


Post-Mortem DNA Extraction from Diverse Storage Methods

NATIONAL ASSOCIATION OF MEDICAL EXAMINERS
Monday, October 15, 2018


Gregory Webster, MD, MPH
Division of Cardiology
Ann & Robert H. Lurie Children's Hospital of Chicago
Northwestern University Feinberg School of Medicine
gregory.webster@northwestern.edu



Sudden unexpected death in the young

- Age 1 to 40 years
- Death in < 1 hour or witnessed alive and well within 24 hours

“What if I don’t have a lavender top tube?”



Gaps in knowledge

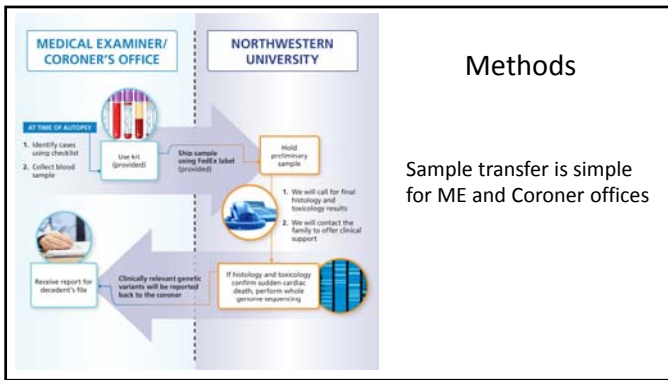
- Pathogenic variants occur in 20-30% of sudden death cases.
 - Not explained by genetic testing in the other 70%
- Our goal is to link molecular autopsy with clinical risk stratification

National Association of Medical Examiners Position Paper:
Retaining Postmortem Samples for Genetic Testing

Debra Walthers MD, Samantha Buckner MD DCC, Kim Deery MD DCC,
Christina Posthumus MD DCC, Jeff Jensen MD, Frank Miller MD,
J. Keith Franklin MD PhD, R. Anne Anderson MD, John Ketting MD DCC,
Carl Henry MD, Heather Weiland MD DCC,
Neil Francis MD
2017 July 19/18

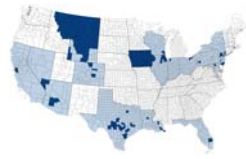
“For the purpose of potential genetic testing and/or DNA banking, an appropriate sample is 5-10 mL of blood collected at autopsy or as part of an external examination that is preserved with K2 EDTA (usually a purple top tube).”

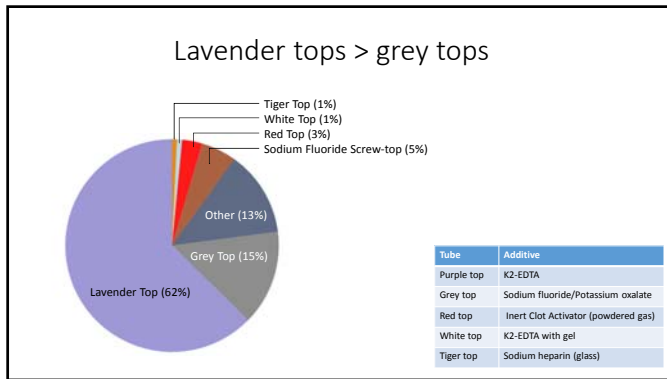


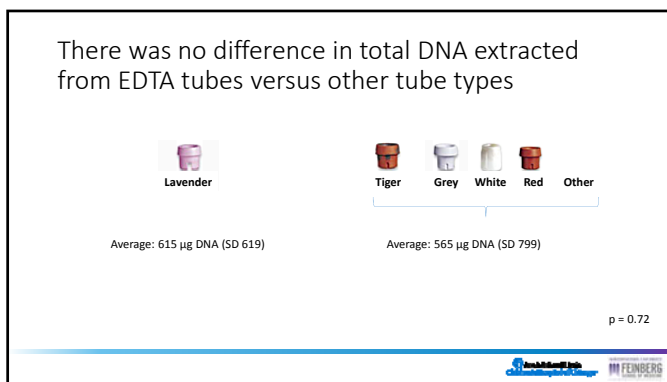


Methods

- Blood storage in EDTA was recommended
- Accepted in any container
- Extraction:
 - RNase treatment (Sigma Aldrich)
 - Protein precipitation (Qiagen)
 - Isopropanol precipitation (Qiagen PureGene)
- DNA was quantified using spectrophotometric analysis (NanoDrop).







Quantity, quality → whole genome sequencing

Using a threshold of 2 µg required for WGS, yield was sufficient in 101/103 cases (98%).

- 1 sample in a lavender top
- 1 sample in a grey top

Limitations

DNA yield per volume of blood could not be quantified reliably in this study.



Summary

1. Genetic sequencing reveals a pathogenic or likely pathogenic variant in 20-30% of cases
2. Lavender-top/EDTA is best
3. Send whatever tube you have.
4. We can often extract from left-over toxicology (e.g. NMS lab)

We would like to collaborate with you!
gregory.webster@northwestern.edu