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

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Abstract

A review of the negative and positive DNA extraction controls processed in the first 6 months of 2008 within the Analytical section of DNA Analysis was conducted. One 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.

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 limit such contamination.

Positive cell extraction controls (FBOT0000278)

88 positive cell extraction controls were profiled. 85 (96.59%) 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. One control failed to profile even after being reworked. 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. Each positive control was prepared by a standard method of applying buccal cells to FTA paper. However, once the cells have dried on the FTA paper, the location of the cells are not visible and there is always a risk that the location a punch was taken from 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 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.

Positive semen extraction controls (FBOT0000281)