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DNA ANALYSIS OF RESOMATION PRODUCT

SUMMARY

Resomation is a new process developed as an alternative to inhumation or cremation. Disposal is achieved using potassium hydroxide and water at a high pressure and temperature to reduce the body to bone ash and a solution of peptides, amino acids, sugars and salts.

Given the nature of the reaction, it is expected that the DNA from the corpse is broken down during resomation, and that no identifiable DNA is present in the remaining solution. Two samples of the solution produced by resomation were provided for DNA analysis to test this expectation.

Both samples were subject to the standard DNA quantification and profiling methods used for human identification in forensic science. No DNA was detected in either sample. No DNA profile was obtained from either sample.

SAMPLE MATERIAL

Two resomation samples were submitted to the SPSA Forensic Services Laboratory, Edinburgh. These samples had their pH adjusted to 8.5 by carbon dioxide prior to submission (see Appendix for method and results). In addition, a water control was provided to confirm that DNA contamination of the samples did not occur during the pH adjustment.

Approximately 900µl of solution was submitted for each sample and the water control. Three 200µl aliquots were taken from each, for processing of the samples in triplicate.

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METHODS

SPSA Forensic Services in Edinburgh processed the samples and the control using the standard DNA profiling procedures employed by the Laboratory for DNA profiling for casework samples. The processing of samples in forensic science casework consists of four processes:

- DNA extraction, using the Qiagen Blood Mini Kit (Cat# 51106);
- DNA quantification, using the Applied Biosystems Quantifiler Human Kit (Cat# 4343895);on the ABI Prism 7500;
- Polymerase chain reaction (PCR), using the AmpF/STR® SGM Plus® PCR Amplification Kit (Cat# 4307133);
- Capillary electrophoresis (CE), on the ABI prism 3130

The SPSA Forensic Science Laboratory in Edinburgh is accredited by the United Kingdom Accreditation Service to supply DNA profiles to the National DNA Database. All the methods used in this exercise are identical to those that have been accredited and are based on the published methodology for the above kits and instrumentation.

The DNA quantification method for detecting human DNA has a published sensitivity of at least 16pg/μl, with DNA routinely detected at concentrations as low as 6pg/μl during the validation study[1]. The instrumentation will report a DNA concentration of 1pg/μl or above, and the SPSA Forensic Science Laboratory has observed DNA concentrations approaching 1pg/μl (data not published). The software reports as "undetected" anything below this threshold.

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The SGM+ DNA profiling method used in the Laboratory is optimised for a total of 1ng DNA in 10µl (*ie* 100pg/µl) added to the reaction in order to obtain a full DNA profile, although the SPSA Forensic Laboratory has obtained complete DNA profiles from samples with considerably less total DNA added (data not published). A complete DNA profile would consist of 20 DNA alleles and one or two more at the sex marker, amelogenin. An incomplete DNA profile could consist of anything from just one to nineteen DNA types (with or without a result for amelogenin).

For samples with DNA concentrations approaching 1pg/µl, no reportable DNA profile would be expected, although indications of a profile might be obtained. The SGM+ profiling method was used in this exercise to confirm that no identifiable DNA profile could be obtained from the sample.

RESULTS

DNA Quantification using the Quantifiler Human Kit: the triplicates for both samples and the water control all gave a result of "undetected". If DNA had been present in the sample at a concentration of greater than 1pg/µl, then the software would have obtained a value for the DNA concentration.

DNA profiling using the SGM+ Kit: no DNA alleles were produced, and therefore no DNA profile was obtained from any of the triplicate samples processed.

DISCUSSION

As outlined in the method, the Quantifiler Human kit software will calculate and report a DNA concentration of just 1pg/µl. All replicates gave a result of "undetected", confirming that none of the samples had DNA present at a concentration greater than 1pg/µl.

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No DNA profile was obtained from any of the samples using the SGM+ method. This confirms the lack of DNA found by the quantifiler method, and provides reassurance that no DNA profile could be obtained from the resomation samples that could be used to identify the corpses.

CONCLUSION

DNA was not detected in the solution produced by resomation.

It is not possible to obtain even an incomplete SGM+ DNA profile, from the solution produced by resomation.

Signed: Steven Fegan Date: 9th July 2010

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REFERENCES

1. Green, R.L., Roinestad, I.C., Boland, C. and Hennessy, L.K. Developmental Validation of the Quantifiler Real-Time PCR Kits for the Quantification of Human Nuclear DNA Samples *J Forensic Sci*, July 2005, Vol. 50, No.4

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APPENDIX - RESOMATION SAMPLE PREPARATION PROTOCOL AND RESULTS

Materials

- Lab coat
- Powderless gloves
- Sealed sample
- CO_2 Canister + regulator
- Tubing (sterilised)
- Filter – sterile (0.45µm)
- Scissors
- Sterile centrifuge tube (x3)
- Storage rack
- Sterile H_2O for control sample in forensics container
- pH papers
- Pipette (1 ml Gilson)
- Pipette tips (1 ml sterilised)
- Waste container
- Marker pen
- Forensics Eppendorf tubes (sterile)
- Blue roll
- Zip lock bags

Methodology

Setup

1. Ensure that laminar flow cabinet is sterilised with UV light and ethanol wipe down if necessary.
2. Wear lab coat and gloves for all work.
3. Place materials in cabinet and set up CO_2 canister on table/bench near work area. Attach tubing to canister. Only connect sterile tubing and filter when ready to begin work. This should remain in cabinet for all work.

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4. Ensure centrifuge tubes and Eppendorfs are labelled appropriately before commencing work.

Sample treatment

H_2O sample to be processed first.

1. Take the sterile H_2O sample and using the sterile tubing, bubble CO_2 into the sample.
2. After 30 sec, using a new sterile tip, take an aliquot of sample (water) and place this on a pH strip. Take a note of pH. Discard tip.
3. Repeat the process until sample pH is 8.5. Seal sample and place in labelled zip lock bag.
4. Use scissors to cut off the tubing exposed to the water sample. Place tubing in waste.

Resomation sample to be processed second.

5. Using pipette set at 1.00 ml, transfer 15-20 ml of sample to first centrifuge tube. Repeat this for the second tube. Seal with lid until CO_2 is to be applied.
6. Ensure CO_2 flow rate is low. Apply CO_2 to the first sample. After 30 sec measure the pH using a fresh tip and pH strip. Note pH.
7. Repeat process until pH reaches 8.5.
8. Transfer between 2 ml of treated sample to Eppendorf tube, seal and store.
9. Cut off end of tubing that has been exposed to sample.
10. Repeat process for second sample.
11. Once all samples are processed and sealed, store Eppendorfs in fridge until delivered to lab. Keep the processed samples in the

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centrifuge tube in the fridge until instructed by forensics lab to dispose of.

12. Clean up laminar flow cabinet.

Results

Sample ID	Starting pH	End pH
Blank H_2O	5.5	5.0
Replicate 1	14	8.5
Replicate 2	14	8.5

- The blank sample was exposed to 15 sec of CO_2.
- Replicates 1 and 2 were exposed to 45 min and 35 min of CO_2 respectively to achieve a pH of approx 8.5.