Direct-to-PCR tissue preservation for DNA profiling

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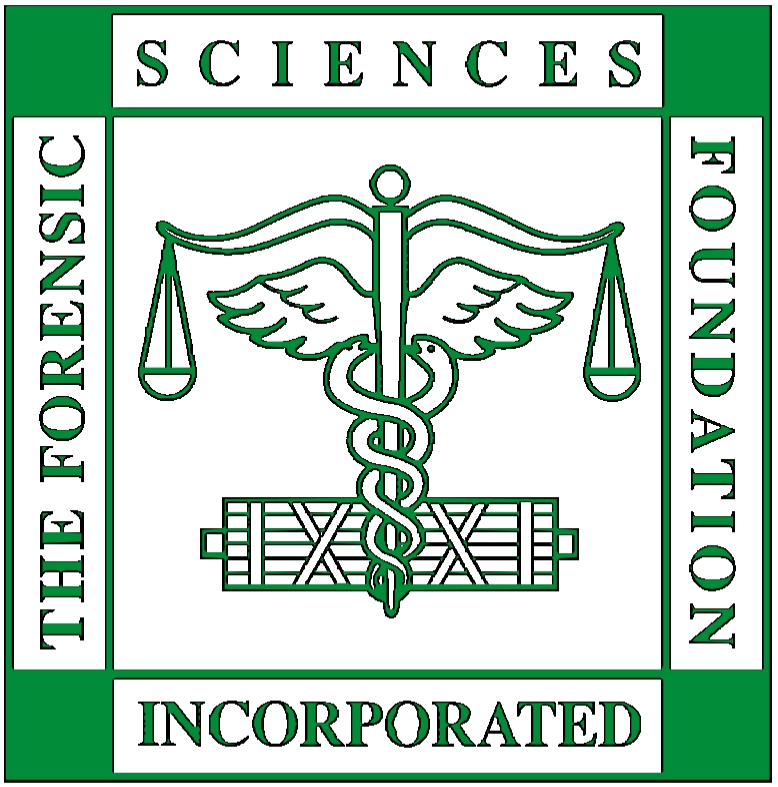

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Warning!

There is some content in this presentation that some people may find disturbing.

DISCLOSURE

- Products used:
  - DNA Genotek Tissue Stabilizing Kit
  - DNAgard®
  - Quantifiler® Human DNA Quantification Kit
  - PowerPlex® 21
  - Globalfiler®

- The authors declare that they have no competing interests.

INTRODUCTION

- Disaster Victim Identification (DVI)
  - Mass fatalities
  - Limited/lack of facilities
  - Loss of electricity
  - Remote locations
  - Extreme temperatures & humidity
- Remote & rural forensic casework

INTRODUCTION

- Successful DNA typing is impacted by:
  - Adverse environmental conditions
  - Speed of collection
  - Preservation/storage of samples
    - Minimizes DNA degradation
    - Inhibits microbial activity
    - Room temp. storage
- Faster methods are desired
  - Tissue preservation releases DNA into solution ready for PCR

PREVIOUS LITERATURE

Allen-Hall, McNevin (2012)

- Fresh muscle
- Short-term storage (4-28 days)
  - 35°C, humidity 9-26%
- Results:
  - DNA recovered from preserved tissues
  - DNA in aliquots of preservatives
    - DESS, DNA Genotek, DNAgard, TENT
  - TENT failed to protect DNA from further degradation

Preservatives	Constituents
Dehydration	Oven drying at 35°C
Solid NaCl	Laboratory grade NaCl
DESS	20% DMSO, 0.25M EDTA, saturated with NaCl, pH 8.0
Ethanol	70% ethanol, 30% ddH_2O
Ethanol + EDTA	70% ethanol, 30% ddH_2O, 0.1mM EDTA
TENT buffer	10mM Tris, 10mM EDTA, 100mM NaCl, 2% Tween 20
RNA*later*®	Proprietary
DNA Genotek Tissue Stabilizing Kit	Proprietary
DNAgard®	Proprietary

INTRODUCTION

Can we perform direct PCR from preservatives?

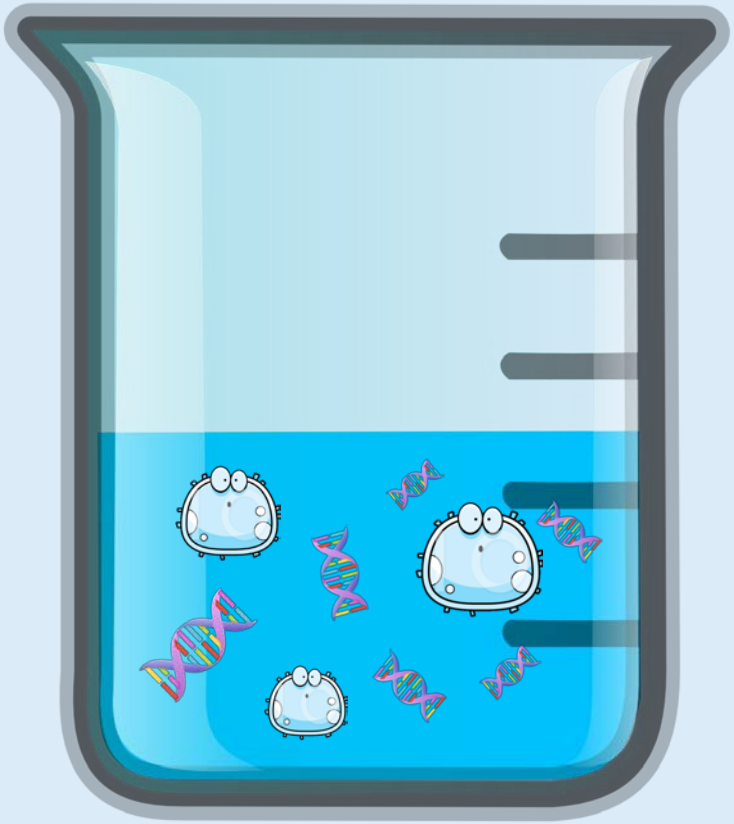
- Advantages to eliminating DNA extraction:
  1. Quicker & less expensive
  2. Less opportunity for loss of DNA
  3. Smaller risk of contamination
  4. DNA can be archived in small volume

- Direct addition of preservatives to PCR poses challenges:
  1. Preservatives contain high concentrations of PCR inhibitors
  2. Components of tissue, by-products of decomposition, & contaminating material from soil also inhibit PCR

MATERIALS & METHODS

Preservative solutions that previously leached DNA into solution (2012)

- DMSO/EDTA saturated w/ salt(DESS)
- Tris, EDTA, NaCl, Tween 20 (TENT)
- DNAgard® [Biomatrica]
- DNA Genotek Tissue Stabilizing Kit [DNA Genotek]



MATERIALS & METHODS

Preservation of Tissue Samples

1. Fresh skeletal muscle (3 donors)
   - 300mg preserved in 1 or 2mL of preservative
   - Stored at 35°C for 3, 7, 14, & 28 days
   - 20 or 50µL aliquot archived at -80°C for four years

2. Decomposing skin & muscle (2 cadavers)
   - Tissue collected at 0, 6, 8, & 10 days of decomposition
   - 30mg in 300µL of preservative
   - Stored at 35°C for 1 month
   - "No preservative" control

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MATERIALS & METHODS

Days of Decomposition

Day 0 **Day 6** **Day 8** **Day 10**

MATERIALS & METHODS

Quantification of DNA in Preservatives:

- Quantifiler® Human DNA Quantification Kit [Thermo Fisher Scientific]
  - PCR inhibition measured by a delay in C_T for the IPC
  - When IPC was undetected → 1:10 or 1:20 dilution
    - Only DESS, DNAgard®, & DNA Genotek from decomposed samples
- 7500 Real-Time PCR System [Thermo Fisher Scientific]

MATERIALS & METHODS

STR Genotyping

- Fresh tissue: 0.5ng of DNA added directly to PowerPlex® 21 System [Promega]
- Decomposed tissue: 0.5ng of DNA added directly to GlobalFiler® [Thermo Fisher Scientific]
- Samples amplified on 9700 & CE on 3500 [Thermo Fisher Scientific]

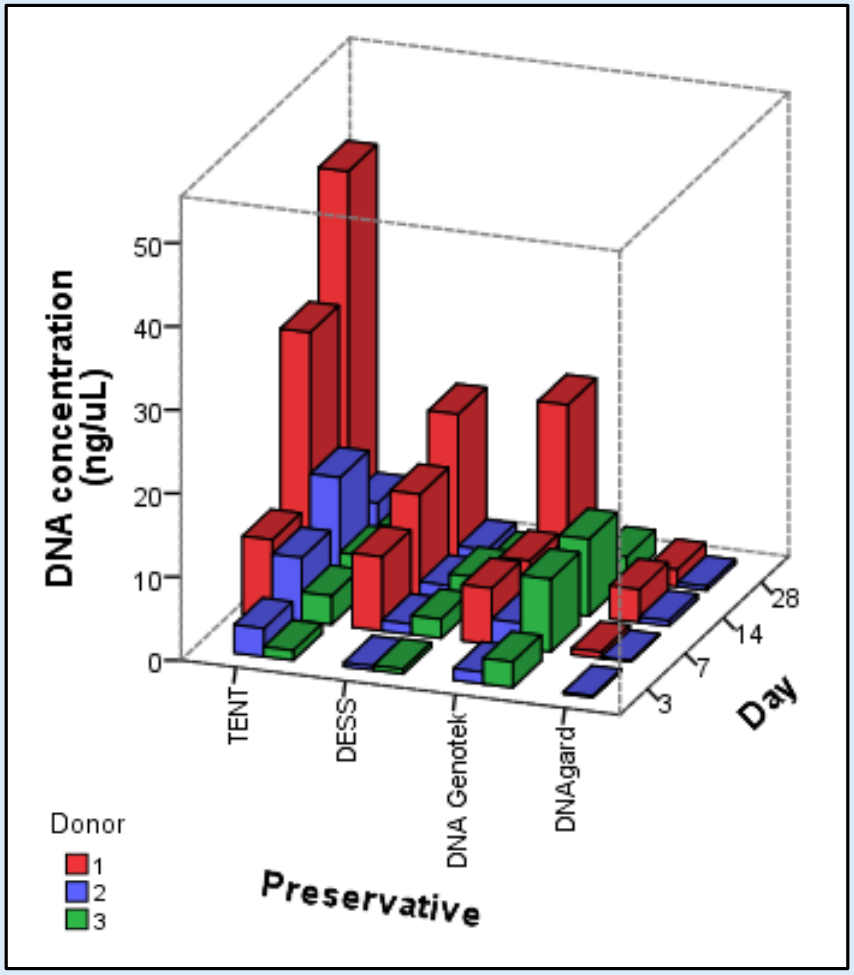
Statistical Analysis using SPSS [IBM]

- Shapiro-Wilk test for normality
- ANOVA (normally distributed data) or Kruskal-Wallis test (non-normal data)
- $p<0.05$

RESULTS - FRESH

DNA Concentrations in Preservatives

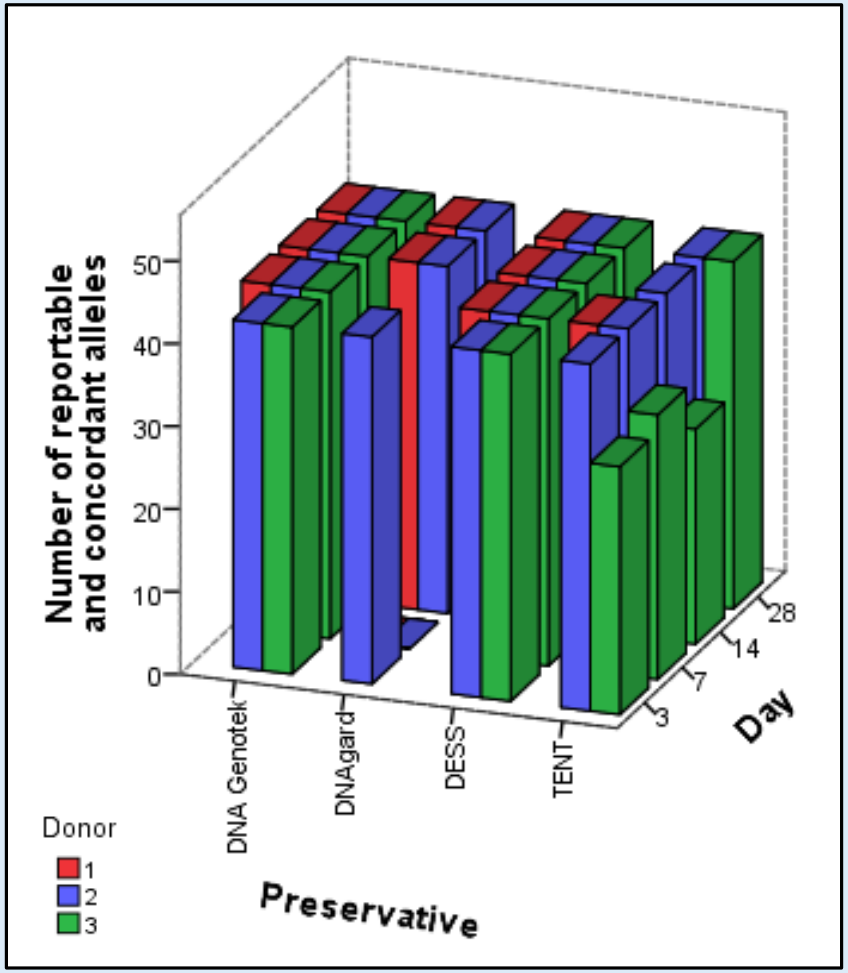
- No PCR inhibition
- DNA yield highly dependent on donor tissue (Kruskal-Wallis *p* value=0.003)
  - Highest [DNA] from donor 1
- Significant difference between preservatives (p=0.005)
  - TENT yielding highest [DNA]
  - DNAgard® with the least
- [DNA] increases for longer storage (up to 28 days)



RESULTS - FRESH

STR Genotyping in Preservatives (PowerPlex 21®)

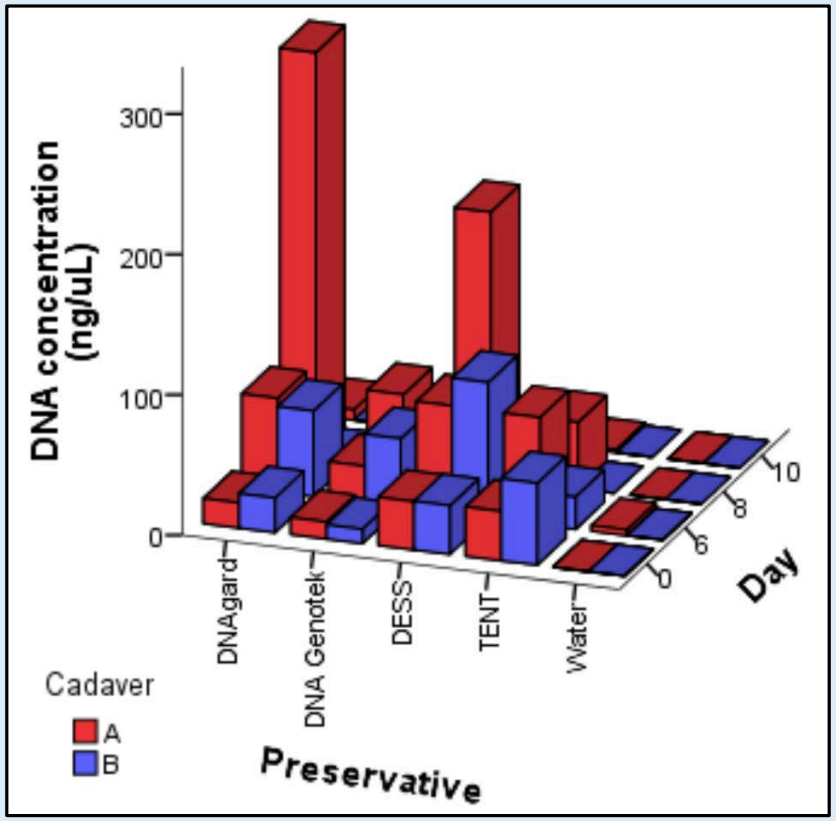
- DESS & DNA Genotek generated full profiles
- DNAgard generated full profiles (except day 7)
- TENT produced profiles with 26-42 alleles, peak heights diminished at higher molecular weight loci (Penta D & E)
- Donor 2 had highest peak heights for all loci & all preservatives



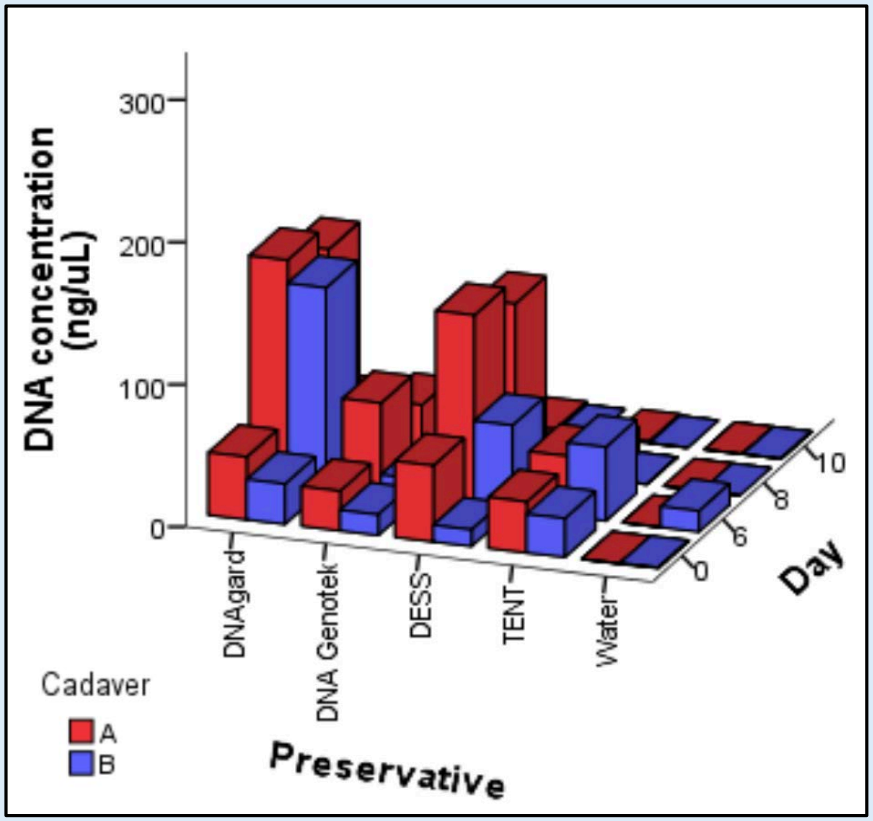
RESULTS - DECOMPOSED

DNA Concentrations in Preservatives

Skin



Muscle



RESULTS - DECOMPOSED

DNA Concentrations in Preservatives

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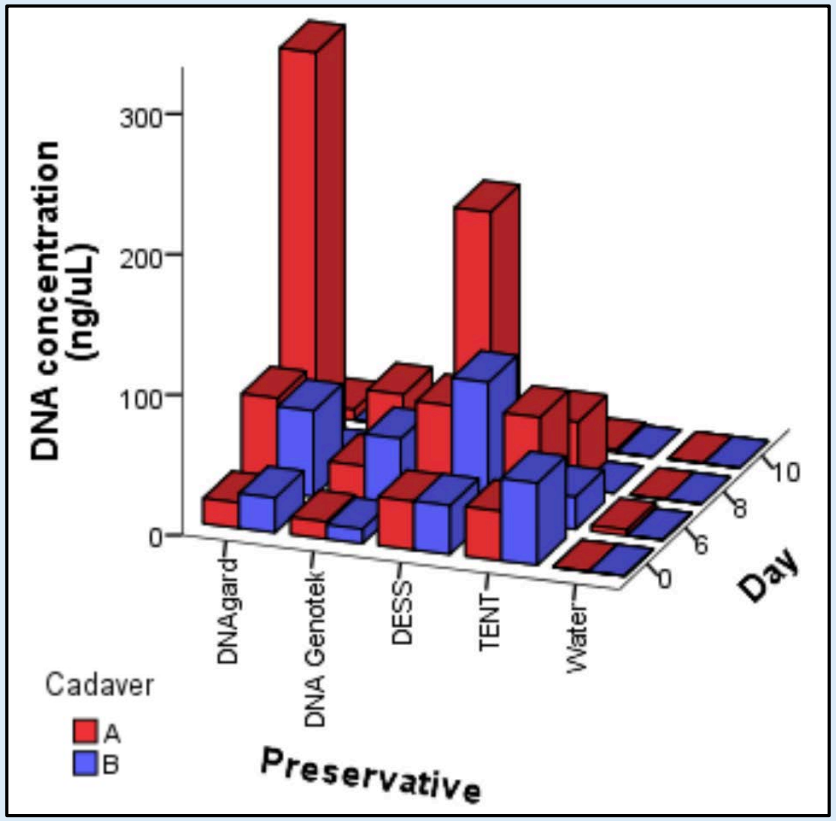
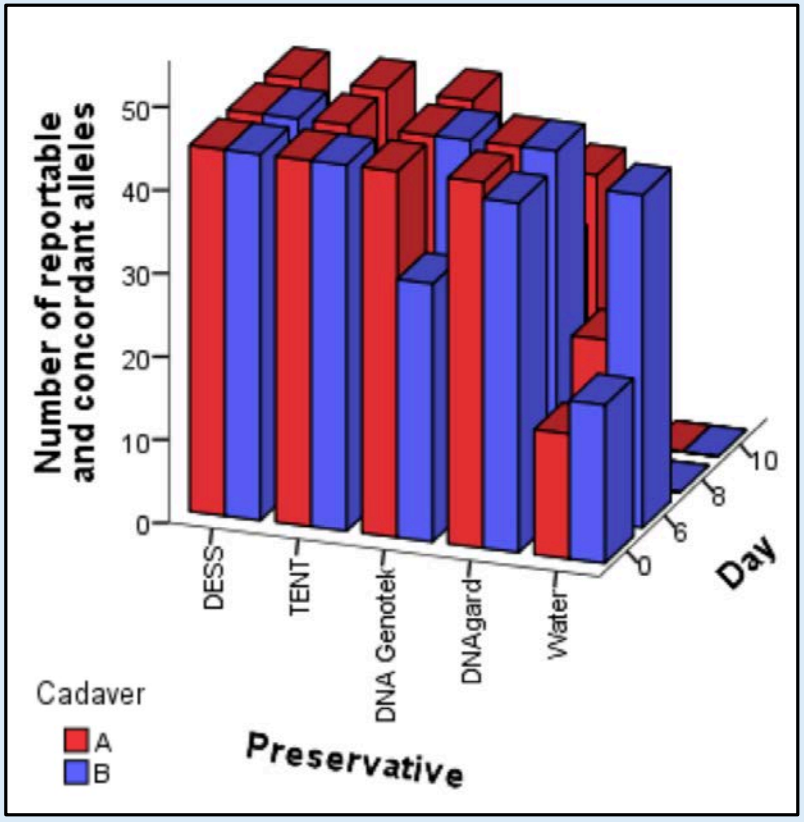


- Inhibition in preservatives surrounding decomp tissues
  - IPC only detected in TENT
  - 1:10 dil for DESS & DNAgard®
  - 1:20 dil for DNA Genotek
- All preservatives yielded more DNA than water control
- [DNA] increased from day 0 to 6, then decreased to nearly 0 at day 8 for B (skin & muscle) & at day 10 for A (consistent with time of bloat)

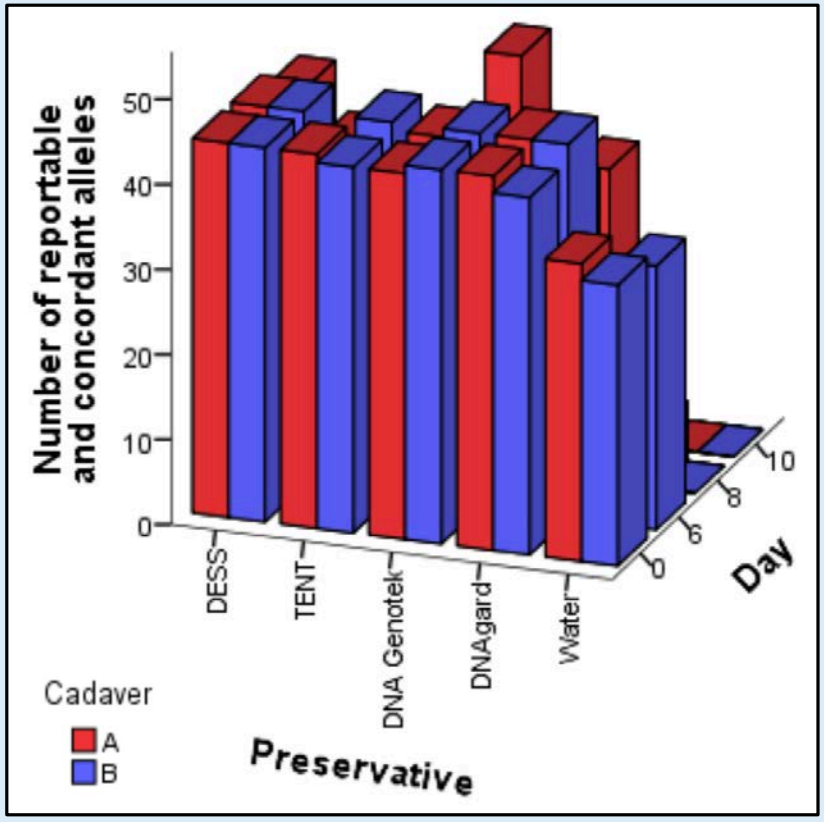
RESULTS - DECOMPOSED

STR Genotyping in Preservatives (GlobalFiler®)

Skin



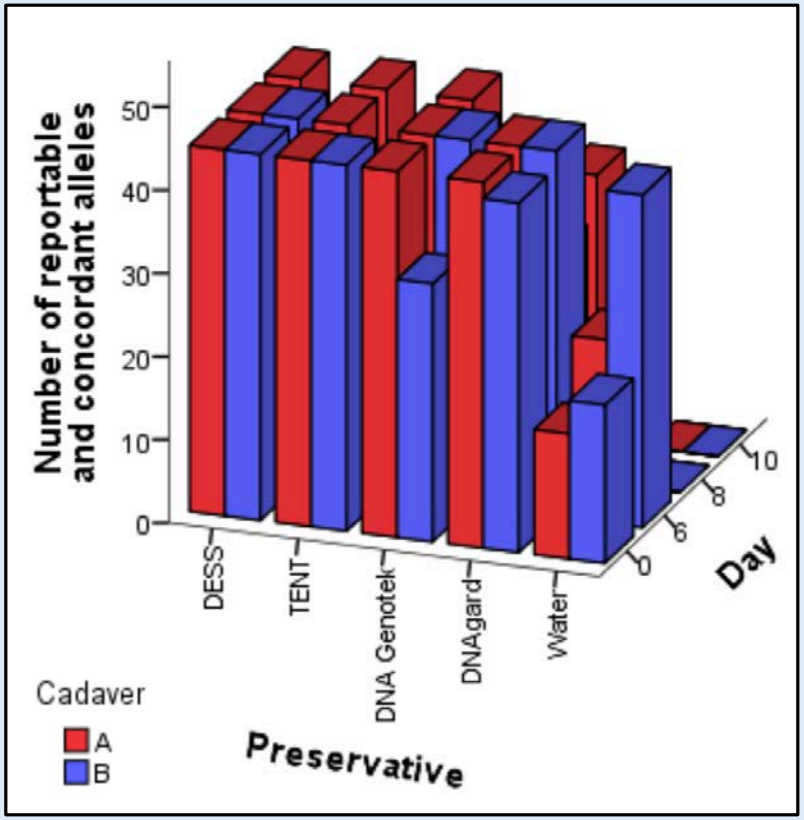
Muscle



RESULTS - DECOMPOSED

STR Genotyping in Preservatives (GlobalFiler®)

Skin



- 1:10 dil for DESS & DNAgard, 1:20 dil for DNA Genotek required to relieve inhibition
- Full GF profiles from DESS, TENT, & DNA Genotek up to 8 days from cadaver A skin
- All preservatives yielded full profiles up to 6 days from cadaver A skin & cadaver B muscle

DISCUSSION

- Aliquots of preservative can be stored at -80°C for up to 4 years:
  - All except TENT are likely to produce full profiles
  - Long term archival does not diminish successful DNA typing
  - Storage at -80°C reduces PCR inhibition (when compared to samples stored at room temp.)

- DNA extraction can be eliminated
  - Faster DNA based DVI
  - Any increase in inhibition (from preservatives) can be easily diluted out

- Direct PCR may be suitable for DVI, provided that:
  - Tissue is collected before full bloat or entering active decay
  - DNA is of sufficient quantity & quality

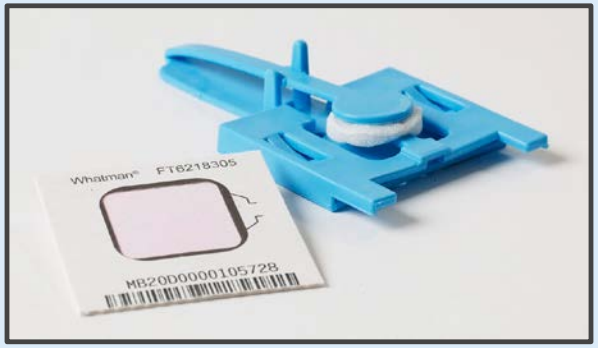
CONCLUSIONS

- Decomposing tissue can be preserved at room temp.
- Tissue digestion & DNA extraction can be avoided
- Direct PCR approach for identifying fresh & decomposed tissue preserved at room temp. is possible
- Complete profiles can be obtained in a more timelier manner
- *Significant impact to address demands for immediate sample preservation & provide faster DNA identification*

FUTURE WORK

- Optimizing TENT buffer to better preserve DNA for storage & facilitate direct PCR

- Collection of DNA directly from decomposing bodies using FTA Elute cards, Whatman EasiCollect devices, Bode Buccal DNA Collection System, & traditional cotton swabs for room temp. storage



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ACKNOWLEDGEMENTS

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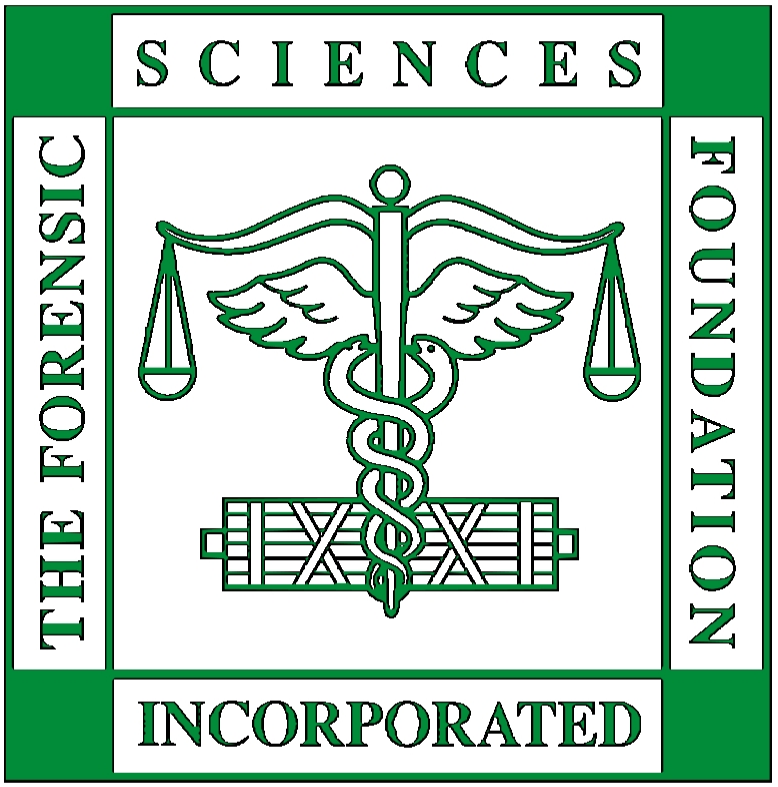
REFERENCES

- Allen-Hall A, McNevin D. (2012) Human tissue preservation for disaster victim identification (DVI) in tropical climates. Forensic Sci Int Genet 6(5):653-657.

For more information, refer to:

- Sorensen A, Berry C, Bruce D, Gahan ME, Hughes-Stamm S, McNevin D. (2015) Direct-to-PCR tissue preservation for DNA profiling. Int J Legal Med



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Questions?

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