

Developing Traditional Forensic Science Exploitation of Contaminated Exhibits Recovered from a Nuclear Security Event

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Abstract. Forensic scientific support to a nuclear security event involving the malicious use of nuclear or other radioactive material outside regulatory control, will aim to maximise the information that can be obtained utilising nuclear forensic analysis and traditional forensic sciences. The latter is potentially challenging to achieve as traditional forensic science laboratories are not designed or designated to handle exhibits contaminated with nuclear or other radioactive material. Equally, analytical laboratories designated to support nuclear forensic analyses are not equipped with the instrumentation or the trained staff to undertake traditional forensic science examinations. The United Kingdom, in response to this technical challenge, has established a purpose-built facility, the Conventional Forensic Analysis Capability (CFAC) at a nuclear licensed site to enable the examination of items contaminated with nuclear and other radioactive material using a range of traditional forensic science examination techniques. The current activities within the CFAC laboratory are focussing on adapting and validating methods and procedures to be used in forensic science examinations in support of a nuclear security event.

1. Introduction

Since the early 1990s, nuclear forensic science has become increasingly recognised as an additional discipline to provide scientific support to law enforcement or equivalent investigations [1]. The development of the discipline was partly in response to illicit trafficking incidents involving nuclear or other radioactive material outside regulatory control, [2] and the potential asymmetric threat of the malicious use of such materials in a terrorism related incident [3]. Nuclear forensic science focuses on the analyses that provide elemental, chemical, isotopic and physical characteristics (e.g. morphology) to aid in the interpretation of the seized “questioned” nuclear or other radioactive material in support of the investigation. Equally important is the information that can provide the classical broader associative, investigative and event/incident reconstruction links of individual(s) to places, events and processes [4]. The ability to undertake traditional forensic science examinations would aid in these

interpretations [5]. Nevertheless it may not be possible to conduct routine examinations in a standard forensic laboratory environment safely, due to the items being contaminated with the nuclear or other radioactive material. As a result in the United Kingdom, the Office for Security and Counter-Terrorism (OSCT) within the Home Office funded the construction of a specialist laboratory at the Atomic Weapons Establishment (AWE).

This paper provides an overview of the method development and validation studies that have been undertaken in the Conventional Forensic Analysis Capability (CFAC) laboratory at AWE, for the safe handling and processing of exhibits or items recovered from a scene of crime involving nuclear or other radioactive material outside regulatory control.

2. An overview of the CFAC laboratory

The CFAC laboratory was designed around the concept that forensic scientists and practitioners from a traditional Forensic Science Service Provider (FSP), Police and other specialist forensic science laboratories, having undertaken suitable training, would perform the examinations on the exhibits contaminated with nuclear or other radioactive material with technical (i.e. glove box operations), radiological monitoring and protection advice provided by AWE specialists.

To ensure that the CFAC laboratory was designed to meet the required technical specification of the various traditional forensic science disciplines, forensic scientists and practitioners from the organisations who would operate in the CFAC laboratory (e.g. Forensic Access Ltd and the Forensic Services Directorate within the Metropolitan Police Service) were involved in the design phase [6]. The housing of multiple disciplines in a single environment generated a number of challenges, such as ensuring that adequate examination capability was provided for each, whilst acknowledging limitations due to the size of the laboratory. For example, there are a number of techniques that can be utilised to aid in the development of a fingerprint, from vacuum metal deposition to using chemical dyes (e.g. SOLVENT BLACK 3 [7]) however, it would not be possible to incorporate all of these techniques into the CFAC laboratory. As a result, and based on technical advice from the forensic science practitioners, it was determined that the CFAC laboratory should be able to enable fluorescence examination, cyanoacrylate vapour, ninhydrin and 1,8-Diazafluoren-9-one (DFO) treatments of exhibits as a minimum.

Two purpose-built glove box units form the main examination capability within the CFAC laboratory. This follows a similar approach to other nations who have developed support to traditional forensic science examinations [8]. The CFAC glove box units have incorporated a dedicated chamber for cyanoacrylate vapour treatment, a digital microscope, fluorescence illumination using a Coherent® TracER 532nm (green) wavelength laser or a 577nm (yellow) wavelength laser depending on which glove box is used, and the ability to download data from cellular devices, Fig 1.

The CFAC laboratory at AWE incorporates the traditional forensic examination requirements for: record photography; fingerprint development; digital data recovery from cellular and other electronic devices; DNA sampling; trace evidence recovery and characterisation; questioned document analysis and physical investigations for explosive-related examination. This is done whilst ensuring that there is no compromise in terms of the radiological safety and operating requirements for a designated radiological controlled laboratory on a nuclear licensed site, Fig. 2.



FIG 1. One of the glove box units housed within the CFAC laboratory. The digital microscope for imaging and the integrated Coherent® TracER laser system used to support fingerprint development are visible in the image. Insert shows the integrated chamber for the cyanoacrylate (CNA) vapour treatment.



FIG 2. An overview montage image of the CFAC laboratory which includes some of the capabilities that would support the traditional forensic science examinations of exhibits contaminated with nuclear and other radioactive material.

3. Validation studies of traditional forensic science methods to be undertaken in the CFAC laboratory

Although the CFAC laboratory was built around the requirement of traditional forensic scientists and practitioners, it still provides a unique operating environment where it is not possible to directly transfer methods and procedures used in the standard forensic laboratories. Therefore the traditional examination methods require adaptation or simplification for use in the CFAC laboratory. The adaptation could be due to the practical limitations of working in a glove box, such as dexterity. Alternatively it may be safety requirements imposed for working in a radiological controlled environment such as removing the potential for a contaminated wound or cut hazard caused by the use of a sharp-edged tool while undertaking an examination, e.g. tweezers for trace evidence recovery. Once the various methods have been adapted for use in the CFAC laboratory, then they must be validated to enable the production of a series of standard operating procedures that can be accredited and used in support of legal proceedings, Fig. 3.

The range of examination techniques that can be undertaken in the CFAC laboratory, specifically in the glove box units, would normally in a standard forensic facility be undertaken in different laboratory areas. Therefore a significant component of the validation work is to develop and subsequently implement suitable environmental monitoring and laboratory cleaning protocols to reduce the potential for forensic cross-contamination.

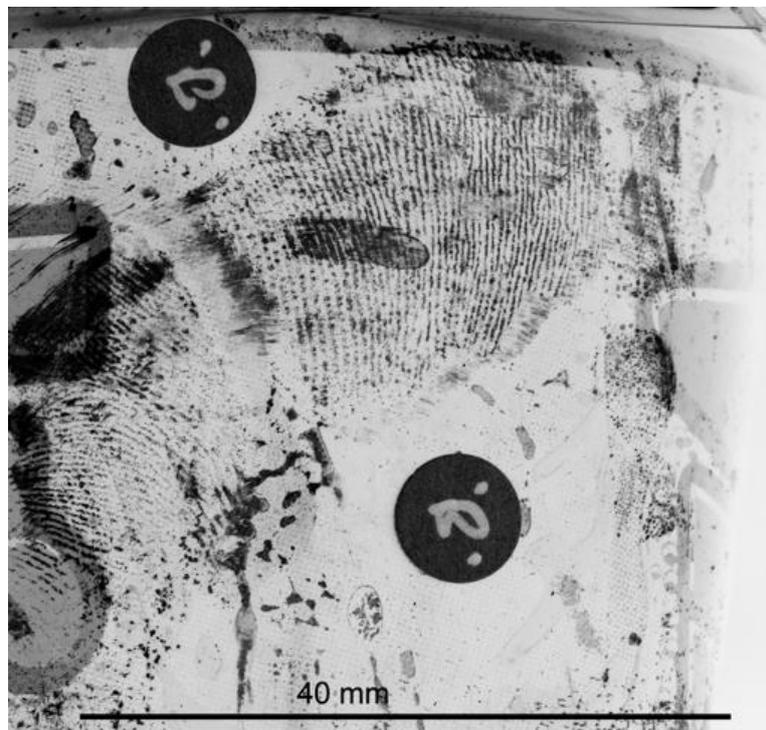


FIG 3. A digital photograph of a fingerprint identified on an exhibit after being processed in the glove box as part of the validation process.

It is likely that a question on the impact of radiation exposure on evidence would be raised during defence council cross-examination of the forensic scientist or practitioner if the investigation leads to a criminal prosecution. Thus an important aspect of the validation studies is to develop an understanding of the potential effects of radiation exposure on forensic evidence, such as the potential colour degradation of hairs or fibres, or the inability to develop a suitable fingerprint from an exhibit [8]. As a result there is a current on-going research effort to study the effects of radiation exposure on evidence, with the initial focus on DNA [9]. Equally important is the need to understand the effects of decontamination [10], which again the current validation studies are exploring, with some preliminary work relating to questioned documents.

In support of the validation studies, the glove box trained forensic scientists and practitioners undertake mock casework examination exercises to demonstrate competence to operate in a radiological controlled environment, Fig. 4. These exercises aim to test the totality of tasks undertaken in the CFAC laboratory, including the application of the validated methods to examine an exhibit; administrative paperwork (such as evidence continuity and the examination strategy / plan); interactions with the radiological protection advisors.



FIG 4. One of the forensic science practitioners trained to operate in the CFAC laboratory demonstrating the ability to perform a taping procedure of an exhibit in one of the glove boxes during a mock casework exercise.

4. Applying the findings from nuclear forensics analysis to traditional forensic science interpretations

Beyond the traditional forensic science examination techniques that can be applied in the CFAC laboratory, it is equally important to utilise the findings from the nuclear forensic analysis that might provide additional context or interpretation. Particle analysis techniques developed for nuclear safeguards environmental sampling and nuclear forensic science [11] can support the traditional forensic interpretation in a similar way to that used for classical trace forensic science. For example, the identification of firearms discharge residue particles (more commonly referred to as gunshot residue (GSR)) provides potential information to an incident involving the use of a firearm [12]. Using that interpretative philosophy, the linkage of an individual to a scene or activity could be supported by the identification of nuclear or other radioactive particulates sampled from exhibits such as clothing or a disposable glove recovered from a crime scene, Fig. 5.

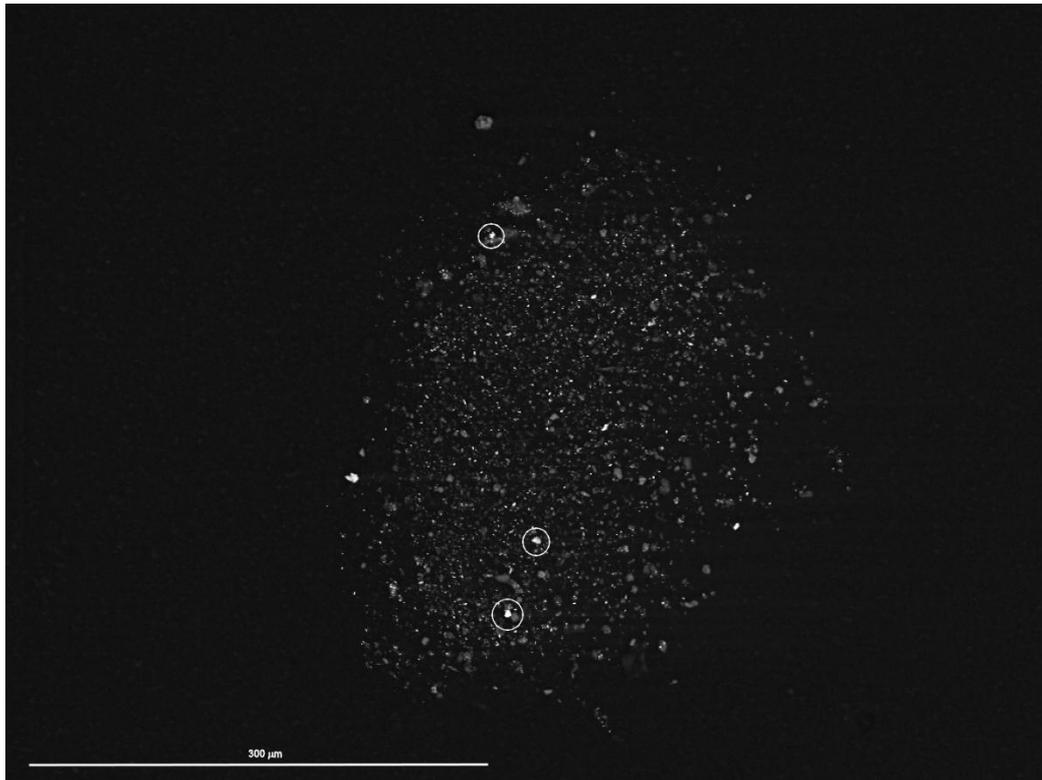


FIG 5. A backscattered electron image of the surface of the carbon adhesive disk mounted aluminium specimen stub that has been used to sample a disposable glove, to mimic potential for particulate transfer if the glove had been worn during an activity or process using nuclear or other radioactive material outside regulatory control. The brightest particles (denoted by the circles) identified in the image would then be subjected to further analysis due to the likely presence of high atomic number elements such as uranium (scale bar is 300 micrometers).

5. Future work

On completion of the validation and method development work for the traditional forensic science procedures to be undertaken in the CFAC laboratory, the next stage will be to gain accreditation to the international standards that apply and are expected to be held by laboratories undertaking forensic science examinations [13]. Furthermore, in the United Kingdom, the Forensic Science Regulator within the Home Office has developed a Code of Practice and Conduct to which, it is expected that FSPs and forensic science practitioners adhere [14].

6. Summary

The scientific support to the investigation following a nuclear security event may include all aspects of forensic science. In addition to the analytical capabilities at AWE for the analyses of the nuclear and other radioactive material [15], the United Kingdom through OSCT within the Home Office has funded the development of a dedicated facility to support traditional forensic science examinations. The CFAC laboratory enables the safe handling and forensic processing of exhibits contaminated with nuclear or other radioactive materials and small quantities of explosive material. The current ongoing method development and validation studies will ensure that the laboratory is able to undertake examinations to the expected standards of the United Kingdom Criminal Justice System.

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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). $(\text{NH}_4)_2\text{IrCl}_6$ (Johnson Matthey, UK) and $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ (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 HNO_3) was used.

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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 μ l 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 μ l Proteinase K and 20 μ l 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 μ l wash buffer and finally eluted with 150 μ l 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 μ mol NaF or 26 μ mol 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

$$D = n(\text{DNA}) \times n(\text{lysis})^{-1} \quad (1)$$

where

$n(\text{DNA})$ is the amount of tracer isotope in the DNA eluate (mole)

$n(\text{lysis})$ 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 $26 \mu\text{mol F}^-$ 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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Translating Research Findings into Operational Capabilities in Nuclear Forensics: The Australian Experience

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Abstract. Research has demonstrated that some evidence types, such as DNA, fingerprints and digital devices, may yield valuable investigative information in spite of ionising radiation exposure but may not be amenable to decontamination. The investigation of a nuclear security event may involve the examination of evidence contaminated with radionuclides. And thus the development of protocols for its safe handling is important. Given the frequency of such events and competing operational and fiscal demands, law enforcement forensic laboratories are disinclined to develop and maintain these capabilities. Equally, it may not be viable for a nuclear or radiological facility to establish full forensic capabilities. Australia has sought to meet this requirement through a collaborative partnership between the Australian Nuclear Science and Technology Organisation's (ANSTO) Nuclear Forensic Research Facility (NFRF) and the Australian Federal Police (AFP). Under this arrangement AFP forensic scientists undertake examination of evidence in the NFRF and NFRF staff, who have experience in both forensic science and nuclear science, form a key part of the analytical team by serving as the 'interface' between these disciplines. ANSTO and the AFP have developed capabilities vital to Australia for the handling of forensic evidence contaminated with radionuclides.

1. Introduction

The Australian Nuclear Science and Technology Organisation (ANSTO) is home to the Nuclear Forensic Research Facility (NFRF), which was commissioned in 2009. The NFRF is the central hub for nuclear forensics in Australia and possesses the unique capabilities required to undertake nuclear forensic examinations in support of investigations. Such capabilities include facilities for handling radioactive and nuclear materials and evidence contaminated with radionuclides, access to a broad range of analytical services, staff with training and experience in fields ranging from radiochemistry to forensic science, and subject matter expertise for data interpretation.

The role of the Australian Federal Police (AFP) is the enforcement of Commonwealth criminal law in Australia and the protection of Commonwealth and national interests from crime in Australia and overseas. The AFP also provides community policing services in the Australian Capital Territory, Jervis Bay, the External Territories and at Australia's major airports. The AFP's Forensic portfolio provides a range of standard and innovative forensic science and technical intelligence services to support national and international operations, regional capacity building and community policing activities. ANSTO and the AFP have a long-standing relationship which includes research collaborations, training and development and exercise facilitation and participation. This relationship has been formalized by a Memorandum of Understanding.

Over a number of years, the NFRF has undertaken research to explore the effects of ionising radiation on forensic evidence and the handling of exhibits contaminated with radionuclides [1-6]. This research has demonstrated that some evidence types, such as DNA and fingermarks, may yield information in spite of radiation exposure but are not amenable to decontamination. Since these evidence types can provide valuable operational intelligence and/or highly probative evidence for court proceedings, the development of capabilities in the safe handling and examination of forensic evidence contaminated¹ with radionuclides is an important step in preparing to respond to and investigate a nuclear security incident.

The International Atomic Energy Agency (IAEA) notes that the examination of forensic evidence contaminated with radionuclides “presents a unique challenge” [7]. Three options were considered in Australia for the carriage of capabilities in the safe handling and examination of forensic evidence contaminated with radionuclides; sole carriage by law enforcement such as the AFP, sole carriage by a radiological or nuclear facility such as ANSTO or joint carriage by law enforcement and a nuclear research facility. The sole carriage of the capabilities by law enforcement or a radiological or nuclear facility, given the requirements for establishment of the infrastructure, training and development of personnel and implementation of a comprehensive regulatory framework, is not feasible given other demands on resources. However joint carriage, utilising the existing capabilities of each organization, is a viable option and in this spirit a series of projects have been undertaken under the Memorandum of Understanding to develop this joint capability. This paper will describe in greater detail the technical aspects of this project, such as the modifications required to make the infrastructure at a nuclear research facility suitable for forensic examinations and the training required for AFP forensic scientists to work in a radiation environment. It will also describe the lessons learned through this project and plans for on-going collaboration.

2. Work to date

2.1. Initial glove box set up

As a component of previous research, two glove boxes in the NFRF (shown in *Figure 1*) were custom fitted for the decontamination and examination of forensic evidence. The purpose of the glove boxes, which operate at negative pressure, is two-fold. The glove box provides an atmosphere for the sample separate from that in which the user operates, providing protection against internal contamination with radionuclides. Furthermore, the glove box serves to protect forensic evidence from DNA or trace material contamination when the provision of more conventional measures (such as designated clean laboratories) for radionuclide contaminated evidence may not be cost-effective.

¹ The term “contamination” may have two meanings in the context of this work, an issue previously identified by the international community [7]. In nuclear science, “‘contaminated evidence’ refers to the presence of radioactive material on or within physical evidence” [7]. In forensic science, “‘contaminated evidence’ refers to the direct or indirect transfer of extraneous material to a forensic sample or scene of a crime”[7]. In this paper, the terms “contaminated” or “contamination” will refer to evidence contaminated with radionuclides, whilst more specific descriptions such as “DNA contamination” will be used as appropriate.

1. Air intake
2. Exhaust
3. Pressure gauge
4. Air-lock portal
5. Windows
6. Glove portal
7. General purpose outlets
8. Lighting
9. Laptop
10. Camera
11. Polilight™

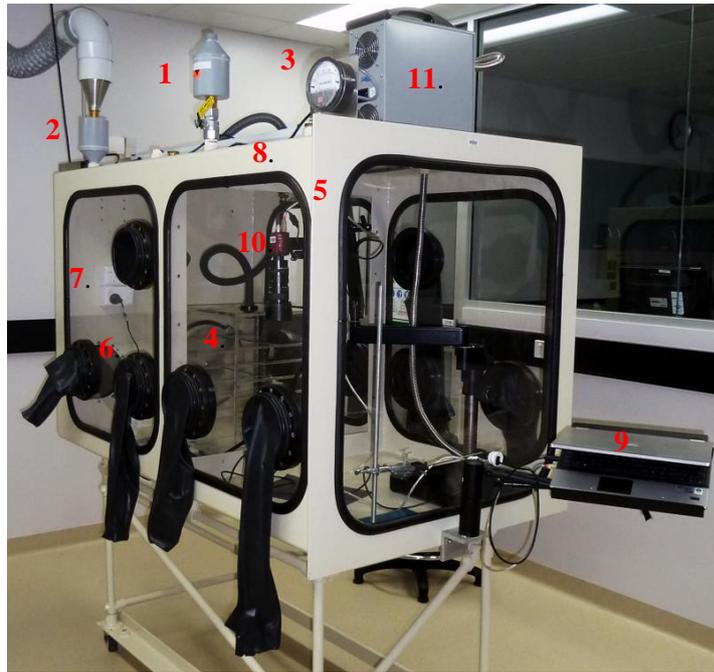


Figure 1 Glove box with fixtures and fittings labelled

Image capture was identified as a high priority for both glove boxes. Images of submitted samples need to be collected for integrity, reference, and evaluation of samples. It is preferable to image contaminated samples prior to processing, as well as utilising a camera for recording developed and enhanced fingermarks. A camera system was installed inside each glove box comprising a small (29 x 44 x 74 mm) FireWire colour camera (Stingray F201C, Allied Vision Technology) with a macro zoom lens (LMZ69M, Kowa) attached to a flexible arm on a heavy base stand (MA242201, View Solutions). The FireWire connection was fed externally from the glove box and connected to a laptop. The small camera was valuable in a space-constrained environment, with the flexible arm enabling allowing a high degree of manoeuvrability and control. Lighting for forensic imaging was achieved through the permanent inclusion of a light guide connected to a suitable light source. This allowed the Polilight® (model PL500; Rofin Australia), a variable wavelength high intensity light source designed for forensic purposes housed externally to the glove box for maintenance and space saving reasons.

One glove box was equipped for fingermark development on both non-porous and porous surfaces. Standard procedures for these techniques were followed but conducted in the glove box rather than as a bench-top procedure which is more typical. On non-porous surfaces such as plastic or glass, one of the most common techniques for the development of fingermarks is cyanoacrylate fuming. The cyanoacrylate (superglue) vapours react with lipid components of the fingermark, producing a hard white polymer which can subsequently be visualized by applying a fluorescent stain such as Rhodamine 6G. A customized cyanoacrylate-fuming chamber was built and introduced to the glove box prior to the final window being fitted. This set-up allows the fuming chamber to be independently ventilated through a DeFumigator™ cyanoacrylate fume extractor (FR300, Sirchie®). Noxious odours and fumes are extracted, assisting in limiting excess build-up of solid cyanoacrylate on the internal surfaces of the glove box. The heat source is a USB powered heating block that provides sufficient heating loads to vaporize the cyanoacrylate. On porous surfaces such as paper, chemical reagents which react with the amino acid components of fingermarks are most commonly used for fingermark visualization. Among these reagents is indanedione-zinc, which upon heating will form a fluorescent product. The standard bench-top procedure uses an ironing press to heat the sample, which was replaced with a commercial hair straightener due to the size constraints of the glove box.

The second glove box was equipped for DNA extraction. The first stage of forensic DNA analysis is extraction, in which the DNA is separated from the substrate and other cellular components. Research has demonstrated that two commercially available extraction kits, the DNA IQ™ System (Promega Corporation) and ChargeSwitch® System (Invitrogen), both of which utilize solid phase extraction

principles, are effective in extracting DNA away from radioactive contaminants [4]. The resultant radionuclide-free DNA extract can then safely be analysed at a conventional forensic laboratory. These findings led to one glove box being fitted with the tools needed to undertake these extractions such as a heat block, microcentrifuge and vortex.

2.2. Training and personal dosimetry program

A custom training program has been developed to prepare AFP staff to undertake evidence examinations in the NFRF glove boxes. This training incorporated standard ANSTO training, such as a site safety induction and face-to-face basic radiation safety training, as well as training specific to the NFRF including a laboratory induction and glove box training. All of the components of this training program are captured in the ANSTO Business Management System, which is certified to ISO 9001. It is intended that these training elements will form, in conjunction with discipline-specific requirements, a casework authorization (approval to independently undertake a specific type of examination on evidentiary samples, gained through training and demonstration of practical competence) for the handling of forensic evidence contaminated with radionuclides in each forensic discipline.

During the development of the training program it was identified that two levels of glove box training, including the topics summarised in *Table 1*, are required:

- Glove Box User training is required for those who will be undertaking operations in the glove boxes, and thus is the level of training required for AFP staff to undertake examinations in the glove boxes.
- Glove Box Operator training is required for those who will be undertaking and supervising operations in the glove boxes and conducting maintenance of the glove boxes and is restricted to NFRF staff.

Table 1 Training topics for Glove Box Users and Operators

| Glove Box User | Glove Box Operator |
|--|---|
| — Pre-use inspection | All Glove Box User topics and: |
| — Contamination testing for glove integrity | — Posting out of waste and other items through bag port |
| — Routine contamination monitoring | — Changing gloves |
| — Posting in | — Changing waste bag |
| — Emergency response (ventilation failure, fire, pinhole or major tear in glove) | — Changing filters |

In addition to training, AFP staff who are to undertake examinations of contaminated evidence must be enrolled in ANSTO’s personal dosimetry program. A baseline internal dosimetry measurement is undertaken, with further monitoring to be scheduled on an as-needed basis. Individual thermoluminescent dosimeters (TLDs) have been issued to the AFP staff authorized to work in the NFRF, and are part of the internal dosimetry program managed by ANSTO.

2.3. Digital evidence

The first forensic discipline to develop operational protocols for the handling of evidence contaminated with radionuclides was digital forensics. Previous work, undertaken in the context of decontamination of evidence exposed to bioterrorism agents, has demonstrated that it may be possible to extract useful data from consumer electronics exposed to gamma radiation [8]. Digital evidence, which covers an enormous range of items such as mobile phones (also known as cell phones), computers, tablets, external data storage devices and personal GPS units may, as well as being valuable during investigations and prosecutions, be a key source of intelligence to prevent or mitigate further incidents.

A key step in a digital forensics examination is the transfer of data from the evidential device to a computer for further analysis. This occurs via a write blocker, which prevents any changes to data on the device, to preserve the integrity of the data. If the device is contaminated, it may be necessary to contain it in a glove box. However, it is not practical to also contain the write blocker and computer for reasons such as space and handling constraints, expense, the difficulty of keeping equipment updated and the need undertake forensic data capture and preservation so that the data can be further analysed in a digital forensics laboratory. Thus, it was necessary to develop an interface which could be used to export data from a device within the glove box via a write blocker to a computer outside the glove box. The requirements of ANSTO and the AFP for this interface which were considered during the design process are summarized in *Table 2*.

Table 2 Requirements for the design of the glove box digital evidence interface

| ANSTO Requirements | AFP Requirements |
|---|---|
| — Maintains negative pressure of the glove box during normal operations and any required change of components | — Provide flexibility to accommodate all common cable types |
| — Does not compromise the integrity of the glove box window to which it is fitted | — Utilizes cables >1.5m long |
| — Is adaptable over time without major modification if new types of cables are developed | — Includes only commercially supplied cables and components |
| — Can be easily used by glove box operators, including change of components | — Does not disrupt signal integrity |
| — Doesn't interfere with other operations in the glove box | — Addresses the possibility of cable failure by redundancy or the ability to replace cables |
| — Can be constructed from commercially available components | |

A range of options were considered, from commercially available high-vacuum data feed-throughs to fixing cables in high vacuum Klein Flange (KF) or ConFlat (CF) feed-throughs using resin to fully custom-built systems. However, the requirement which ultimately came to have most weight in selecting the most appropriate system was the need to accommodate a wide range of cables (twenty one different cables were considered in the initial design phase) and future cable forms as consumer electronics technology evolves. Thus, flexibility of the system became a key feature.

The final design, shown in *Figure 2*, is remarkable for its simplicity. Cables are threaded through a length of PVC conduit and fixed in place with resin. This conduit is then passed through a cable gland to secure it though the glove box window. As the opening of the cable gland is relatively small, an experienced operator can replace the conduit without compromising the negative pressure of the glove box by utilising the ventilation controls. All components are commercially produced and are readily available and easily used, meaning that a feed-through for a new cable type could be fabricated within hours if required during a casework examination.



Figure 2 Glove box feed through for digital evidence cables

Other challenges in the examination of contaminated digital evidence were also identified. The first was the size of the glove box port – it would simply not be possible to transfer a desktop computer tower or large laptop computer into the glove box. This can be overcome through in-field removal of the hard drives from their casings; although this approach is generally not preferred it may be required in these circumstances. A further issue was the use of touch-screen devices, which are largely unresponsive when wearing neoprene glove box gloves. This was readily overcome by the use of a stylus. Other fine manipulation of components and tools in the glove box was found to be challenging, an issue which can ultimately only be overcome through experience and practice. A final challenge, which remains outstanding, is that of getting an active mobile network device into a forensically safe state (i.e. SIM removed, in airplane mode, or turned off) inside the glove box. Items can be packaged for transport in such a way that they are shielded from radiofrequency signals, which will prevent their connection to the network. However when this packaging is removed the device may connect to the network, allowing remote alteration or deletion of data, before it can be put in a forensically safe state. In conventional digital evidence examinations this is overcome by the handling of the device at this stage in a Faraday box; however, this is clearly not feasible when the device has to be handled in a glove box. Solutions such as the construction of a glove box which acts as a Faraday box, the conversion of the laboratory structure into a Faraday box or erecting a temporary Faraday cage around the glove box and users as required were determined not to be possible. Various options, including the use of a broadband radiofrequency noise generator (“mobile phone jammer”), femtocell² or Faraday tent within the glove box, are currently being considered, although all pose regulatory and/or practical challenges.

3. Planned Work

3.1. Further training

The training and personal dosimetry programs described in Section 2.2 will continue to be implemented as new forensic disciplines work to develop protocols for in-glove box examinations, with refresher training provided as appropriate. In addition, it has been identified that there is a need to extend training beyond those analysts who will work in the NFRF laboratories. Training is to be provided to enhance the confidence of other AFP Forensics staff (such as forensic biologists,

² A femtocell is a small low-power cellular base station which will connect to a service provider’s network via broadband. Mobile network devices in the vicinity will connect to the femtocell in preference to the broader network, and the femtocell can in turn be configured to not pass these connections to the broader network, isolating the mobile network device from the network.

fingerprint examiners and forensic exhibit registry staff) who may be involved in the handling or examination of evidence arising from a nuclear security event. Two distinct audiences have been identified:

- Analysts receiving samples, such as DNA extracts, which have been decontaminated in the NFRF
- Analysts receiving samples exposed to radiation but not contaminated.

Custom training will be developed and delivered by ANSTO's Radiation Protection Advisors, in collaboration with NFRF staff, focusing on areas of radiation safety applicable to these audiences. For example, those handling decontaminated samples will be educated about the ways in which radiation can be measured and thus decontamination effectiveness can be verified, whilst the focus for those handling samples exposed to radiation will be the distinction between radiation exposure and contamination.

3.2. Operational implementation of fingermark and DNA examinations

Plans have been devised for the development of operational capabilities for the examination of contaminated fingermark and DNA evidence. This may require the development of new work practices; for example, fingermark examiners and biologist generally work alone on a given exhibit but experience has demonstrated that a team of two examiners working opposite each other is most efficient when operating in the glove box environment.

As previously noted in Section 2.1, the two glove boxes were equipped for examination of fingermarks and DNA at the time at which they were originally retrofitted. However, after consultation with operational law enforcement a number of modifications have been undertaken. The first is an upgrade of the camera system, installing a system identical to that used in the AFP laboratories (Nikon D600 with Nikon AF-S Micro 60mm f2.8G ED (1:1) lens, run with Nikon Camera Control Pro 2). This will have several benefits; image quality will be equal to that produced by the AFP laboratories, the training required for operators will be significantly reduced as they will already be familiar with the system, and the new system is more user-friendly with features such as auto-focus. In addition, the camera stands have also been modified, eliminating the need for a heavy base by affixing them to the base and roof of the glove box. This has created more floor space in the glove box, which has been identified as a need during method development.

Further modifications are also planned. In addition to the still cameras, video cameras are to be fitted to both glove boxes, with feeds to screens situated outside the laboratory. These will allow forensic experts who are not trained in glove box operations to observe and provide feedback and assistance during complex examinations, enable oversight of examinations by investigators and assist with the implementation of As Low As Reasonably Achievable (ALARA) radiation protection principals by allowing non-essential personnel to be located outside the laboratory. Finally, ultraviolet (UV) lights are to be installed in both glove boxes and the cyanoacrylate fuming chamber. UV lights are commonly used in forensic laboratories, including the AFP, for the DNA decontamination of rooms and fingerprint fuming chambers. The UV lights will be fitted into quartz sleeves attached to the glove box, allowing their easy removal for maintenance without compromising the glove box integrity.

DNA decontamination poses a challenge which may not be addressed by UV lights alone. A major problem with their use as a decontamination tool is shadowing; that is, any areas in shadow will not be decontaminated. An alternative means of decontamination is wiping surfaces with a disinfectant such as hypochlorite; however, this technique will also be limited by the reduced flexibility and dexterity of glove box operators. Given these issues, it has been determined that an alternative sample environment is required for the search stage of DNA analysis, where an area of interest is isolated from a large item (e.g. a blood stain on an item of clothing), which is the stage when the risk of contamination of items and/or the work environment is greatest. The proposed solution is the use of a disposable glove bag (CaptAir Pyramid, erlab) customized with a bag out port. This will allow the searching and sub-sampling of items to be done in the glove bag, with samples placed into microcentrifuge tubes and bagged out. The glove bag, still containing the larger item of evidence, can then be stored. The extraction of the sample, a stage at which the risk of DNA cross-contamination between samples is lower, can then be conducted in the glove box. This transfer from a glove bag to a glove box is

necessary as there is not sufficient space to accommodate the tools or manoeuvrability within the glove box to complete the extraction process. Although the risk of contamination is lower at this stage, vigorous decontamination protocol utilizing both the UV lights and hypochlorite solutions will be implemented.

A further requirement for the handling of DNA samples in the glove boxes has been the implementation of biosafety protocols in the NFRF as ideally human specimens should be handled in a Physical Containment 2 (PC2) laboratory [9]. Many of the engineering controls required for laboratories handling radioactive material meet or exceed those required for a PC2 laboratory, so major structural changes were not required. However, significant new procedures for the handling of these materials, waste management and spill clean-up were implemented, changes were made to personal protective equipment, staff will undergo training and a health management program (including relevant vaccination) will be applied to all staff that may have contact with these samples. All changes were made in accordance with best practice guidelines (such as Australian Standards) and based on careful risk assessment of relevant activities.

3.3. DNA research

As noted in Section 2.1, previous research conducted in collaboration with the NFRF has demonstrated that the commercially available DNA IQ™ System and ChargeSwitch® System extraction kits are effective in removing radioactive contamination from DNA samples [4]. However, this research was limited in scope, considering only caesium-137. More recent research has demonstrated that the ChargeSwitch® System is also effective at removing americium-241, cadmium-109, cobalt-60, uranium and thorium from DNA samples [10]. However, the combined body of research to date does not cover the full range of radioisotopes of security concern, nor has the extraction kit currently used by the AFP, the QIAamp DNA Investigator Kit (Qiagen), been assessed for its effectiveness in removing radioactive contaminants from forensic DNA samples. Thus it was identified that to support the development of operational capabilities for extraction of DNA from contaminated forensic evidence, research should be done to validate the suitability of the QIAamp DNA Investigator Kit for decontamination of DNA samples across a broad range of radioisotopes. Validation studies such as these are vital in ensuring the reliability of forensic evidence and its defensibility in court. The DNA IQ™ System and ChargeSwitch® System will also be included in this study in order to facilitate comparison with previous work and to offer operational alternatives should the QIAamp DNA Investigator Kit be found unsuitable.

The general study design is as follows:

- A known quantity of blood, fixed on filter paper, will be spiked with a standard radioisotope solution. The radioisotopes to be included in this study are those previously identified in the literature as being of security concern: americium-241, cobalt-60, caesium-137, iodine-131, iridium-192 and strontium-85 (a gamma emitting surrogate for strontium-90) [11]. Samples will be prepared in combinations of high and low DNA and radioisotope concentrations. Appropriate blanks will also be prepared to quantify background radiation levels in the samples.
- The DNA will be extracted from samples using manual protocols (i.e. without automation) for the QIAamp DNA Investigator Kit, DNA IQ™ System and ChargeSwitch® System.
- Gamma spectrometry will be performed on extracts to quantify the residual radioactive contamination within samples.
- If radioactivity levels are below safety thresholds, quantification and profiling of the DNA will be performed by the AFP in their conventional forensic laboratories.

4. Lessons Learned

4.1. *Understanding of organizational cultures*

This project has required and continues to require extensive collaboration between ANSTO, a research organization, and the AFP, a law enforcement organization. Research and law enforcement cultures differ in innumerable ways, and an understanding and acceptance of these differences was vital to the success of this project. An important tool for acquiring this cultural understanding is cross-training; as previously described in Section 2.2 AFP staff participated in ANSTO safety and radiation protection training, and in addition a NFRF staff member undertook the AFP's forensic induction training. The existence of a Memorandum of Understanding between the two organizations, and the Project Arrangements developed under it, provides a formal structure which guided relationships and communications between the organizations. Finally, the participation in the project of staff from both agencies with long-term collaborative relationships is of benefit.

4.2. *Potential applicability to non-nuclear security applications*

In considering the value of this capability, it became apparent that the ability to handle forensic evidence contaminated with radionuclides may be of value in the investigation of a range of incidents beyond those traditionally considered "nuclear security events" (although they may technically be such). Of the two main "branches" of nuclear forensics – the examination of radioactive or nuclear material and examination of contaminated evidence – the latter is more likely to be called upon in such cases. Radioactive materials are becoming more widespread as more applications are discovered and developing countries become increasingly industrialized, and thus more likely to be encountered by law enforcement. Examples of such incidents which may require this capability are:

- The death of an individual who has undergone brachytherapy [12]
- Violent crime involving a victim who has recently had a nuclear medicine procedure
- An offence at a nuclear medicine clinic
- An accident during the transport of industrial or medical radioisotopes
- Radioactive or nuclear material encountered incidentally at a crime scene [13].

In considering this potential broader range of applications, it becomes clearer that the capability to handle forensic evidence contaminated with radionuclides is of importance to law enforcement.

4.3. *Importance of a diverse project team*

The diverse range of activities required to deliver the outcomes of this project could not be achieved by scientists from one discipline, or even by scientists alone. At the core of the project team is staff that have expertise in both nuclear science and forensic science, who act as an 'interface' between these two, at times, divergent disciplines. However, this is supplemented by forensic scientists from law enforcement, ensuring operationally validated methods are applied that would withstand court testimony. The NFRF is also supported by a wide range of services which provide support across ANSTO, as a nuclear organization, at large; health physics, workplace health and safety, training, regulatory affairs, security and engineering are all crucial to the success of this work. Making use of these existing capabilities also significantly reduces the fiscal, personnel, infrastructure and regulatory demands of this project. However, it remains important for individuals within each organization to be delegated responsibility for coordination of their organization's capability development activities in order for projects to proceed in a timely manner.

4.4. *Value of hands-on experience*

A key component of this project was creating opportunities for AFP staff of all levels to get hands-on experience conducting forensic examinations in the glove box environment. This allows the full spectrum of staff, from senior decision makers to operational teams, to gain a greater appreciation of the importance of training and experience in this environment. This is far more effective than simply describing the limitations on dexterity and flexibility and ergonomic challenges posed by the glove

box. It was recognized that implementation of *ad hoc* procedures in response to an incident may cause significant safety hazards and regulatory non-compliance as well as compromise the integrity of the evidence.

4.5. Accepting limitations

The project seeks to provide a facility for the forensic examination of radionuclide contaminated evidence. Currently capability is focussed on the three highest demand forensic services, namely digital forensics, fingerprints and DNA analysis. All of these disciplines have approached this project with the understanding that the limitation of space, dexterity and access within the glove box will limit the range of procedures that can be undertaken and will require modifications from standard operating procedures, for example, whilst DNA extractions are now automated in most forensic laboratories manual protocols are necessary in the glove box due to space. Hence, in their development of procedures, disciplines have focussed on key procedures and/or those of highest strategic value.

4.6. Need for common language

Working at the interface of two scientific disciplines, nuclear science and forensic science, means that there is often no common language. At times, as illustrated in this paper by the word “contaminated”, the same word may have very different meanings. International efforts are moving to combat these challenges; for example, the Nuclear Forensics Lexicon which formed part of the Netherland’s gift basket at the 2014 Nuclear Security Summit is now widely available as a smart phone application. However, practitioners from both disciplines must work together on a smaller scale to develop a workable vocabulary, as well as be alert to the risk of miscommunication which may come with this issue.

5. Conclusions

The development of a national capability for the handling of forensic evidence contaminated with radionuclides is not a trivial task. As this paper has demonstrated, significant commitment and collaboration by law enforcement (AFP) and research (ANSTO) has been, and continues to be, required to develop credible processes in Australia, with work ongoing in the disciplines of DNA and fingerprints. This project draws upon the full spectrum of expertise in both agencies, and provides learning opportunities that can be applied to future collaborations between the AFP and ANSTO and other agencies.

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