Post-Mortem DNA Extraction from Diverse Storage Methods

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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
"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

Sample transfer is simple for ME and Coroner offices

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).
There was no difference in total DNA extracted from EDTA tubes versus other tube types

- Lavender tops > grey tops
- Average: 615 μg DNA (SD: 616)
- Average: 565 μg DNA (SD: 799)

p = 0.72

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!
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