CaSS | Forensic and Scientific Services
A CLINICAL AND STATEWIDE SERVICE

A review of DNA extraction control results obtained in the first six months of 2008

Megan Harvey, All[redacted]
Analytical section, DNA[redacted]S

Abstract

A review of the negative and positive DNA[redacted]trols processed in the first 6 months of 2008 within the Analytical section of DN[redacted]s conducted. One OQI (Opportunity for Quality Improvement) was raised [redacted]e audit process and all controls were validated in AUSLAB laboratory infor[redacted]n.

Introduction

For each DNA extraction batch processed within the Analytical section of the DNA Analysis department at FSS for 2008, a positive and negative extraction control was included. Each negative extraction control consisted of the reagents and lab-ware that were used for the process with the exception of no substrate. Each positive extraction control consisted of a mock sample, which was created using DNA from staff member/s that did not routinely work within the laboratory area and whose DNA profile was known. Positive and negative extraction controls were processed identically to samples on the same batch.

In addition to the positive and negative extraction controls from DNA extraction batches, negative controls were included in two post-extraction processing batches, namely DNA extract concentration via centrifugal filtration with a Microcon YM-100 (Millipore) filter and DNA extract clean-up via a modified extraction using the Macherey-Nagel NucleoSpin Tissue kit. Negative controls for the Microcon and Nucleospin batches consist of lab-ware used for the process and 100µl of nanopure water in place of DNA extract. Negative extraction controls for both the Microcon and NucleoSpin cleanup were processed identically to DNA extracts on the same batch.

Table 1 below shows the various control types used for each of the DNA extraction and post extraction processing procedures. The extraction type, control type and assigned case number for each control. Samples from a single case are grouped using a single identifier, the same procedure using an FSS DNA Analysis derived code was used to group controls of a similar type.

[redacted]

Table 1. Various control types used within FSS DNA Analysis Analytical section through 2008

Control type	Extraction type	Extraction Method	Case number	Control type
Negative Control	all	all	[redacted]	
Positive Control	Cell	Chelex NucleoSpin		Buccal cells on FTA paper
Positive Control	Blood & Bone*	Chelex Organic NucleoSpin DNA IQ †		Blood on FTA paper or Blood on Swab‡
Positive Control	Differential Lysis sperm fraction	Chelex		Buccal cells on Swab combined with Sperm on Swab
Positive Control	Differential Lysis epithelial fraction	Chelex		
Positive Control	Semen	Chelex		Sperm on Swab
Positive Control	Hair	Chelex		Plucked hair (scalp & eyebrow)

* Bone extractions were performed using an Organic extraction procedure.
† Blood and Cell extractions were combined into one method for extractions carried out using the DNA IQ extraction method.
‡ Blood on FTA paper positive control were used for Chelex, NucleoSpin & Organic extractions, Blood on Swab positive control was used for DNA IQ extractions.

Results & Discussion

For each control type, results were exported from the AUSLAB laboratory information system and imported into Excel. The exported results were then reviewed for expected results. Any controls where no results were exported or the expected result was not obtained from AUSLAB laboratory information system the control was re-processed and a reason given for no result obtained. All control results in AUSLAB were completed, validated and appropriate specimen notes and batch audit entries made as required.

Negative extraction controls (FBOT0000277)

906 negative extraction controls were profiled. 897 (99.01%) failed to show any evidence of amplifiable DNA. For nine negative extraction controls showing amplified DNA an OQI (Opportunity for Quality Improvement) was raised in the QIS quality system and the source of the contamination was investigated. 62 negative extraction controls displayed quantification values, with one having a value above the limit of reporting (see OQI# 20231) whereas 61 controls had quantitation values below the level of detection. 59 of the extraction controls with quantitation values below the level of detection did not display profiles or the presence of any suspected peaks below detection threshold when reviewed, no further investigation was performed. 3 extraction controls with quantitation values below the level of detection displaying partial profiles resulted in an OQI being raised and the source of the contamination was investigated. A summary of the nine negative extraction controls that failed is show in Table 2.

Table 2. Summary of failed negative extraction controls

Sample ID	OQI#	Investigation outcome
[redacted]		Sample contamination during automated IQ extraction process
		Sample contamination during automated IQ extraction process
		Sample contamination during automated IQ extraction process
		Sample contamination during automated IQ extraction process
		Sample contamination during automated IQ extraction process
		Sample contamination during automated IQ extraction process
		Below threshold profile, not attributable to any source, profile added to staff matching table
		Positive and negative control juxtaposed
		Sample contamination during automated IQ extraction process

For each of the negative controls where contamination was not attributable to human error or an identified procedural problem, the source of the contamination could not be located. At each stage, reviews of the lab-ware and lab environment were conducted in order to locate possible process improvements that may [redacted] tamination.

Positive cell extraction controls (FBOT0000278)

88 positive cell extraction controls were pr[redacted]9%) controls displayed the expected full DNA profile with no evidence of conta[redacted] controls displayed partial profiles with all alleles present being consistent wit[redacted] profile. One control failed to profile even after being reworked. The partial DNA[redacted]e the result of reduced extraction efficiency, or due to a reduced level of D[redacted] the positive control. Each positive control was prepared by a standard metho[redacted]uccal cells to FTA paper. However, once the cells have dried on the FTA pa[redacted]n of the cells are not visible and there is always a risk that the location a punch[redacted]m on the FTA paper may have been from an area where little or no buccal cells were applied. The amount of sample applied to the FTA paper cannot be accurately controlled.

Positive blood extraction controls (FBOT0000279)

281 positive blood extraction controls were profiled. 277 (98.58%) showed the expected profile with no evidence of contamination. One control displayed a partial profile with all alleles present being consistent with the expected profile. The incomplete removal of heme or the over-digestion of the sample during the extraction process could explain controls resulting in partial profiles. One control failed to profile due to the juxtaposition of the positive and negative control (see negative extraction controls). An OQI was raised in the quality system for this extraction control. Two controls were registered as blood extraction controls however there specimen type was sperm lysate and epithelial lysate. The profiles obtained from these controls were consistent with the profiles used for differential lysis.

Positive differential lysis extraction controls (FBOT0000280)

94 positive extraction controls were profiled (47 sperm lysate and 47 epithelial lysate) controls. Of the 47 sperm lysate controls, 42 (89.36%) display the expected DNA profile with no evidence of contamination. Of the remaining 5 controls, 2 contained extra peaks consistent with the epithelial lysate control, most likely representing carry-over of the female fraction during the extraction procedure and 3 displayed partial profiles with all alleles present being consistent with the expected profile.

Of the 47 epithelial lysate controls, 6 (12.76%) amplified the expected DNA profile with no evidence of contamination. Of these 4 were partial DNA profiles. These samples may have resulted from processing errors during the extraction method. In particular during the procedure, when a portion of extraction material was removed, leaving behind the sperm pellet, if excessive amount of liquid was left with the sperm pellet, epithelial DNA will have been lost (i.e. retained with the sperm pellet to be digested by washes prior to lysis of the sperm cells).

Each of the 37 remaining epithelial lysate controls contained peaks consistent with the sperm lysate positive control. This indicates that either male epithelial cells were present [redacted] n co-extracted, or it is also possible that the sperm used for creation of [redacted] degrades somewhat with successive cycles of freeze and thaw (each time [redacted] rols is made) and therefore some sperm DNA is un-intentionally released [redacted] process.

[redacted] no positive differential lysis controls that contained peaks that were not [redacted] r the epithelial or sperm lysate control profiles, therefore no [redacted] detected.

Positive semen extraction controls (FBOT0000281)

A total of four semen positive extraction controls were registered and profiled. All four controls displayed profiles that were consistent with the expected DNA profile with no evidence of contamination.

[redacted]

Positive hair extraction controls (FBOT0000282)

35 positive controls were profiled. 6 (17.14 [redacted] splay the expected full DNA profile with no evidence of contamination. 8 (22.86 [redacted] splay partial profiles with all alleles present consistent with the expected profile [redacted] controls display partial profiles that could not be compared to the expecte [redacted] extraction and reworking. One control displayed no DNA profile (NSD) after e [redacted] reworking.

Conclusion

As a result of reviewing the extraction cont [redacted] t six months of 2008 one OQI was raised and all controls were validated and [redacted] t entries were added in AUSLAB laboratory information system. This revie [redacted] cumented as Audit 9177 in QIS.

[redacted]