Kingdom of Bahrain Ministry of Interior General Directorate of Criminal Investigation & Forensic Science Forensic Science Laboratory



مملكة البحرين وزارة الداخلية الإدارة العامة للمباحث والأدلة الجنائيـة مختبر البحث الجنائي

THE RECOVERY OF TOUCH DNA FROM RDX-C4 EVIDENCES: CASE STUDY

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- RDX (Research Department Explosive) is the organic compound with the formula (C3H6N6O6).
- It is a white solid material without smell or taste, widely used as an explosive.
- Chemically, it is classified as a nitramide. It is more energetic explosive than TNT and it was used widely in World War II.
- RDX is stable in storage and is considered as one of the most energetic military explosives . An explosion can only be initiated by a shock wave from a detonator.
- RDX is also known as 1,3,5-trinitro-1,3,5triazinane.







- The term *composition (C4)* is used for any explosive material compounded from several ingredients to create the *plastic explosives*.
- RDX-C4 consists of the following:
 RDX (91%),
 - a plasticizer (which can be dioctyl adipate {DOA}, or dioctyl sebacate) (5.0%),
 - a binder, which is usually polyisobutylene (2.0 %),
 - Non-detergent motor oil (1.6%).
- RDX-C4 can be molded by hand for use in demolition work and packed by hand into shaped charge devices.
- Hypothesis; If molded by hand, can we have contact DNA?







- Many terrorism cases have shown the presence of RDX-C4 in samples:
 - such as real IEDs (Improvised Explosive Device);
 - Bombs;
 - Pipes;
 - and some packed in bags or wrapped in adhesive film in warehouses (C4 block).



• 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 as described below:

Siezed & Explosive RDX-C4 in Bahrain





The <u>effect</u> of explosive RDX-C4 is very massive and can cause many causalities 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 use of *DNA profiling technology* is the most suitable way to identify the terrorists and keep an end to their violence.



Samples Collection

- Total number of *five cases* were selected and brought to the Forensic Science Laboratory (Bahrain) during the years 2017 and 2018 after their disposal.
- These cases consisted of adhesive film with tapes wrapped around RDX-C4 substances (blocks) and bags containing the RDX-C4.







• Black battery and pipes







• Magnetic improvised explosive device (IED).



 All of the samples were positive for RDX-C4 by *GC-MS technology*. The samples were seized and found within hidden warehouses and in some roads, and they were ready to be deployed.



Samples Collection

• *Tape lifts and Single or double nylon swabs/ flocked swabs* (swabs moistened with DNA grade purified water) were used to collect from the samples with RDX-C4 such as handles and zipper of bag, internal parts of magnetic (IED), pipe's opening and battery.





Samples Collection

- *Direct cutting of samples* was done for pieces of tapes endings of the C4 block, small parts, and wire twists inside the magnetic (IED).
- In some occasion swabbing along with direct cutting of samples can be joined along.





Samples Extraction

• Touch DNA was extracted and *purified using magnetic beads chemistry* (i.e. Lyse & Spin in EZ1 Advanced XL– Qiagen and AutoMate Express DNA Extraction System– Thermo fisher Scientific) with increase time of incubation in EZ1 to one hour at 56°C using 475µl of undiluted G2 buffer and 25µl pk and 400µl of buffer MTL and 1µl RNA Carrier.



• We have modified the protocol of sample lysate transfer in EZ1 Advanced XL by cutting the caps of Lyse & Spin tubes after incubation and install them directly into the instrument to decrease the sample loss.





- **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 PCR System (Applied Biosystems) following the manufacturers protocols.

Most of the samples were subjected to concentration step using *vacuum dry technique* (i.e. Concentrator Plus – Eppendorf), to obtain a reliable quantity for a successful PCR.





Amplification and Detection

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



Amplification and Detection

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





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 GlobalFiler PCR Amplification Kit.



Results Interpretation

- Single source samples were checked directly into Bahrain DNA database containing ~ 60,000 DNA STR profiles.
- The accepted RFU values of the samples were high and approved by our internal validation for GlobalFiler PCR Amplification Kit (~85 for each loci).

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	Y indel			TH01				D1S1656		
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- Full profiles were generated from the different exhibits containing RDX-C4.
- All of the samples generated were *DNA mixtures* except for the tape wrapped around the *adhesive film of the RDX-C4 block*.
- DNA mixtures of 2-3 contributors are supported with Likelihood values using *LRmix Studio* available online.

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







28.2 122 29.2 702

Results and Discussion

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





The results obtained from RDX-C4 inside the magnetic IED showed DNA mixtures (0.01ng/μl).





 The results obtained from black battery (external surface) contaminated with RDX-C4 showed DNA mixtures (0.75ng/µl).





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





- 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 through metals and electronics.

- We have discovered that *C-4 cannot bind* to the *DNA neither to the solutions* used in all of the previously described protocols. Thus, it does not cause inhibition nor degradation to the DNA.
- From this point of view, we were successful in obtaining accepted and fit results using the above described methods.



Conclusion

- This study was *very useful and informative* to assist the forensic community in *terrorism cases* applications worldwide.
- The ability to generate *investigative leads* after an explosion or when discovering terrorists' warehouses is *critical* to stop terrorism.
- The findings of this study emphasize the need to continuously *re-evaluate* standard operating *protocols* with empirical studies for such type of cases.



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