platforms. Five OQI's (18580, 19349, 19477, 19768 and 20231) had previously been raised to address a dropped Slicprep™ 96 Device plate and profiles in the negative controls. The management team convened and a decision was made to conduct an internal audit of the entire extraction process.

Initial investigations were unable to determine whether the contamination events originally identified were due to the MultiPROBE® II platforms alone or a combination of the platforms and the DNA IQ™ process. The management team subsequently decided to audit the initial results obtained from every casework sample processed through DNA IQ™ and on the MultiPROBE® II PLUS platforms. This covered the period from the 23 October 2007 to the 28 July 2008, when both processes were temporarily halted. A working party was later established to manage the reporting of all results, with a focus on the release of urgent statements.

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Laboratory Information Systems and Solutions (LISS) in association with Thomas Nurthern (DNA Analysis, Forensic Scientific Services (FSS)) created an export file from AUSLAB. This function exported the initial results obtained from an extraction batch to a text file that could then be imported into Microsoft Excel. Timothy Gardam (DNA Analysis, FSS) developed a results comparison macro that identified any matches of >12 alleles between samples from within an extraction batch. This matching stringency was determined by the management team in alignment with the upload stringency of the National Criminal Investigation DNA Database (NCIDD).

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4. Equipment and Materials

- i. AUSLAB function to export the initial results from an extraction batch
- ii. AUSLAB function to export the details of samples from an extraction batch (eg sample/case number, position, priority)
- iii. Batch Comparison Macro
- iv. Microsoft Excel

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5. Methods

5.1. Extraction Audit Progress

- i. The status of each extraction batch was recorded in an Excel spreadsheet titled Batch Audit Progress, located in I:/Extraction Audit (See Figure. 1 – Batch Audit Progress).

	A	B	C	D	E	F	G	H
	Extraction Batch	Date Started	Date Complete	Outcome	Batch Allocated To	Specimen Note Added	Batch Audit Entry	Comments
2	CWIGEXT20071023_02	15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
3	CWIGEXT20071023_03	15/08/2008	15/08/2008	Plate OK		Yes	Yes	All matches checked, no queries
4	CWIGEXT20071025_02	15/08/2008						
5	CWIGEXT20071026_01	15/08/2008						
6	CWIGEXT20071030_01	15/08/2008						
7	CWIGEXT20071031_01	15/08/2008						
8	CWIGEXT20071031_02	15/08/2008						
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Figure 1. – Screen shot of Batch Audit Progress

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5.2. Virtual Platemap of Extraction Batch

- i. AUSLAB was opened.
- ii. Options 5. (Workflow Management) and 2. (DNA Batch Details) were selected.
- iii. The Extraction Batch number was entered (eg. CWIQEXT20071023_02). The DNA Extraction Batch Details were displayed (See Figure 2. – AUSLAB DNA Extraction Batch Details).

DNA BATCH DETAILS

Batch Type: Casework DNA 10 Ext
 Batch Number: CWIQEXT20071023_02
 Date Created: 15:58 23 Oct-07
 Status: Completed

Pos	Lab No.	CRISP No	Priority	Request	Request Date
1	33383-6624	FB0T0000035	3	9PLEX	23 Oct-07
2	33383-6683	FB0T0000035	3	9PLEX	23 Oct-07
3	23486-8604	QPS060418345	3	9PLEX	13 Mar-07
4	23486-8610	QPS060418345	3	9PLEX	13 Mar-07
5	25235-4838	QPS060409092	3	9PLEX	13 Mar-07
6	25933-1236	QPS060409092	3	9PLEX	13 Mar-07
7	12935-7499	QPS030230225	3	9PLEX	13 Mar-07
8	12935-7488	QPS030230225	3	9PLEX	13 Mar-07
9	12935-7477	QPS030230225	3	9PLEX	13 Mar-07
10	12935-7466	QPS030230225	3	9PLEX	13 Mar-07
11	12935-5988	QPS030279753	3	9PLEX	13 Mar-07

[F5] Sequence Check [F6] Reagents [F7] Complete Batch [FB] Print Menu
 [SF5] Reload Batch [SF6] Files [SF7] Remove Menu [SF8] Audit

Figure 2. – Screen shot of AUSLAB DNA Extraction Batch

- iv. Reload Batch (Shift F5) was selected to export the extraction batch results from AUSLAB. These results were saved as a text file to C:/AUSLAB, which was then moved into the I:/Extraction Audit/Work in Progress/AUSLAB Shift F5 folder.
- v. The save function (Ctrl F11) was selected to export all of the extraction batch details from AUSLAB. The prompt "OK to save table to disk (y/n)?" was displayed, "y" was selected for yes and the file name I:/Extraction Audit/Work in Progress/CWIQEXT20071023_02.xls was entered.
- vi. The extraction batch details spreadsheet was opened, a header row was inserted titled Position, Lab no, Case no, Priority, Test Code, Date, Extracton Batch, Sample ID, Sex, D3, D8, D5, vWA, D21, D13, FGA, D7 and D18.
- vii. The extraction batch number was entered for all samples.
- viii. A new worksheet titled "Comparison" was inserted into the extraction batch details spreadsheet.

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5.3. Extraction Batch Comparison

- i. The Batch Comparison Macro in I:/Macros/testing was opened.
- ii. Compare Profiles was selected.
- iii. A prompt window "Choose a file for match comparison" was displayed. The extraction batch results text file to be compared (located in the Shift F5 folder) was selected. This generated the batch profile comparison results (See Figure 4. – Batch profile comparison results).
- iv. This page was copied and pasted into the comparison worksheet of the virtual plate map, and then printed to include the extraction batch number as a hard copy record.

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R
	Sample ID	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score	Compare Profiles				
2	265218185 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR, NR	15, NR	15					
3	269331245 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21, NR, 24	NR, 11	15,16	15					
4																	
5	265218185 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR, NR	15, NR	15					
6	265218240 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15					
7																	
8	269331245 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21, NR, 24	NR, 11	15,16	17					
9	265218240 9PLEX		15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17					
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See Figure 4. – Screen shot of the batch profile comparison results

The batch comparison macro identified any matches of >12 alleles between samples from within an extraction batch. Matching alleles were highlighted in yellow. Light yellow indicated one matching allele, and dark yellow indicated at least two matching alleles. The number of matching alleles between samples was listed in the column titled Match Score.

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5.4. Checking Matches

- i. AUSLAB was opened.
- ii. Options 5. (Workflow Management) and 2. (DNA Batch Details) were selected.
- iii. The Extraction Batch number was entered (eg. CWIQEXT20071023_02).
- iv. The first matching sample highlighted by the Macro was selected. Sample details were noted (eg. exhibit type). This was repeated for the second matching sample. If both samples were from the same exhibit and/or case this match was passed. Comments for each match were entered into the comparison worksheet within the virtual plate map (See Figure 5. – Batch profile comparison results/comments).

Sample ID	Amel	D3	D8	D5	vWA	D21	D13	FGA	D7	D18	Match Score	Comments
265218185	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
269331245	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	15	Straw, same case
265218185	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,24	NR,NR	15,NR	15	Cigarette butt, same case
265218240	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	15	Cigarette butt, same case
269331245	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,NR,24	NR,11	15,16	17	Straw, same case
265218240	9PLEX	15,16	10,12	11,12	17,18	29,30	12,12	21,24	10,11	15,16	17	Cigarette butt, same case

Figure 5. – Batch profile comparison results/comments

- v. If the extraction batch was released at this stage, Audit (Shift F8) and Insert Audit Trail (F5) was selected from the extraction batch and “Extraction batch audit complete. Plate released” was entered. A specimen note was also entered for each sample on the extraction batch.
- vi. Any matches that could not be passed were highlighted in red and the entire batch was placed on hold pending further investigation.
- vii. The Batch Audit Progress spreadsheet was updated with all outcomes.
- viii. Both the Batch Audit Progress spreadsheet and all finalised virtual plate maps were password protected to ensure the integrity of these documents.

5.5 Further Investigation

- i. For matches requiring further investigation, Genotyper printouts were obtained and case details were noted eg. address, date and type of alleged offence.
- ii. Nominated case scientists performed both mixture and single source interpretations.
- iii. If matches were excluded, the paperwork was returned for filing, and both AUSLAB and the Batch Audit Progress spreadsheet were updated.
- iv. If possible, re-sampling was conducted to confirm matches between single source profiles from different cases. Where re-sampling was not possible, the original spin baskets were re-extracted.

5.6 Contamination Events

Mixed or single source matches that were identified as contamination events were brought to the attention of the team leader. An attempt was made to establish the source of the contamination through the reconstruction of a plate map. An OQI was then raised by a senior scientist.

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6. Results

It was identified that there were 278 extraction batches processed through DNA IQ™ and on the MultiPROBE® II platforms from the 23 October 2007 to the 28 July 2008 (See Table 1. - Current status of audited extraction batches). These extraction batches were filed in six extraction folders (Volumes 28-33) located in the Analytical Section.

Table 1. – Current status of audited extraction batches

Total number of extraction batches	278
Extraction batches released	202
Extraction batches on hold	14
Extraction batches removed	62

Extraction batches were released:

- where no matches were returned after the use of the Batch Comparison Macro, or
- where any matches could be explained by samples originating from the same exhibit or from exhibits within the same case.

Matches that could not be eliminated included partial and full profiles from both single and mixed source samples. Nominated case scientists performed interpretations to determine if the matches could be excluded or whether further investigation was required before the batch could be released.

Extraction batches were placed on hold if they were part of previously raised OQI's (five), new OQI's raised as a result of this audit (three), or if re-sampling was required to confirm initial results (nine).

- Previous OQI's included 18580, 19349, 19477, 19768 and 20231. These reports may be accessed through QIS.
- The three new OQI's 20351, 20422 and 20437 identified three previously unknown contamination events in three different extraction batches. These batches are on hold pending further investigation. For further details please refer to Appendix 1. - OQI 20351, Appendix 2. - OQI 20422 and Appendix 3. - OQI 20437.
- Matches between different cases were identified on nine extraction batches. Re-sampling or re-extraction of the original spin baskets has been conducted and results are still pending for six batches.

There were five extraction batches where 53 samples had not progressed to Quantification. To finalise initial results a new test code entry was required in AUSLAB.

Extraction batches were removed in AUSLAB for several reasons. These included:

- controls not being added
- incorrect sample type (tapelifts or manual extraction)
- incorrect batch type (off-deck lysis)
- requiring smaller batch sizes (urgent samples)

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7. Discussion

From the 278 extraction batches included in this audit, a total of 202 batches were released. Of these:

- 22 batches returned no matches after the use of the Batch Comparison Macro.
- 157 batches returned matches that could be explained without further investigation.
- 20 batches were initially identified as requiring further interpretation by a nominated case scientist, but were subsequently passed as there was no evidence of contamination.
- 3 batches were re-sampled and passed as the profiles obtained from re-sampling matched the original profiles.

A total of 14 extraction batches were placed on hold. These included:

- Five batches involved in previous OQI's
 - Four of these OQI's (19349, 19477, 19768 and 20231) were from previously identified contamination events. The initial results were reported as "Quality Control Failure".
 - The fifth OQI (18580) resulted from a dropped Slicprep™ plate.

If possible re-sampling was conducted.

- Three batches involved in new OQI's that identified previously unknown contamination events
 - OQI 20351 involved extraction batch CWIQEXT20080402_01. This batch had a partial minor DNA profile present in the positive extraction control - 346792908 (position 1). This profile matched to the corresponding components present in the profiles obtained from samples of a sexual assault case - 209039621 (position 25), 209039610 (position 26), 209039596 (position 27), 209039585 (position 28), 209039604 (position 31) and 209039579 (position 32). This profile also matched to the profiles obtained from samples of four different volume crime cases - 209066683 (position 42), 209066674 (position 43), 209066660 (position 44) and 259718144 (position 51). The contaminating profiles appeared to be distributed in both directions across the plate.
 - OQI 20422 involved extraction batch CWIQEXT20080506_02. This batch had a mixed DNA profile present in sample - 365296308 (position 7). The major component of this DNA profile matched to the profile obtained from the deceased in this case. The minor component of this profile matched to the profile obtained from the complainant in what appears to be an unrelated sexual assault case - 320124514 (position 23) and 320124503 (position 24).
 - OQI 20437 involved extraction batch CWIQEXT20080630_01. This batch had a bent pipette tip at position 6 which appeared to have made contact with the horizontally adjacent well at position 14. No DNA profile was obtained for sample - 323288136 (position 6), whilst a partial profile was obtained for sample - 365366424 (position 14). In addition, a full profile obtained for sample - 320126679 (position 5) from a sexual assault case matched to sample - 323288127 (position 7) a case involving the unlawful use of a motor vehicle.

These adverse events did not appear to have a uniform distribution across the plate.

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- Six batches involved matches that required the re-sampling of exhibits to confirm the initial profiles and exclude any potential contamination events. If re-sampling was not possible, other items from within the case not previously examined were sampled or the original spin baskets were re-extracted. Results are pending.

A total of five extraction batches contained 53 samples that had not progressed to quantification, and therefore the initial results were still outstanding. A new test code entry was required in AUSLAB for these samples to progress. This was either due to the processing comments not being checked by the MultiPROBE® II platform operator or a previously identified system fault in AUSLAB.

8. Miscellaneous

An outcome of the working party was to recommend the use of the filter function in the virtual plate map in conjunction with normal quality processes to manage the reporting of all results.

9. Summary

Evaluation of the casework data obtained from extractions using DNA IQ™ and the MultiPROBE® II platforms involved 216 extraction batches. 202 batches were released, 14 batches were placed on hold (six batches still awaiting results) and 62 batches were removed.

This audit identified three new contamination events and three OQI's were raised to address these issues in accordance with the laboratory quality system. The new methodology developed and applied in this audit provides scientists with a strengthened quality system that may prevent similar adverse events from occurring in future and simultaneously increase the confidence scientists have in reporting results.

10. Recommendations

Possible recommendations from this audit are listed below:

- ✓ • The Batch Comparison Macro could be applied to all new extraction batches to assist in the identification of any adverse events prior to the release of results.
- If an adverse event is identified, a streamlined process needs to be in place to address the issue effectively with efficient laboratory communication (eg. e-mail alert system).
- Availability of AUSLAB functions to export all results from any batch type, facilitating other types of audits and quality measures.
- ! [• A previously identified system fault in AUSLAB needs to be addressed to ensure that all samples progress from extraction to quantification.

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APPENDIX 1. – OQI 20351

big volume across plate

OQI No.: 20351

What Stage?: INVESTIGATION OF ROOT CAUSE

Created By: RIKA, Kylie

On: 08-AUG-2008

Corporate Unit*: Forensic and Scientific Services

Site/Location*: Coopers Plains

Centre/Group: Forensic Sciences (FS)

Department*: Forensic Biology (FSS)

Work Area: Forensic Biology - Major Crime

Description: CWIQEXT20080402_01 was found to have a partial minor DNA profile present in the extraction positive control (346792908). This partial minor DNA profile matches alleles present in samples 209039621(position 25), 209039610 (26), 209039596 (27), 209039585 (28), 209039604 (31), 209039579 (32). These samples are from a sexual assault case and the profile from these samples is the same. This profile also matches profiles from four separate volume crime cases located on this extraction batch. It appears as though the contaminating profiles have not only contaminated from right to left across the plate but also from left to right. Part of the investigation into this event has been researched by the Extraction Audit Team and a word document with all the details is being drafted and sent to the receiver of this OQI so that it can be included in the investigation part of the OQI process.

Source of OQI: Audit

What Needs Fixing?: Process

Action By Whom?: MCNEVIN, Allan

In Corporate Unit: Forensic and Scientific Services

In Site/Location: Coopers Plains

In Centre/Group: DNA Analysis

In Department: Forensic Biology (FSS)

In Work Area: Forensic Biology - Analytical

Root Cause: Other

Date Actioned or Due: 18-AUG-2008

How Fixed?: Other

Accepted?: Pending

Approval Type: Pending

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APPENDIX 2. – OQI 20422

OQI No.: 20422
What Stage?: INVESTIGATION OF ROOT CAUSE
Created By: HOWES, Justin
On: 20-AUG-2008
Corporate Unit*: Forensic and Scientific Services
Site/Location*: Coopers Plains
Centre/Group: Forensic Sciences (FS)
Department*: Forensic Biology (FSS)
Work Area: Forensic Biology - Major Crime
Description: An unexpected mixed DNA profile was obtained for lab no. 365296308 (QP800235382) that had a major DNA profile matching the deceased in the matter, and a minor DNA profile matching a DNA profile obtained from a complainant in an apparently unrelated sexual assault matter (QP800088413).
Source of OQI: Internal Problems (QHPSS)
What Needs Fixing?: Process
Action By Whom?: MCNEVIN, Allan
In Corporate Unit: Forensic and Scientific Services
In Site/Location: Coopers Plains
In Centre/Group: Forensic Sciences (FS)
In Department: Forensic Biology (FSS)
Root Cause: Other
Date Actioned or Due: 30-AUG-2008
How Fixed?: Other
Accepted?: Pending
Approval Type: Pending

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APPENDIX 3. – OQI 20437

OQI No.: 20437
What Stage?: INVESTIGATION OF ROOT CAUSE
Created By: STORER, Amanda
On: 21-AUG-2008
Corporate Unit*: Forensic and Scientific Services
Site/Location*: Coopers Plains
Centre/Group: Forensic Sciences (FS)
Department*: Forensic Biology (FSS)
Work Area: Forensic Biology - Major Crime
Description: CWIQEXT20080630_01 : Whilst this process was running on the robot the operator noted that the plate was misaligned on the deck, resulting in the 8tip arm contacting the lysate plate. The operator noted that the disposable conductive tip at position 6 was bent and appeared to have made contact with the well of position 14 (adjacent).

Position 6 did not result in a DNA profile. (QT700518732, UUMV, Greenslopes). Position 14 resulted in an incomplete male DNA profile. (QT800323269, burglary, Noosaville)

During further checks of this extraction batch, it was discovered that the DNA profile in position 5 matched the DNA profile in position 7.

Position 5 contained extract from the oral swab of a SAIK (QT800131925, Townsville), and this DNA profile matches to the DNA profiles obtained from the other intimate samples from the SAIK of that case, so it appears to be a true result. Position 7, however, was supposed to contain extract from a swab from the right throttle of a motorbike involved in the UUMV case mentioned above (QT700518732). The other sample for this case was in position 6, which did not result in a profile, and no reference samples were received in relation to this matter for further comparison.

Summary : position 5 (sample 320126679) from a sexual assault case has matched to position 7 (sample 323288127) from an

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apparently unrelated unlawful use of motor vehicle case in another part of the State.

Source of OQI: Internal Problems (QHPSS)
What Needs Fixing?: Process
Action By Whom?: MCNEVIN, Allan
In Corporate Unit: Forensic and Scientific Services
In Site/Location: Coopers Plains
In Centre/Group: DNA Analysis
In Department: Forensic Biology (FSS)
Root Cause: Other
Date Actioned or Due: 31-AUG-2008
How Fixed?: Other
Accepted?: Pending
Approval Type: Pending

