**Strategies for DNA Analysis from Contaminated Forensic Samples**

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**Abstract.** The capability of a standard forensic DNA purification kit (Charge Switch Forensic DNA Purification Kit, Invitrogen, USA) to decontaminate DNA samples from radionuclides typical for nuclear security scenarios was investigated with the aim to achieve sufficient decontamination for further processing the purified DNA sample in a standard forensic laboratory. It could be shown that measurement of the radionuclide in the initial lysis solution in combination with the decontamination factor for the investigated radionuclide allows demonstrating compliance with the necessary clearance limits for the purified DNA samples. Thus, we propose a strategy for analysing R/N contaminated DNA samples by separating DNA using the well-established Charge Switch method in a nuclear laboratory and then transferring the DNA to a forensic laboratory for further analysis.

**1. Introduction**

Crime scene management and the analysis of forensic evidence get significantly more complex if radioactive contamination is present [1]. For analysis of classical forensic evidence, different strategies can be chosen such as establishment of a forensic laboratory which is licensed and equipped to handle radioactive materials or decontamination of evidence for subsequent analysis in a classical forensic laboratory [2, 3].

In this work, the capability of a standard forensic DNA purification kit (Charge Switch Forensic DNA Purification Kit, Invitrogen, USA) to decontaminate DNA samples from radionuclides typical for nuclear security scenarios (see Table I) was investigated with the aim to achieve sufficient decontamination for further processing the purified DNA sample in a standard forensic laboratory.

**2. Experimental**

A mixed radionuclide reference standard (QCY48, Nuclitec GmbH, Germany) was used for Am-241, Cs-137 and Co-60. A Po-209 reference solution was obtained from NIST (NIST SRM 4326). (NH4)2IrCl6 (Johnson Matthey, UK) and SrCl2.6H2O (Merck, Germany) were dissolved in MilliQ water to prepare stock solutions for Sr (25 mg/ml) and Ir (2 mg/ml). For Pu-242, a well characterized in-house standard solution (750 Bq/g in 8 M HNO3) was used.

Table I. Isotopes of greatest concern for radiological dispersal devices [4] and isotopes used in the present work.

|  |  |  |
| --- | --- | --- |
| Isotope | Common use | Isotopes measured |
| Am-241 | Well logging, gauges | Am-241 |
| Cs-137 | Medical, rad. sources | Cs-137 |
| Sr-90 | RTGs | Sr nat. |
| Po-210 | Static eliminators | Po-209 |
| Co-60 | Medical, rad. sources | Co-60 |
| Ir-192 | Radiography | Ir nat. |
| Pu-238 | RTGs | Pu-242 |
| Pu-239 | α or α,n sources | Pu-242 |
| Cm-244, Cf-252 | n sources | Am-241 |

An aliquot of tracer solution was added to 1 ml of lysis solution and after addition of 200 µl purification buffer to the sample, the pH was adjusted to pH 6 with dilute NaOH. The remaining purification procedure was done according to the manufacturer's instructions. 10 µl Proteinase K and 20 µl magnetic beads to bind the DNA were added, then the magnetic beads were fixed in the Eppendorf vial using a magnetic rack and the lysis solution was removed. The beads were washed twice with 500 µl wash buffer and finally eluted with 150 µl elution buffer. All solutions (lysis solution, washing steps and final eluate) were analysed for the concentration of the tracer initially added.

For Plutonium the enhancement of separation by complexation of Pu with F– and Diethylenetriaminepentaacetic acid (DTPA) in the lysis solution was also investigated. In this case, 26 µmol NaF or 26 µmol CaNa3DTPA (Heyl, Germany) were added to the lysis solution.

Strontium and Iridium were analysed with an Element 2 ICP-MS (Thermo Finnigan), Am-241, Cs-137 and Co-60 by gamma spectrometry with a BEGe detector (Canberra, 50% relative efficiency, with DSA-1000, calibrated with LabSOCSTM and evaluated with Genie 2000). Po-209 and Pu-242 were measured by LSC with Ultima Gold AB (PerkinElmer) liquid scintillation cocktail and alpha/beta separation using a Quantulus (PerkinElmer) liquid scintillation counter.

**3. Results**

A decontamination factor D was defined as

D = n(DNA) × n(lysis)–1 (1)

where

n(DNA) is the amount of tracer isotope in the DNA eluate (mole)

n(lysis) is the amount of tracer isotope in the lysis solution after DNA sorption (mole)

Decontamination factors are summarized in Table II. The final DNA eluate was found to contain a fraction from 5.6×10–2 for Ir to 2.4×10–5 for Sr of the activity of the lysis solution. For Plutonium, a smaller decontamination factor of 1.1×10–2 could be observed.

The separation of Pu could be enhanced by addition of 26 µmol F– or DTPA to the lysis solution. The results of these experiments are summarized in Table III.

Table II. Decontamination factors.

|  |  |  |
| --- | --- | --- |
| Isotope | measured | Decontamination factor D |
| Am-241, Cm-244, Cf-252 | Am-241 | 2.6×10–4 |
| Cs-137 | Cs-137 | 1.5×10–4 |
| Sr-90 | Sr nat. | 2.4×10–5 |
| Po-210 | Po-209 | 1.3×10–3 |
| Co-60 | Co-60 | 2.9×10–4 |
| Ir-192 | Ir nat. | 5.6×10–2 |
| Pu-238, Pu-239 | Pu-242 | 1.1×10–2 |

Table III. Enhanced Pu removal by complexation of Pu in the lysis solution.

|  |  |  |  |
| --- | --- | --- | --- |
| Experiment | Pu | Pu + F– | Pu + DTPA |
| Decontamination factor D | 1.1×10–2 | 2.6×10–3 | 1.3×10–4 |

**4. Discussion**

The feasibility of removing the isotopes of greatest concern for radiological dispersal devices by application of a standard forensic DNA purification kit could be demonstrated. Depending on the chemical element, contamination can be significantly reduced in the DNA eluate sample compared to the initial lysis solution. In case of Pu, addition of a complexing agent like DTPA to the lysis solution enhances decontamination.

The chemical elements studied in this work were added in dissolved form to the lysis solution, thus representing a worst case scenario for decontamination attempts as this would mean complete leaching of the contamination from the forensic sample during the initial lysis step. In real cases, an easily soluble contamination could be expected e.g. for Cs-137 (CsCl), in many cases the chemical form of the radionuclide used in radiation sources would be metal (e.g. Co) or ceramic (e.g. SrTiO3) [5].

The decontamination factors established in this work could also be used to determine the radionuclide content of the purified DNA sample from the analysis of the lysis solution for clearance of the sample for further processing in a standard forensic laboratory. This is especially important if direct non-destructive measurement of the purified DNA sample is not possible e.g. for Pu or Sr-90.

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