

The Recovery of Touch DNA from RDX-C4 Evidences:

Case Study

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Abstract. RDX (Research Department Explosive) is the organic compound with the formula (O₂NNCH₂)₃. It is a white solid material without smell or taste, widely used as an explosive. It is more energetic explosive than TNT, and it was used widely in World War II. The estimated number of RDX-C4 cases in Bahrain ranged between the years 2015-2018 (May) with a total quantity of 370.72 KG is a total number of 38 cases. The effect of explosive RDX-C4 is very massive and can cause many casualties and fatalities among civilians and policemen. It can penetrate through metals and buildings. These cases consisted of adhesive film with tapes wrapped around RDX-C4 substance (blocks), black battery, pipes, black bag which contained RDX-C4 and in magnetic improvised explosive device (IED). Touch DNA recovery utilized different collection methods, such as nylon swabbing, tape lifting and direct cutting of certain parts of the samples that were positive of RDX-C4 through GC-MS technology. Samples were extracted and purified with magnetic beads chemistry and quantified. Low copy DNA extracts were subjected to a concentration step. DNA extracts were amplified and processed for detection to obtain reliable results using GlobalFiler Amplification PCR kit and run through ABI 3500xL Genetic Analyzer for fragment length determination. We have discovered that RDX-C4 cannot bind to the DNA nor to the solutions used in DNA typing. Thus, it does not cause DNA inhibition or degradation. From this point of view, we were successful in obtaining accepted and fit results using advanced techniques. This study will be very useful and informative to assist the forensic community in terrorism cases applications worldwide as terrorists do not respect geographical boundaries nor ethnicities of the victims and the use of DNA profiling technology is the most suitable way to identify the terrorists and keep an end to their violence.

Keywords: RDX-C4, IED, Touch DNA, Kingdom of Bahrain, Magnetic beads chemistry, Terrorism, GlobalFiler Amplification PCR kit, ABI 3500xL Genetic Analyzer

1. Introduction

RDX (Research Department Explosive) is the organic compound with the formula (O₂NNCH₂)₃. It is a white solid material without smell or taste, widely used as an explosive. Chemically, it is classified as a nitramide and like HMX. It is more energetic explosive than TNT, and it was used widely in World War II (1). It is often used in mixtures with other explosives and plasticizers or phlegmatizers (desensitizers). RDX is stable in storage and is considered one of the most energetic military explosive; and an explosion can only be initiated by a shock wave from a detonator (2).

Many terrorism cases have shown the presence of RDX-C4 in samples such as real IEDs, bombs, pipes and some packed in bags or wrapped in adhesive film in warehouses.

The estimated number of RDX-C4 cases in Bahrain in between the years 2015-2018 (May) with a total quantity of **370.72 KG** and a total number of 38 cases (**Fig. 1**).

The effect of explosive RDX-C4 is very massive and can cause many casualties and fatalities among civilians and policemen. It can penetrate through metals and buildings. Terrorists do not respect geographical boundaries nor ethnicities of the victims and the uses of DNA profiling technology are the most suitable way to identify the terrorists and keep an end to their violence.

2. Materials and Methods

2.1 Samples Collection, Extraction and Normalization

Total number of five cases were selected and brought to the Forensic Science Laboratory (Bahrain) during the years 2017 and 2018 (**Fig. 2**) after their disposal. These cases consisted of adhesive films with tapes wrapped around RDX-C4 blocks, black battery, pipes loaded with RDX-C4, black bag which contained RDX-C4 and finally magnetic improvised explosive device (IED). The samples were positive for RDX-C4 by GC-MS technology. Samples were seized and found within hidden warehouses and some roads, ready to be deployed.

Tape lifts, single or double nylon swabs (moistened with DNA grade purified water) were used to collect from the samples with RDX-C4 such as from handles and zipper of bag, battery body, pipe's opening and from the internal parts of the magnetic (IED). Direct cutting of samples was done to small pieces of tape endings, wire twists inside of the magnetic (IED) (3).

Touch DNA was extracted and purified using the magnetic beads chemistry (i.e EZ1[®] Advanced XL – Qiagen (4) and AutoMate Express[™] DNA Extraction System (5) – Thermo fisher Scientific) with increase time of incubation in EZ1 to 60 minutes at 56°C; 850 rpm using 475µl of undiluted G2 buffer and 25µl PK. All the samples were added to Lyse and Spin tubes (Qiagen) prior to incubation (6).

Quantification was done through Investigator Quantiplex Hyres[®] Kit – Qiagen or Quantifiler HP DNA Quantification Kit (Life Technologies/Thermo Fisher Scientific) using a 7500 Real-Time System (Applied Biosystems) following the

manufacturers protocols (7). Most of the samples were subjected to various concentration steps using vacuum dry technique (i.e using Concentrator® Plus – Eppendorf (8)), to obtain a reliable quantity for a successful PCR.

2.2 Amplification and Detection

DNA extracts were amplified using GlobalFiler PCR Amplification Kit - Thermo Fisher Scientific following the manufacturer protocol (9). Previously genotyped DNA Control 007 available in the kit along with negative controls were used during amplification.

The amplicons were analyzed by capillary electrophoresis by an ABI 3500xL Genetic Analyzer for fragment length determination. Samples were prepared by adding 1µl of the PCR product or allelic ladder to the corresponding well on the CE plate which contained a mixture of formamide and size standard (9.6 µl formamide and 0.4 µl GeneScan LIZ600 size standard, v2.0, of Life Technologies) (9).

The plate was prepared and denatured at 95°C for 3 min and then placed on ice for 3 mins prior loading for capillary electrophoresis. PCR products were separated and detected using POP-4™ polymer and an ABI 3500xL Genetic Analyzer (Life Technologies) (9).

2.3 Data Analysis

Data was captured by 3500 Series Data Collection v3.1. The raw data was then analyzed using GeneMapper ID-X v1.4. RFU values were obtained through in-house validation of the GlobalFiler PCR Amplification Kit. Single source samples were checked into the Bahrain DNA database containing ~ 60,000 DNA STR profiles. DNA mixtures of 2-3 contributors are supported with Likelihood values using LRmix Studio available online (10).

3. Results and Discussion

Full profiles were generated from the different exhibits containing RDX-C4 as shown below (Fig. 3-7). The RFU of the samples were high and approved by our internal validation for GlobalFiler PCR Amplification Kit (~85 for each loci). All the samples generated were of DNA mixtures except for the tape wrapped around the adhesive film of RDX-C4 block.

To comment upon the obtained results, RDX-C4 is a sticky solid substance that can retain some of the cells from the shedders or from sweating while assembling the explosive inside compartments of IEDs or bombs. Also, C-4 is very stable and insensitive to most physical shocks and can withstand different physical properties such as welding and molding thru metals and electronics (11).

We have discovered that C-4 cannot bind to the DNA nor to the solutions used in the protocols. Thus, it does not cause inhibition or degradation to the DNA. From this point of view, we were successful in obtaining accepted and fit results using the above described methods.

In addition, this study will be very useful and informative to assist the forensic community in terrorism cases applications worldwide. The findings of this study emphasize the need to continuously re-evaluate standard operating protocols with empirical studies for such type of cases.

4. Figures and Tables

4.1 Figures

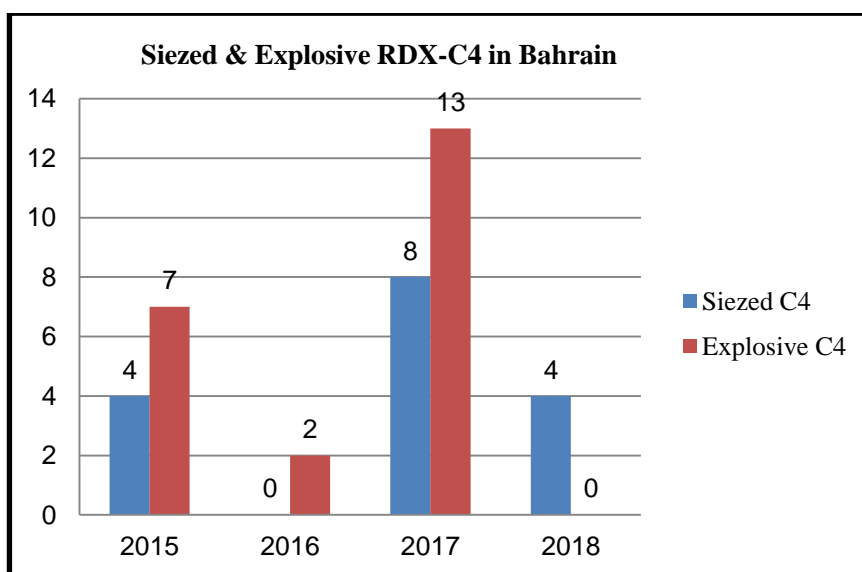


Figure 1 Number of RDX-C4 cases in Bahrain from 2015-2018 (May)



Figure 2 Some of the processed samples of different terrorism cases

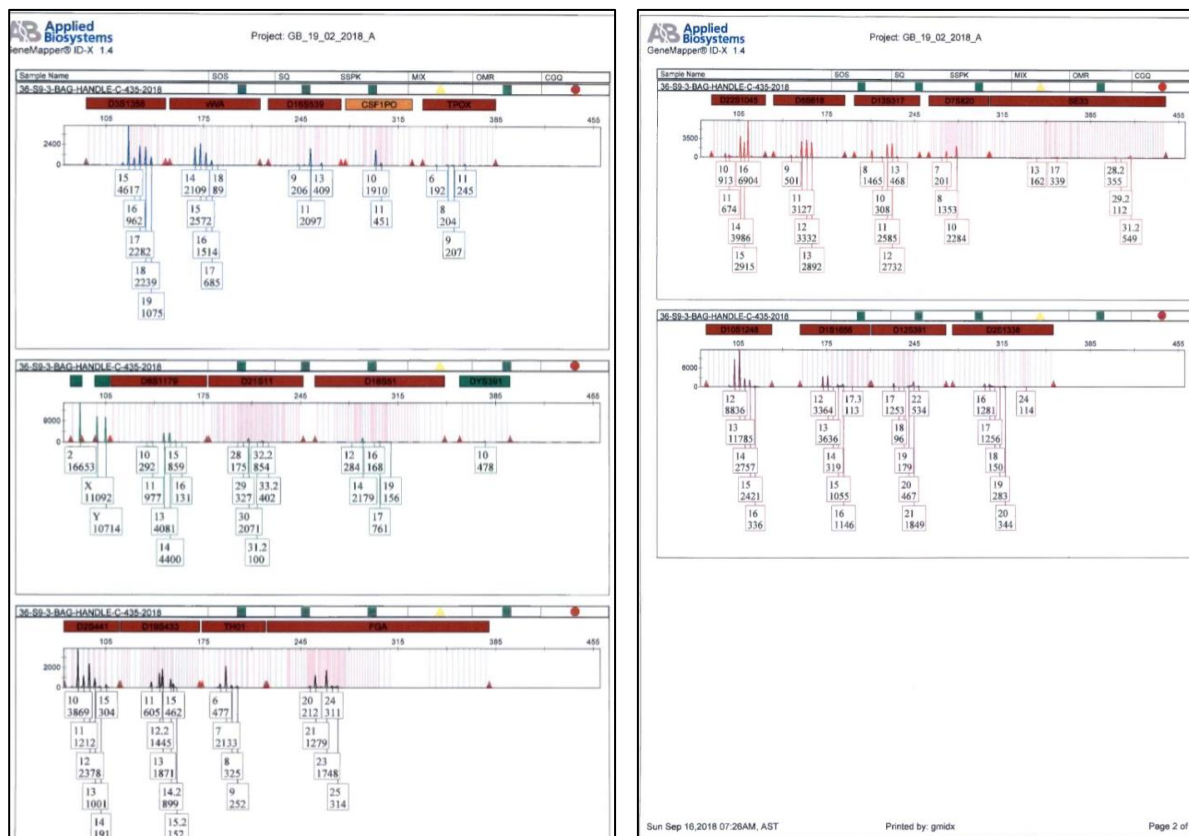


Figure 3 The results obtained from handles of black bag contaminated with RDX-C4 showed DNA mixtures (0.75ng/μl)

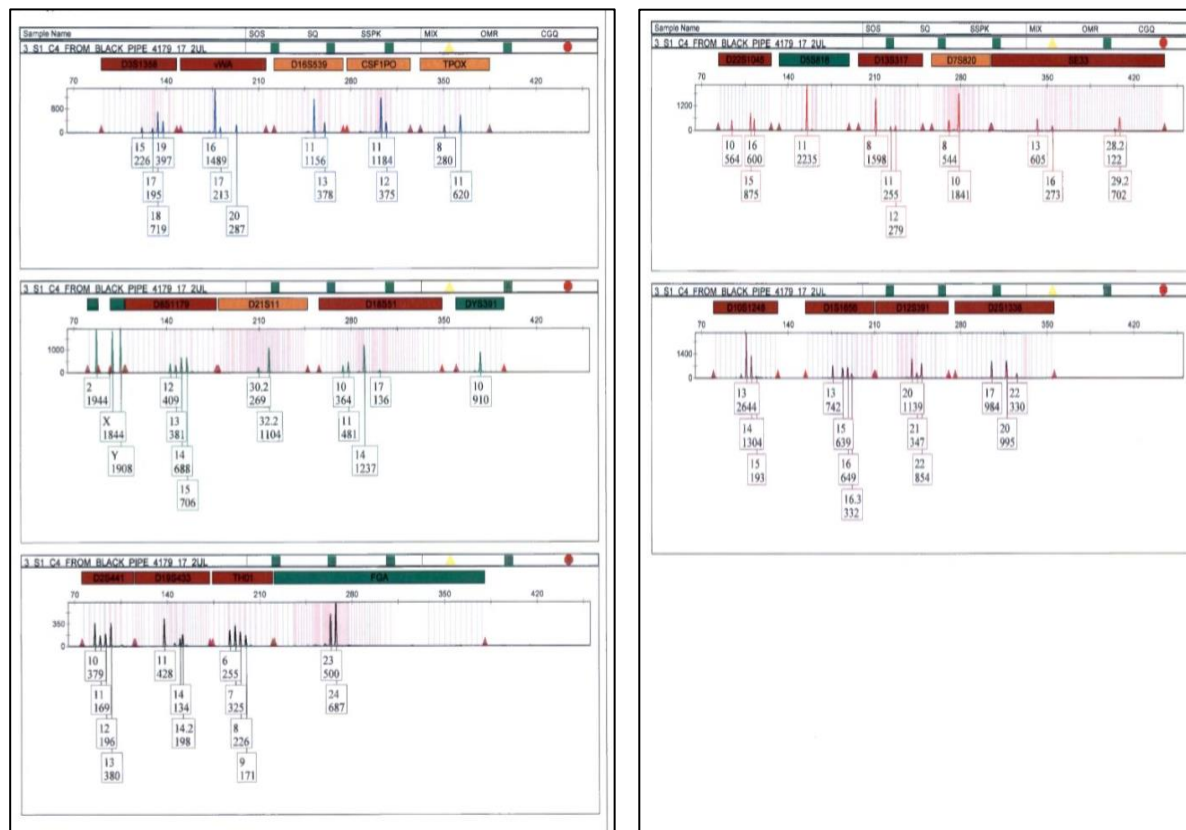


Figure 4 The results obtained from RDX-C4 inside the black pipe showed DNA mixtures(0.01ng/μl)

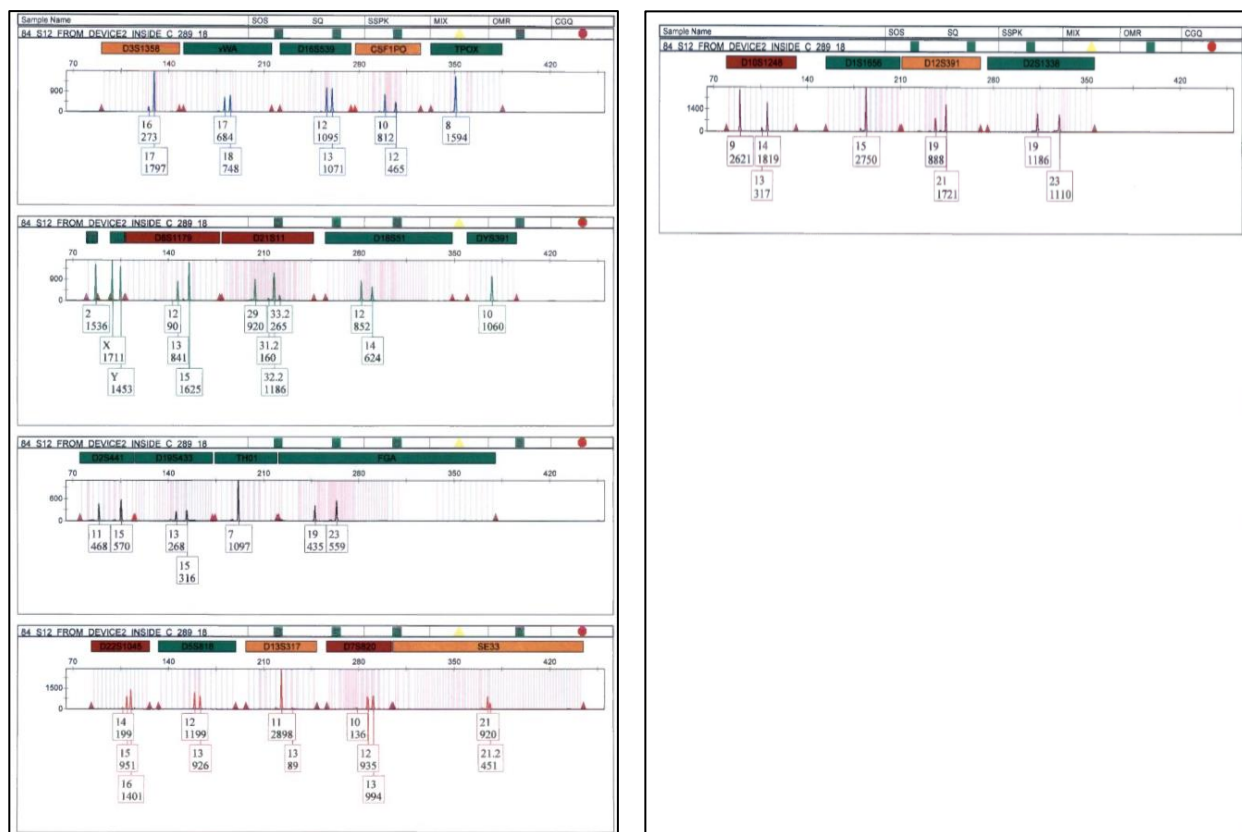


Figure 6 The results obtained from RDX-C4 inside the magnetic IED showed DNA mixtures(0.01ng/ μ l)

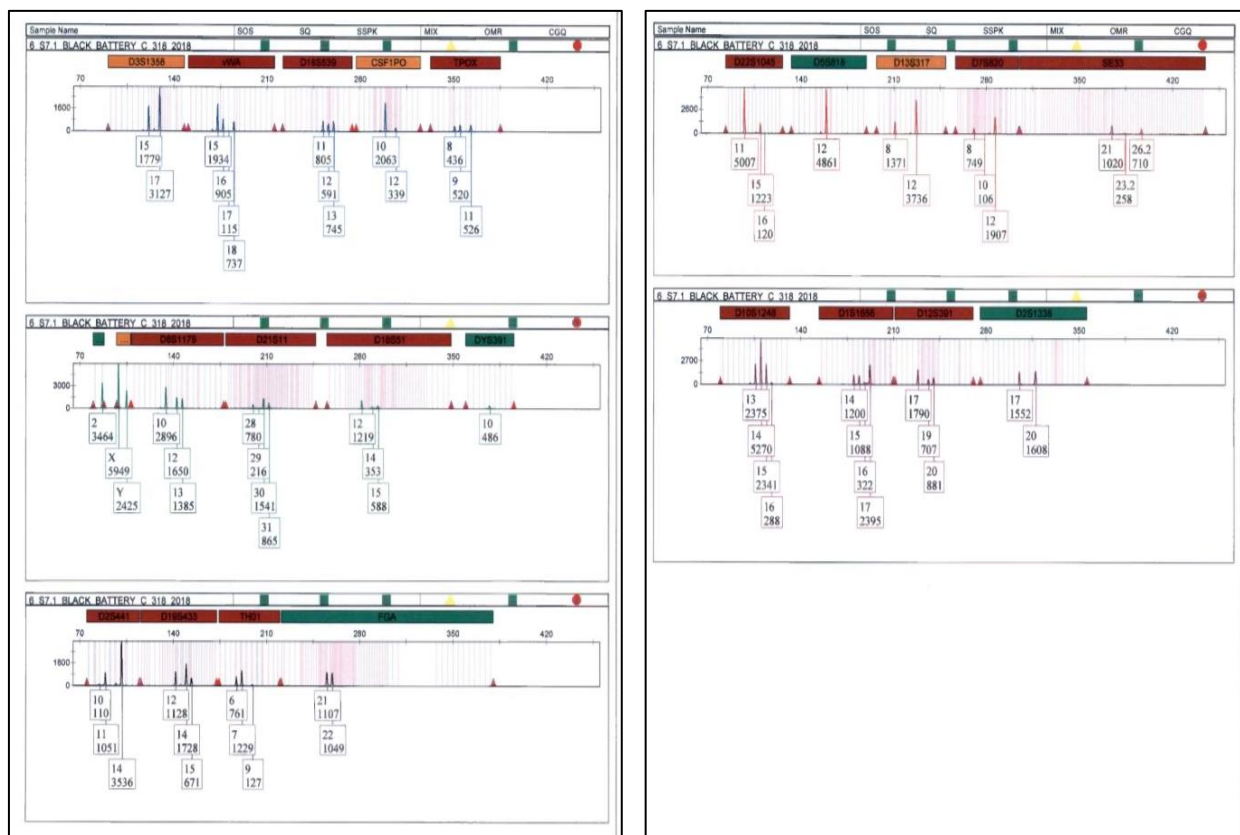


Figure 5 The results obtained from black battery (external surface) contaminated with RDX-C4 showed DNA mixtures (0.75ng/ μ l)



Figure 7 The results obtained from tape wrapped around C4 block showed single profile of DNA (0.5ng/μl)

5. Conflict of interest

None

6. Acknowledgment

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7. References

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