EXTRACTION BATCH CONTAMINATION

Affected Batch: [redacted]

Contaminated profile:

- Plate position: 22
- Lab No. : [redacted]
- Case: [redacted]
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- Offence details : Complainant assaulted by 4 unknown offenders (no indications of def'd/comp. bleeding; blood stained shirt and jeans submitted for analysis
- Profile details : A subsample from the inside front left pocket of the jeans, barcode [redacted], produced a mixed partial DNA profile with indications of 2 contributors (27 alleles/6 NR's). It was not possible to separate this profile into major and minor components – no reference samples were submitted for this case. Other subsamples from the same item gave the same partial and full unknown male 1 profile which was observed in the mixed DNA profile (NR peaks were 'hovered' over to determine allele calls)

Contaminating profile(s):

- Plate position: 28,29,30,31
- Lab No(s): [redacted]
- Case : [redacted]

- Offence details : Offender(s) have assaulted complainant, with the victim receiving minor head injuries (lacerations), 4 blood-stained T-shirts submitted.

- Profile details : 4 subsamples from one item, 'Nixon' t-shirt (various stained regions), gave the same partial and full DNA profiles (ukm1) - [redacted]. Reference sample from the complainant (SHAW) does not match the unknown profiles obtained.

Points to consider:

- Possibility of these cases being related, both involving assault with multiple offenders, relatively close locations (Brisbane, Gold Coast) and similar time periods (Feb/March 2008)
- Mixtures were not an unexpected result from the initial case, given the nature and details of the offence. Of note, a sample from the inside front right pocket gave a single source profile.
- Initial results of the mixed profile (GEN9CW20080422_02) was 6 alleles/12 NR peaks; this was reworked and confirmed on GEN9CW20080710_02.
- The extraction batch had previously been checked and released as part of the extraction audit – no other indications of detectable contamination was observed.

- Owing to the incomplete nature of the mixed profile initially obtained, and the stringency level of profile matching (12 alleles), it was not unexpected that this potential contamination would be missed. This plate has now been placed on hold, pending further investigations.

Recommendations:

- Initial extraction audit to flag all mixed partial DNA profiles for careful review (particularly when reworks likely), prior to release of results.
- Sub-threshold allele values examined closely, if available, particularly when reported as 'Partial profile, insufficient for comparison..'
- Interpretation/acceptance of final results undertaken with caution with reference to results obtained from other samples/items within case, comparison to EVD samples where possible and consideration of resampling affected exhibits.

Al's notes
Investigations:
Sample [redacted] was extracted on extraction batch [redacted] in position 22. This sample gave a mixed DNA profile that was shown to be reproducible after concentration and re-amplification of the DNA extract. This result was expected due to the nature of the sample and the case. It was not possible to separate this profile into major and minor components and there were no reference samples were submitted for this case. Other sub-samples from the same item gave the same partial and full unknown male 1 profile which was observed in the mixed DNA profile.

During the peer review of this result, a potential for contamination of this sample from other samples on the same extraction batch was indicated and this was investigated. The samples identified as potential sources of the contamination were [redacted] from positions 28-31 respectively on the same extraction batch.

During the investigation, the stored lysate for all five samples we re-extracted, as well as the stored substrate. Throughout this investigation, both the results from the initial extraction and any re-extracted material was analysed using Genemapper-IDX software with a peak detection threshold of 20RFU in order to gain the most information.

The re-extraction of the stored lysate (i.e. material retained after removal from para-magnetic resin during the automated DNA IQ extraction process) for each of the five samples gave results consistent with that obtained from the initial extraction process. From these results, it can be concluded that the contamination of sample [redacted] from either sample [redacted] must have occured prior to the separation of the lysis solution and the para-magnetic resin.

The re-extraction of the stored substrate (i.e. the material originally submitted for DNA extraction that passed through the initial stages of the original extraction process) gave differing results. The profile obtained from the substrate of [redacted] was consistent across almost all alleles contained within the mixture, however single alleles at the FGA, D18, D5 and D13 loci that were consistent with the potential source profiles were not found. The profiles obtained from re-extraction of substrates from samples [redacted] yielded profiles consistent to that obtained from the original extraction (sample 3 [redacted] only yielded 4 alleles, however all were consistent with the original profile obtained).

When assessing the results obtained from the original extraction and re-extraction of the substrate from sample [redacted] (with the assistance of the Reporting and Intelligence team leader), it can be seen that the mixture ratios are not conserved. When viewed in combination with the additional alleles obtained, it can be seen that contamination of sample [redacted] with a profile consistent with that obtained from samples [redacted] has occurred.

Since sample [redacted] has a ten-fold higher level of DNA concentration than either of the other four samples under investigation (in all samples tested, including the

original DNA extract, stored lysate and re-extracted substrate) and lies in the adjacent well to the contaminated sample [redacted]) it can therefore be concluded that there has been contamination of sample [redacted] with sample [redacted]. The results obtained from the investigation therefore indicate that contamination of sample [redacted] from sample [redacted] has occurred after the manual lysis of the substrates (off-deck lysis procedure), but prior to completion of the removal of lysed material from para-magnetic resin during the automated portion of the extraction procedure.

The potential steps at which contamination may have occurred are outlined in "Actions" below.

1. During the transfer of the lysate obtained from manual lysis into the deep-well plate via the use of the Storstar. This however is considered unlikely. This is because lysate was added to well 22 prior to the addition of wells 28-31 and the nature of the pipetting process whereby individual care is taken whilst performing the process.

2. During the removal of the adhesive seal used to seal the deep-well plate containing stored lysates awaiting automated DNA IQ extraction. This is the most likely as it was noted during Audit 8227 that condensation on the seal that could not be removed by centrifugation was a contamination risk. Additionally, it was observed in one instance that seepage across the adhesive seal (from a positive to a negative control in adjacent wells) appeared to have occurred. Samples [redacted] and [redacted] were extracted in adjacent wells (22 and 30 respectively).

3. There may have been operator error during the manual addition of DNA IQ para-magnetic resin during the start of the automated extraction procedure (i.e. incorrect pipetting procedure), however this is unlikely as staff are trained to perform such pipetting steps with due diligence and care.

4. During the mixing of the deep-well plate (containing 1.5mL of buffers and resin within a 2.2mL well) on the extraction platform DPC shaker. This had not been observed, however has been proposed as a possible mechanism during close scrutiny of the automated procedure.

5. During the 1st stage of removal of lysate from the para-magnetic resin to the storage plate. This procedure occurs twice during the automated extraction protocol (fresh disposable tips for each step). If there was drippage of the lysate containing unbound DNA and this was to drip from either well 30 into well 22 (or bubble burst), this may account for the contamination event. However, the physical movement of the 8-tip arm during this liquid transfer makes this an unlikely proposition (i.e. once the tips retract from well 30, it does not move directly over well 22).

Actions:
As a result of previous OQI's raised and concerns identified around the automated DNA IQ extraction process, the extraction of samples using the automated DNA IQ procedure was halted on the 28-7-2008.

Prior to this Audit 8227 had been commissioned and carried out. A number of areas

for improvement were identified through the audit, and these have been implemented or are under investigation as outlined in OQI's 20367, 20368 and 20369.

After the cessation of the automated DNA IQ extraction protocol, a review of all batches processed through this protocol was carried out by a specially commissioned team. A number of potential contamination events were identified and each is to be investigated on batch-by-batch basis.

Additionally, careful review of results obtained from samples processed through the automated DNA IQ extraction procedure prior to reporting will be carried out. Every DNA result obtained from these samples will be interpreted with caution.

Modifications have been made to the automated DNA IQ extraction procedure (including the use of an alternative to the adhesive seal and an alternative resin mixing procedure). This modified procedure will undergo extensive verification and approval from the DNA Analysis management team prior to re-introduction.

The contamination events and concerns and improvements etc. that surround the automated DNA IQ extraction procedure have been discussed at various departmental and team meetings.