TOOLS FOR IDENTIFICATION: FORENSIC RADIOLOGY AND NEW DEVELOPMENTS IN DNA SAMPLE TYPES FOR DECOMPOSED AND BURNT HUMAN REMAINS

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ICRC Workshop: Management and Identification of Decedent Migrants
No Conflicts of Interest to declare

The following presentation contains images of deceased persons
TO BE DISCUSSED

Identification Phases

1. Scene
2. Mortuary/laboratory
   • Radiology/Molecular
3. Ante-mortem
   • Information/reference sample
4. Reconciliation-contemporaneous or deferred
5. Debriefing
TYPES OF REMAINS

Preserved-intact

Decomposed

Fire-affected

Fragmented
IDENTIFICATION

Primary

- Dental
- DNA
- Fingerprints

- Medical implants
IDENTIFICATION METHODS

Secondary/supportive

• Visual
• Clothing
• Documents
• Jewellery
• Circumstances
• Scars, tattoos, deformities
• Others- modelling, superimposition
RADIOLOGY MODALITIES

- Plain X-ray
- Image intensifier
- CT
- (MRI)
CT ADVANTAGES

- Digital permanent record
- Remains in body bag
  - minimise hazard risk
  - minimise evidence loss
- Data for deferred/remote pathological, odontological, anthropological examination
  - Reconciliation/re-allocation
CT- ANALYSIS

Scan once, post-process many times

- 1º survey- initial radiological CT report
- 2º survey- specific dental, anthropological assessment
- 3º survey- retrospective radiological review
CT REPORTING

Sex
Age
Natural Disease

Specific identifiers
• Dentition
• Surgical implants

• ISFRI-DVI*
Age estimation (non-anthropological)
AGE ESTIMATION
MULTI-MODALITY
Jewellery and other objects
ID

Disease/deformity
ID

Medical devices
ID

Dental- Plain Xray
RADIOLOGY

Dental- CT
PROBLEMS

- Artefacts
- Positioning- non orthogonal
- Small fragments/building materials
- Expensive*
- Limited portability/availability
- Radiological/radiographic expertise
- Servicing
- Data Storage- PACS
CT RADIOLOGY SUMMARY

Rapid processing of remains
Permanent record
Supplements physical examination
Minimise tissue loss/hazards
Aid in primary and secondary identification
Can be resource intensive-money, personnel
MOLECULAR BIOLOGY
NEW APPROACHES
MOLECULAR BIOLOGY APPLICATIONS

Routine identification:
• Nuclear DNA (nDNA) – 16 autosomal markers including sex determination
• Mitochondrial DNA (mtDNA)

Disaster Victim Identification (DVI):
• Multiple fatalities- few to hundreds

Missing persons investigations:
• Unidentified remains reconciled with missing persons

DNA testing in old specimens:
• mtDNA analysis
NUCLEAR DNA (nDNA)

- Nucleus (one per cell)
- One copy of nDNA per cell
- Large
- Packaged into structures
  - Chromosomes
    - 23 pairs of chromosomes in a human cell
    - Including the sex-determining X and Y chromosomes
- Mode of inheritance
  - ½ from mother
  - ½ from father
MITOCHONDRIAL DNA (mtDNA)

- Small
- Circular genome
- Mitochondria (many per cell)
- Several copies mtDNA per mitochondria
- 100s copies per cell
- Relatively stable – compartmentalisation
- More mtDNA compared to nDNA
- mtDNA is exclusively inherited from the mother
**DNA IDENTIFICATION**

**Kinship:**
- nDNA analysis
- Compare profiles to establish if individuals are related
  - Parent/child relationships
  - Sibship (same parents)

**Direct comparison with ante-mortem data:**
- Self to self (e.g. deceased compared to Guthrie cards, histology blocks, hair etc.)

**Challenges:**
- Incinerated remains
- Decomposed remains
INCINERATED REMAINS

Range of body types

- Intact charred remains to fragmented burnt and calcined bones

Varied success of DNA analysis- 2009 experience

- Good for bone/muscle/blood from charred remains
- Poor from fragmented burnt bones
## DNA TRIAGE PROCESS - 2009 FIRES

### Post-mortem sample collection

<table>
<thead>
<tr>
<th>Condition of body</th>
<th>Sample to be collected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not decomposed, whole body</td>
<td>Blood (on FTA card or swab) and buccal (mouth) swabs</td>
</tr>
<tr>
<td>Not decomposed, fragmented</td>
<td>If available, blood</td>
</tr>
<tr>
<td></td>
<td>And</td>
</tr>
<tr>
<td></td>
<td>Deep red muscle tissue (≈1.0 g)</td>
</tr>
<tr>
<td>Decomposed, whole bodies and fragmented remains</td>
<td>Long compact bone samples</td>
</tr>
<tr>
<td></td>
<td>(cut 4–6 cm, using window cut without separating the shaft) And/or</td>
</tr>
<tr>
<td></td>
<td>Healthy teeth without fillings (molars preferable) And/or</td>
</tr>
<tr>
<td></td>
<td>Any available bone (≈10 g, if possible; dense cortical bone preferable)</td>
</tr>
<tr>
<td>Severeely burnt bodies</td>
<td>Any of the samples above</td>
</tr>
<tr>
<td></td>
<td>Or</td>
</tr>
<tr>
<td></td>
<td>Swab from inside the urinary bladder (see Ref. [32])</td>
</tr>
</tbody>
</table>


FSI Genetics (1) 3-12

VICTORIAN INSTITUTE OF FORENSIC MEDICINE
2011 PNG FLIGHT 1600: 28 DECEASED

• Variation in preservation - many victims severely burnt
• Bladder preserved intact
• Bladder swabs collected for DNA analysis in addition to routine specimens - AFP
• Full DNA profiles obtained from all samples
• ? Applicability to routine case work - research
BLADDER SWABS: SAMPLE COLLECTION PROTOCOL

Standard sample

- Dependent on the degree of incineration- bone, blood, muscle

Bladder swab sample

- Small incision (~1 cm) in the anterior wall of the bladder
- Dry cotton swab inserted
- Bladder wall wiped
- If delay (>12 hrs) – aeration required
RESEARCH - BLADDER SWABS 2013

- All fire deaths admitted to SCO - January - November 2012
- House fires; car accidents; aviation; self immolation; homicide.
- 28 cases - wide variability in preservation
- Routine specimens for comparison - blood, muscle, bone depending on case
**BLADDER SWAB RESULTS**

- nDNA extracted regardless of condition of swab (yellow to red)
- Extraction techniques - almost identical to buccal swabs. Easy and robust
- 95% of bladder swabs showed greater nDNA yields (compared with blood or muscle)
- 2 cases showed lower nDNA yields (compared with bone)- still adequate for ID
- Overall 1-10x more DNA from bladder swab samples
BLADDER SWAB STUDY- CONCLUSIONS

- Bladder swabs are a reliable source of DNA for STR analysis
- Ideal for IDs involving incinerated cases
- Minimally invasive techniques
- Simple extraction, good DNA yield
- Reduce the time and complexity in identification.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Sample preparation (hours)</th>
<th>Results available (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>6-12</td>
<td>2</td>
</tr>
<tr>
<td>Muscle tissue</td>
<td>2-6</td>
<td>1-2</td>
</tr>
<tr>
<td>blood</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Bladder swab</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
DECOMPOSED CASES- TOENAIL DNA RESEARCH- 2016

- Typical sample: compact bone or head of femur
- Previous focus on nail scrapings in criminal investigations: few ID studies
- Nails similar to bone: hard material resistant to environmental damage and decomposition
- Toenails: lower numbers of mixed profiles
- Easily accessible: minimal training required
- Decreased processing time

- Study to develop and validate technique for nail analysis
TOENAIL DNA RESEARCH

30 decomposed cases 2013-14
• Average PM interval 3 weeks (2 days to 9 months)
• unsuitable for visual ID
• toenail + conventional sample (bone)

Extraction methods optimised (e.g. washing and digestion times) by parallel live donor study (buccal swab and toenail clippings- 46 cases )
• Adaption of hair extraction technique
• 2 methods validated – silica based column purification (Qiagen) and organic (lab)
• > 0.01g nail material required for full profile
RESULTS

Volunteer specimens
• Both methods yielded sufficient DNA for ID purposes
• Optimized Qiagen method better for more complete profile

Decomposed cases
• Required additional decontamination step (scraping)
• Overall, toenails comparatively more degraded than bone
• nDNA extracted from all 30 toenail cases
• 2/30 bone samples failed to produce adequate nDNA
• 38% of toenail cases produced higher yield than bone in the same case
CONCLUSION- BENEFITS OF USE OF TOENAIL MATERIAL

• Significant reduction in sample preparation time - 2 hours as compared with 6-12 hours for bone and 2 – 6 hours for muscle
• Reduced occupational health and safety risks for staff
• Less invasive/technically demanding + faster sample collection (15’ v 2’)
• Faster overall processing time
• Easier to store (smaller samples, no refrigeration)
PUBLICATIONS

Forensic Science International 233 (2013) 14-20

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Post mortem sampling of the bladder for the identification of victims of fire related deaths

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Forensic Science International 258 (2016) 1–10

Contents lists available at ScienceDirect

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Toenails as an alternative source material for the extraction of DNA from decomposed human remains

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WHERE TO FROM HERE?

- **Massive parallel sequencing (MPS)**
  - Determine the DNA sequence of many (1000s) DNA fragments at once

- **DNA sequences that predict physical appearance**
  - Phenotypic features
    - Eye colour; hair colour; baldness; skin tone
  - Geographical ancestry
    - E.g. European, Asian, or African
QUESTIONS?

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