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Investigation into contamination of a Negative Extraction Control with a Positive Extraction Control

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Background

For each extraction batch carried out in FSS DNA Analysis, a positive and negative extraction control is used. For the majority of casework samples, DNA is extracted using the Promega DNA IQ™ kit. At the time of this investigation, casework samples were placed into a Slicprep™ 96 device (Promega) using *automate.it* STORstar system (Process Analysis & Automation Ltd. Hampshire. UK). The STORstar is an X-Y Cartesian instrument that allows samples to be bar-coded and samples placed through an orifice and into a receptacle (in this case the Slicprep™ 96 device) according to a previously created plate map (or a plate map can be created as desired). Batches and plate maps are created and output by the AUSLAB batch functionality enhancement in use with DNA Analysis. The Slicprep™ 96 device is a 96-deep well plate with a spin basket insert that allows for substrates to be incubated within a solution, and then separated from the solution by centrifugation.

Once samples have been placed within the Slicprep™ 96 device, it is placed on the deck of one of two dedicated PerkinElmer MultiPROBE® II PLUS HT EX with Gripper™ Integration platforms and the extraction procedure is carried out. In the final stages of the procedure, DNA extracts are placed within Nunc™ Bank-It tubes and stored frozen (-20°C). DNA is then quantified using the Applied Biosytems Quantifiler™ Human DNA Quantification kit, prepared on a MultiPROBE® II dedicated to PCR set-up. The real-time PCR is then performed on an Applied Biosystems Prism® 7500 Sequence Detection System. Once the DNA quantification has been determined, an appropriate amount of DNA template is added to the STR amplification reaction. STR analysis is carried out by amplification with Applied Biosystems AMPFℓSTR® Profiler Plus® PCR Amplification kit (prepared on the above mentioned dedicated MultiPROBE® II and amplified on a GeneAmp® PCR System 9700 thermalcycler. Fragment analysis is done by capillary electrophoresis on a Applied Biosystems Prism® 3130*xl* Genetic Analyser, in combination with Genescan (version 3.7.2) and Genotyper (version 3.7.1) software. During preparation of quantification and amplification batches, samples are storage is tracked using AUSLAB storage functionality, samples are stored frozen (-20°C) and at 4°C whilst awaiting processing. Sample tubes are uncapped and recapped using a LifeTool™ RECAP 96M automated capper.

[redacted]vestigation

[redacted]ring the Genescan analysis of the negative extraction control [redacted]) of extraction batch CWIQEXT20080225_02, it was noted that there was the potential presence of a DNA profile, however the peaks observed were below the routine analysis threshold of 75RFU (relative fluorescence units). The peaks observed are representation of light intensity over time, and analysis of the DNA profile occurs by sizing each fragment (the size of each fragment within a single capillary is compared to a an internal sizing standard co-injecteded with the PCR product) with the Genescan software, and by having alleles designations (by comparison of fragment size and dye colour with an external allele designation ladder, 1 ladder per 15 samples) determined by Genotyper software. This negative extraction control was re-amplified immediately to confirm the presence of DNA within the sample. Both the original amplification and re-amplified samples were re-analysed with a reduced RFU threshold (30RFU). Although lowering the RFU threshold makes it difficult to determine true peaks from background fluorescence, this method is useful when investigating laboratory incidents, but is not used for reporting where caution must be observed. The profiles as observed at lowered RFU thresholds are shown in figures 1 and 2 below.

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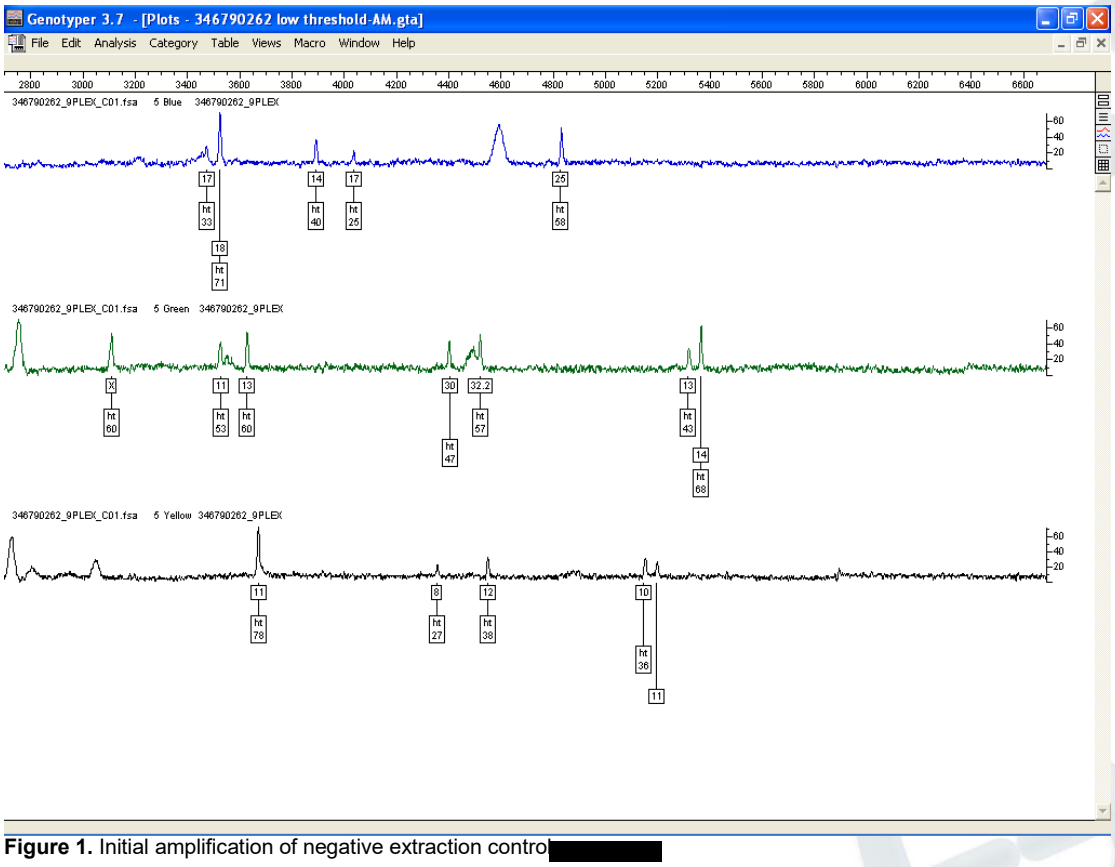

Figure 1. Initial amplification of negative extraction control [redacted]

[redacted]

[redacted]

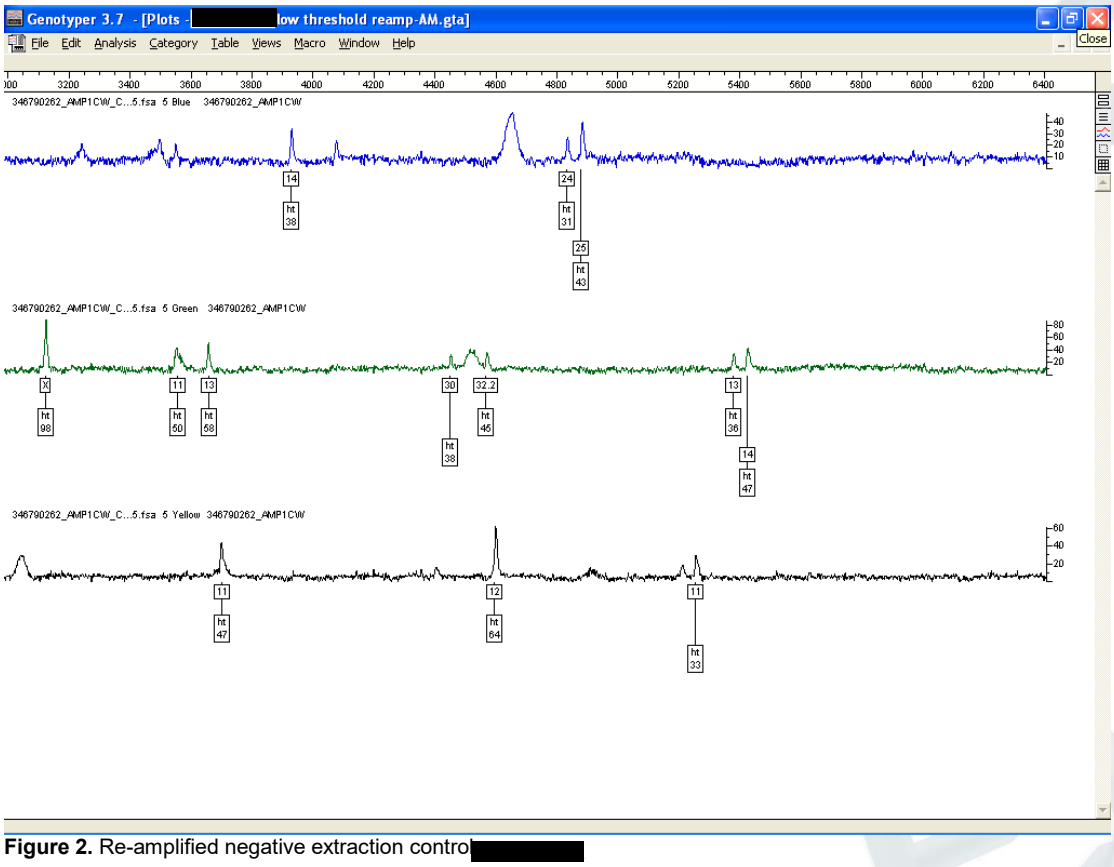

Figure 2. Re-amplified negative extraction control [redacted]

When analysed, the partial DNA profiles contained within the negative extraction control were consistent with the positive extraction control used. All alleles within the positive extraction control were present in at least one of the amplifications of the negative extraction control. This is shown in table 1 below.

Table 1. Summary of results obtained from negative & positive extraction controls

Barcode	D3	vWA	FGA	AMEL	D8	D21	D18	D5	D13	D7	Run
[redacted]	17,18	14,17	25,NR	X,X	11,13	30,32.2	13,14	11,11	8,12	10,11	9PLEX*
	NSD	14,NR	24,25	X,X	11,13	30,32.2	13,14	[redacted]	[redacted]	11,NR	AMP1CW**
	17,18	14,17	24,25	X,X	11,13	30,32.2	13,14	[redacted]	[redacted]	10,11	9PLEX*

* 9PLEX = initial amplification, ** AMP1CW = repeat amplification

The capillary electrophoresis batch that was under analysis at the time the potential contamination was noted was 3100CW20080228_01. At this stage the capillary electrophoresis batch was re-prepared to confirm the presence of the potential contamination and a re-amplification ordered. As soon as both of these actions confirmed the presence of a below threshold profile, AUSLAB batch audit entries were made against the extraction batch (CWIQEXT20080225_02) and AUSLAB specimen notes for every sample within the batch. The negative extraction control had an undetermined (zero) quantification value, indicating the amount of DNA transferred was very low.

The two samples, positive and negative extraction controls, were processed in adjacent well positions at each stage of the process up to completion of the first amplification of the negative extraction control. Therefore contamination of one into the other may have occurred at any stage from use of the STORstar, through extraction on the MultiPROBE® II, to processing of the quantification batch (including decapping and recapping) up to the addition

of DNA extracts during the preparation of the amplification batch on the MultiPROBE® II. At each of these st[redacted]extensive validation and large numbers of routine samples have been processed with no problems detected. It is therefore not possible to determine the exact point where the contamination has occurred. In addition, the level of transference has been very low. When considering the DNA concentration of the DNA extract from positive extraction control (2.59ng/µL), a very small amount of this DNA extract (approximately 0.25µL) may have been sufficient to have been transferred to the DNA extract of the negative extraction control to display the low level of DNA profile observed. This event has been documented in the FSS quality information system (QIS) as OQI#19349. In response to possible issues with the extraction procedure, a review is currently underway. It must be stressed that there has been no firm evidence of any actual problems with any particular part of the process at this stage. Some changes have been made to the processing of extractions since this event has occurred. The Slicprep™ 96 device and STORstar instrument are no longer used for the preparation of casework samples for extraction, in place samples are pre-lysed in individual tubes and the lysate is added to a 96-deep well plate and stored at -20°C prior to extraction on the MPII. The pre lysis (also known as off-deck lysis) necessitates the use of two positive and two negative extraction controls. This has the added benefit of increased monitoring of each batch.

[redacted]

[redacted]

[redacted]