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

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Abstract

A review of the negative and positive DNA extraction controls processed within the Analytical section of DNA Analysis over the second 6 months of 2008 was conducted. No OQI (Opportunity for Quality Improvement) was raised directly from the audit process and all controls were validated in AUSLAB laboratory information system.

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.

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 procedu
† 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)

1252 negative extraction controls were profiled. 1250 (99.84%) failed to show any evidence of amplifiable DNA. Two negative extraction controls showing amplified DNA, were investigated and it was determined that the positive control and negative control labels had been switched at extraction stage. 63 negative extraction controls displayed quantification values, with two having a value above the limit of reporting, these were the samples that had been switched with the positive controls. 61 controls had quantitation values below the limit of detection and did not display profiles or the presence of any suspected peaks below detection threshold when reviewed, no further investigation was performed.

Positive cell extraction controls (FBOT0000278)

286 positive cell extraction controls were profiled. 278 (97.20%) controls displayed the expected full DNA profile with no evidence of contamination. Two controls displayed partial profiles with all alleles present being consistent with the expected profile. The partial DNA profiles may be the result of reduced extraction efficiency, or due to a reduced level of DNA present on the positive control. One control displayed a partial profile that did not display enough alleles to be compared with the expected profile, therefore the extraction performed below expectation. Three controls failed to profile even after being reworked, one of these was determined to be a negative extraction control that had labels switched at the extraction stage. Two controls displayed profiles with more than one contributor the profiles obtained are consistent with the Promega DNA used for validation procedures.

Positive blood extraction controls (FBOT0000279)

220 positive blood extraction controls were profiled. 199 (90.45%) showed the expected profile with no evidence of contamination. Nine controls displayed partial profiles with all alleles present being consistent with the expected profile. Eight controls displayed a partial profile that did not display enough alleles to be compared 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. Three controls displayed profiles of NSD, two still remained to have a NSD profile after being reworked and one was registered incorrectly and was actually a negative extraction control.

Positive differential lysis extraction controls (FBOT00002[redacted]

116 positive extraction controls were profiled (58 [redacted] and 58 epithelial lysate) controls. Of the 58 sperm lysate controls, 53 (91.3[redacted]e expected DNA profile with no evidence of contamination. Of the remainin[redacted]3 contained extra peaks consistent with the epithelial lysate control, most lik[redacted]ing carry-over of the female fraction during the extraction procedure and 2 dis[redacted]profiles one with all alleles present being consistent with the expected profile.

Of the 58 epithelial lysate controls, 15(25.86%) a[redacted]xpected DNA profile with no evidence of contamination. 13 controls displayed [redacted]s with all alleles present being consistent with the expected profile. 18 controls displayed partial profiles that did not display enough alleles to be compared with the expected profile. 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 12 remaining epithelial lysate controls contained peaks consistent with the sperm lysate positive control. This indicates that either male epithelial cells were present and these have been co-extracted, or it is also possible that the sperm used for creation of the positive control degrades somewhat with successive cycles of freeze and thaw (each time a new batch of controls is made) and therefore some sperm DNA is un-intentionally released during the extraction process.

There were no positive differential lysis controls that contained peaks that were not consistent with either the epithelial or sperm lysate control profiles, therefore no contamination was detected. A small test was carried out whereby the semen control was submitted for a straight cell extraction. One of three samples showed a full DNA profile consistent with the expected profile, and one sample showed a partial DNA profile. It is postulated that, in the absence of epithelial cells, sufficient digestion of sperm cells by the action of Proteinase K may occur. This was also observed during the evaluation of an alternative differential lysis kit (Promega Differex kit) in previous in-house studies. Therefore, re-evaluation of the number of epithelial cells included in the differential lysis positive control is warranted, and may explain why 12 of 58 epithelial lysate controls showed the presence of DNA from the semen portion of the control.

Positive semen extraction controls (FBOT0000281)

One semen positive extraction control was registered and profiled. The control displayed a profile that was consistent with the expected DNA profile with no evidence of contamination.

Positive hair extraction controls (FBOT0000282)

48 positive controls were profiled. 8 (16.67%) controls display the expected full DNA profile with no evidence of contamination. 8 (16.67%) controls display partial profiles with all alleles present consistent with the expected profile. 14 (29.16%) controls display partial profiles that could not be compared to the expected profile after extraction and reworking. 18 controls displayed no DNA profile (NSD) after extraction and reworking indicating that extractions performed below expectation.

Conclusion

As a result of reviewing the extraction controls for the second six months of 2008 all controls were validated and relevant audit entries were added in AUSLAB laboratory information system.

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