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NATIONAL POLICE SERVICES

FS&IS FORENSIC SCIENCE AND IDENTIFICATION SERVICES

Applying Rapid DNA technologies to CBRNE events

Chantal Frégeau, Ph.D.; 15th Annual Green Mountain Conference, July 22-24, 2024

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Definition

Chemical (C) Radiological (R) Explosive (E)
Biological (B) Nuclear (N)

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Statistics for R/N Events

GTD GLOBAL TERRORISM DATABASE

University of Maryland by the National Consortium for the Study of Terrorism and Responses to Terrorism (START) <https://www.start.umd.edu/gtd>

Information on more than 200,000 Terrorist Attacks

From 2000-2020:
10 R incidents
0 N incidents

Year	Country	Attacker	Target	Attacker Type	Attacker Motivation	Attacker Ideology	Attacker Religion	Attacker Ethnicity	Attacker Nationality	Attacker Residence	Attacker Age	Attacker Gender	Attacker Education	Attacker Employment	Attacker Status	Attacker Other	Attacker Note	
2000-2020																		


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Statistics for R/N Events

International Atomic Energy Agency

IAEA Incident and Trafficking Database (ITDB)





2023 Factsheet

<https://www.iaea.org/sites/default/files/22/01/itdb-factsheet.pdf>

From 2000-2020:
244 R/N incidents
Trafficking/Malicious use

From 2000-2022:
256 R/N incidents

143 participating States/Countries

Radiological Threat

Weapons that could be used

- Radiological Exposure Devices (REDs; hidden radiation source)
- Radiological Dispersal Devices (RDDs or Dirty Bombs)



[Radiological Exposure Device \(cdc.gov\)](http://www.cdc.gov/radiation)



<http://emergency.cdc.gov/radiation>




<http://www.pbs.org/wgbh/howdirtybomb/images/ctpo-1995.11.jpg> from: "The cesium-filled package uncovered in a Moscow park", Nov. 1995





Top Five Isotopes of Concern



Nuclide (Half life)	Radiation Emission	
¹³⁷ Cs (30 years)	γ	0.5 g ¹³⁷ CsCl = 10 curies
⁶⁰ Co (5 years)	γ	Each tiny pellet ⁶⁰ Co = 3 to 8 curies
⁹⁰ Sr (30 years)	β	
¹⁹² Ir (74 days)	γ	
²⁴¹ Am (432 years)	α	



50 g



In 2009:
Authorities in Ukraine seized a container filled with 3.7 Kg of radioactive material = 10 million US\$

First Responders Mandate #1



- Identify the contaminant
- Determine the extent of contamination
- Contain contaminant
- Decontaminate site

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First Responders Mandate #2

- Collect biological samples at the scene
- Submit to forensic laboratories/DNA analysis



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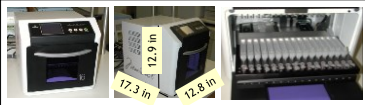
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Mobile Forensic Laboratory



Promega Maxwell[®] 16



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Validation of Experimental Design

Forensic Science International 259 (2016) 161–178
 Contents lists available at ScienceDirect
Forensic Science International
 Journal homepage: www.elsevier.com/locate/forensicint

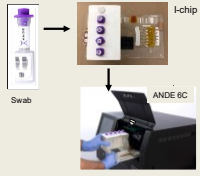
Simulated radioactive decontamination of biological samples using a portable DNA extraction instrument for rapid DNA profiling
 Chantal J. Frigman^a, Claude Dalpé^a
^aReal/Canadian Forensic Police, National Forensic Laboratory Services—Ottawa, 1300 Water Valley, Ottawa, ON, Canada K1A 0K2

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 Contents lists available at ScienceDirect
Forensic Science International
 Journal homepage: www.elsevier.com/locate/forensicint

Evaluation of commercial forensic DNA extraction kits for decontamination and extraction of DNA from biological samples contaminated with radiocesium
 Kaitlyn Toole^a, Paul Roffley^a, Emma Young^a, Kaymann Cho^a, Timothy Shaw^a,
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Rapid DNA Instruments for CBRNE


ANDE 6C (ANDE Corporation)



Swab → I-chip → ANDE 6C

- 4 samples per run
- 110 min
- I-chip
- FlexPlex™ 27 (23a, 3Y, Amel)
- 100% lysate/conc. step
- 29 PCR cycles

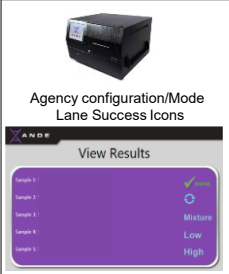
RapidHIT ID (ThermoFisher)



RapidHIT ID, INTEL Sample Cartridge, Primary Cartridge, Secondary Cartridge

- 1 sample per run
- 95 min
- RapidINTEL cartridge
- GlobalFiler™ Express (21a, 2Y, Amel)
- <100% lysate/ reduced lysis vol. (300 µL)
- 32 PCR cycles

Rapid DNA Primary Analysis




Agency configuration/Mode
Lane Success Icons

View Results

Sample 1: Success
Sample 2: Success
Sample 3: Success
Sample 4: Success
Sample 5: Success

Metrics: Low, High

ANDE Expert System / ANDE FAIRS™ software
 .png, .fsa, .xml files, .csv table (No peak height, size)



RapidINIK™ Expert System / GeneMarker™ HID
 GM_Analysis_PeakTable.bt (allele calls, height, size)

Primary vs *Simplified* Secondary Analysis

"Manual Review"

I-chip

INTEL cartridge

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Experimental Design

- 1 µL and 10 µL saliva in triplicates
- Galvanized steel plate & PVC caps (sewer pipes)
- Three concentrations (0.01 M, 0.05 M, 0.1 M) of non-radioactive cesium, strontium and cobalt salts

Stock solution	Cs (CsCl)	Sr (SrCl ₂)	Co (CoCl ₂)
Level A_0.01 M (high)	4 mCi	4 mCi	23 mCi
Level B_0.05 M	20 mCi	21 mCi	117 mCi
Level C_0.10 M (extremely high)	40 mCi	43 mCi	234 mCi

- Saliva stains collected from surfaces using swabs (ANDE swab and BBL cotton swab)
- I-chip (ANDE 6C), INTEL cartridges (RapidHIT ID)

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First Pass Success Rate

Profiles with green check mark

Sample	ANDE 6C/I-chip				RapidHIT ID/INTEL cartridge				No Flag
	Primary Analysis		Manual Review		Primary Analysis		Manual Review		
	%	Count	%	Count	%	Count	%	Count	
10 µL saliva	61.7%	29/47	68.1%	32/47	44.7%	21/47	89.4%	42/47	
1 µL saliva	30.0%	18/60	46.7%	28/60	18.3%	11/60	70.0%	42/60	

Profiles with ≥ 10 autosomal STRs

Sample	ANDE 6C/I-chip				RapidHIT ID/INTEL cartridge				Flags: Imbalances & Low intensity
	Primary Analysis		Manual Review		Primary Analysis		Manual Review		
	%	Count	%	Count	%	Count	%	Count	
10 µL saliva	100%	47/47	100%	47/47	87.2%	41/47	93.6%	44/47	
1 µL saliva	75.0%	45/60	86.7%	52/60	78.3%	47/60	95.0%	57/60	

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First Pass Success Rate

All data combined

Profiles eligible to be searched against a national DNA database

Sample	ANDE 6C/i-chip		RapidHIT ID/INTEL cartridge	
	Primary Analysis	Manual Review	Primary Analysis	Manual Review
Canada (NDBB)	80.4%	86/107	73.8%	92.5%
United States (NDIS)	80.4%	86/107	69.2%	92.5%

Current search criteria:

Canada: ≥ 9 loci from the original 13 CODIS core loci

United States: ≥ 8 loci from the original 13 CODIS core loci with a match rarity estimate of at least 1 in 10 million

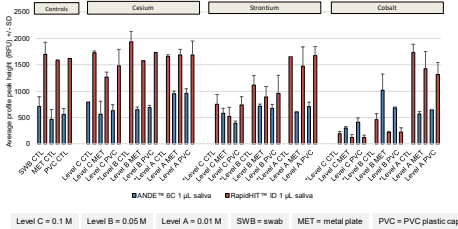


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Nouvelles Pratiques de l'Identification



Average Profile Peak Height

1 µL Saliva



- CsCl has no impact on average profile peak height
- Average profile peak height decreases with an increase in SrCl₂ and CoCl₂ salt concentration for the RapidHIT ID (red color)
- Impact not as pronounced for the ANDE 6C (blue color)

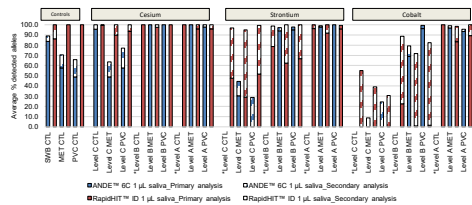


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Average Percentage of Detected Alleles

1 µL Saliva

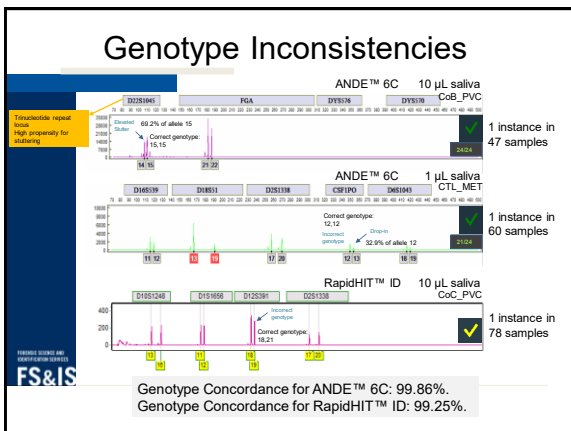
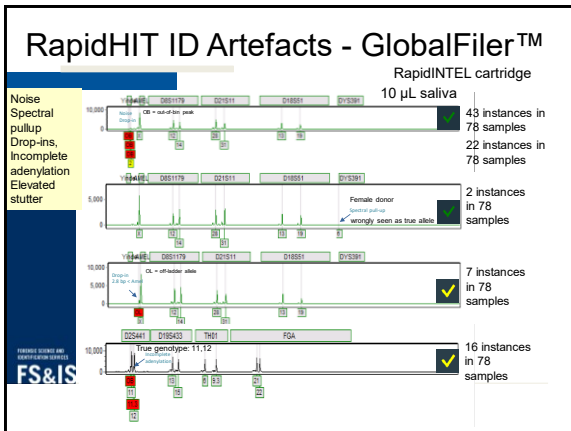
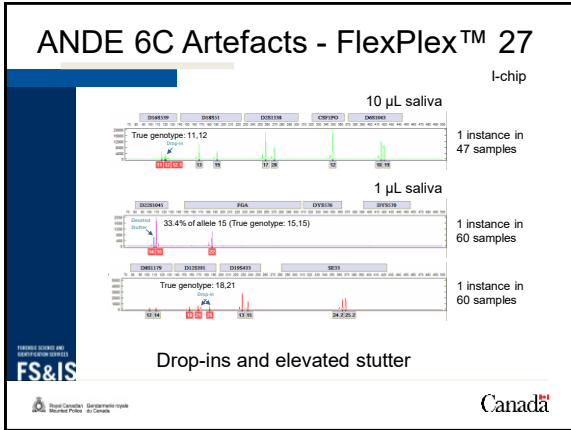


- CsCl has no impact on average % detected alleles (complete profiles in majority of samples processed)
- Average % detected alleles decreases with an increase in SrCl₂ and CoCl₂ salt concentration for the RapidHIT ID (red color)
- Impact not as pronounced for the ANDE 6C (blue color)



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Nouvelles Pratiques de l'Identification





Reprocessing of Swabs

- Obtain DNA profiles in case of instrument or cartridge/chip failure
 - 9/155 (5.8%) RapidINTEL™ sample cartridges with no possible sizing; all from the same lot number
- Enhance quality of initial DNA profile

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Reprocessing of Swabs on RapidHIT ID

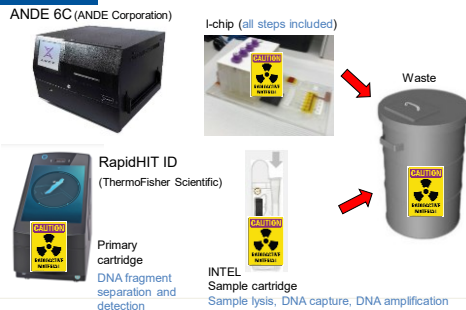
- 73.7% (14/19) had higher fluorescence signals compared to their initial profiles
- 63.2% (12/19) had 1 to 20 additional alleles compared to their first pass profiles
- 36.8% (7/19) had > 80% alleles compared to 26.3% (5/19) for the first pass profiles and 57.9% (11/19) ≥ 70% alleles compared to 36.8% (7/19)
- Reprocessing increased the number of DNA profiles eligible to search a national DNA database (+3 profiles for Canada and +4 for the US)

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Considerations Before Adoption

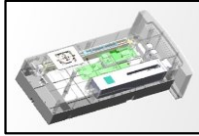


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RapidHIT ID Primary Cartridge

- Single capillary/detection of amplicons
- Contains bulk reagents
 - Lysis buffer
 - Water
 - Polymer syringe
 - Electrophoresis buffer
 - Liquid waste bag
 - Polymer waste bag
- For 150 samples
- 5 samples per day (25 samples per week)
- 1.5 month to use up polymer
- 12 months shelf life (from manufacture)
- 6 months shelf life once installed



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Gamma Radiation Dose Rates

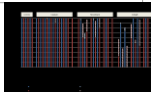
<http://www.radprocalculator.com/> Distance from source (Primary Cartridge): 30.5 cm (1 foot)

	mSv/hr	mSv/hr	mSv/5 min	mSv/5 min
	Cs-137	Co-60	Cs-137	Co-60
1.5 month → After 150 samples	← After 25 samples 1 week			
0.01 M - Level A activity				
Without shielding	18.5	416	0.26	5.8
Lead blanket (0.635 cm solid lead equivalence)	9.9	272.7	0.13	3.8
3 cm thick lead	0.4	94.5	0.01	1.3
6 cm thick lead	10 μSv/hr → 0.01	17.3	0.0003	0.24
0.05 M - Level B activity				
Without shielding	92.7	2079.8	1.3	28.9
Lead blanket (0.635 cm solid lead equivalence)	40.9	1363.3	0.69	18.9
3 cm thick lead	2	472.6	0.05	6.6
6 cm thick lead	40 μSv/hr → 0.04	86.3	0.002	1.2
0.10 M - Level C activity				
Without shielding	184.6	4159.6	2.6	57.8
Lead blanket (0.635 cm solid lead equivalence)	81.4	2726.7	1.4	37.9
3 cm thick lead	4	945.2	0.1	13.1
6 cm thick lead	90 μSv/hr → 0.09	172.5	0.003	2.4

Gamma radiation dose limit for the general public:
1 mSv/yr (20 μSv/week, 0.5 μSv/hr, 0.042 μSv/5 min)

Mechanism of Action of Sr⁺² and Co⁺²

Transition metals: Co⁺², Cu⁺², Mn⁺²
Alkaline earth metals: Sr⁺², Ca⁺², Mg⁺²



- Affinities to DNA (nucleic acid phosphate groups and bases)
 - Liu and Ran (2019) *Colloids Surf, B: Biointerfaces* 19: 111117-10
 - Zhang et al. (2021) *ACS Omega* 7: 33530-33536
- Affinities to DNA polymerases
 - Kuffel et al. (2020) *Int. J. Leg. Med.* 135: 63-72
 - Wu et al. (2022) *Adv. Synth. Catal.* 364: 2760-2771
- Dye fluorescence quenchers
 - Hartzell and Matuszewska (2005) *Electrophoresis*, 26:1046-1056

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Mechanism of Action of Sr⁺² and Co⁺²

RapidINTEL Sample Cartridge

Lysis Chamber

- 300 µL Prep-N-Go Buffer
- Bubbling agitation,
- Heat: 10 min/85°C

PCR Zone

- 1.2 mm FTA paper disc
- < 12 µL/32 cycles,
- Platinum™ Taq polymerase

PCR reaction mix
Primer mix
Size Standard iLS

5 mM and 10 mM CoCl₂ impacts DNA polymerases
3 mM CoCl₂ results in loss STR allele detection

ANDE 6C I-chip

Swab Chamber

- 300+ µL Guanidium-based Buffer
- Chaotic bubbling, no heat
- Silica membrane/DNA purification

PCR Zone

- Conc. Module
- 100% lysate
- < 10 µL/29 cycles

Conclusions for Mock “R” Event

Both Rapid DNA instruments:

- Showed similar first pass success rates (profiles with a minimum of 10 autosomal STR loci)
- Produced complete STR profiles from 1 µL and 10 µL saliva on metal plates or PVC caps in the presence of non-radioactive Cs, Sr and Co salts (Level A/10 mM for CoCl₂ and SrCl₂; Levels A-C 10-50-100 mM for CsCl₂)
- Had profiles adversely impacted using CoCl₂ and SrCl₂ at Levels B and C; interference at pre-PCR/during PCR
- Showed a similar success in generating DNA profiles eligible to be searched against a national DNA database

Conclusions for Mock “R” Event

- Reprocessing of contaminated samples may increase allele calls and number profiles eligible to be searched against national DNA databases
- The ANDE™ 6C offers quick disposal of all radioactive materials associated with contaminated biological samples into appropriate shielded containers
- The ANDE™ 6C does not require recalibrations upon deployment and reagent refrigeration
- In a true “R” event, the production of complete Rapid DNA profiles will depend on the amount and type of radiation received by the biological samples. (Level A)

Acknowledgements

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Processing biological samples from simulated radiological terrorist events using Rapid DNA instruments

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Thank you chantal.fregeau@rcmp-grc.gc.ca

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