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# Postmortem Blood Genomics Biorepository

Hilary A. Uyhelji
Christopher J. Tracy
Vicky L. White
Scott J. Nicholson

Civil Aerospace Medical Institute (CAMI)

Federal Aviation Administration

Oklahoma City, OK 73169

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**12. Abstract**

The Federal Aviation Administration Civil Aerospace Medical Institute Bioaeronautical Sciences Research Laboratory (BSRL) collects, processes, and analyzes forensic fluid and tissue samples from fatal civil aviation accidents in the United States. The BSRL issues standardized forensic sample collection kits (ToxBoxes) for medical examiners and coroners to collect and ship the samples needed for toxicological analysis. The BSRL Forensic Sciences section receives and assays incoming forensic samples to determine if the pilot consumed or was exposed to known drugs or toxic substances. This information is collected on behalf of the National Transportation Safety Board to assist in accident investigation. The BSRL Functional Genomics Research team previously examined gene expression patterns in such forensic samples. However, ToxBox forensic samples were not routinely preserved in a manner that prevented the degradation of those patterns during collection and storage. This report details establishment of protocols and selection of tubes to supplement ToxBox collections with preservation of blood for functional genomics analyses. To preserve ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in forensic samples and establish a biorepository of samples suitable for future gene expression analyses, a supplemental whole blood research specimen collection kit is now included in ToxBoxes. These kits have been distributed in ToxBoxes since August 2022.


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## List of Abbreviations

| | |
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| ACD | Acid Citrate Dextrose |
| BSRL | Bioaeronautical Sciences Research Laboratory |
| CAMI | Civil Aerospace Medical Institute |
| DIN | DNA Integrity Number |
| DNA | Deoxyribonucleic Acid |
| DOT | United States Department of Transportation |
| FAA | Federal Aviation Administration |
| IRB | Institutional Review Board |
| $K_2$EDTA | Ethylenediaminetetraacetic Acid Dipotassium |
| NTSB | National Transportation Safety Board |
| RNA | Ribonucleic Acid |

## Abstract

The Federal Aviation Administration Civil Aerospace Medical Institute Bioaeronautical Sciences Research Laboratory (BSRL) collects, processes, and analyzes forensic fluid and tissue samples from fatal civil aviation accidents in the United States. The BSRL issues standardized forensic sample collection kits (ToxBoxes) for medical examiners and coroners to collect and ship the samples needed for toxicological analysis. The BSRL Forensic Sciences section receives and assays incoming forensic samples to determine if the pilot consumed or was exposed to known drugs or toxic substances. This information is collected on behalf of the National Transportation Safety Board to assist in accident investigation. The BSRL Functional Genomics Research team previously examined gene expression patterns in such forensic samples. However, ToxBox forensic samples were not routinely preserved in a manner that prevented the degradation of those patterns during collection and storage. This report details establishment of protocols and selection of tubes to supplement ToxBox collections with preservation of blood for functional genomics analyses. To preserve ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in forensic samples and establish a biorepository of samples suitable for future gene expression analyses, a supplemental whole blood research specimen collection kit is now included in ToxBoxes. These kits have been distributed in ToxBoxes since August 2022.

## Introduction

The Federal Aviation Administration (FAA) investigates fatal aviation accidents within the borders of the United States in cooperation with the U.S. National Transportation Safety Board (NTSB). Investigations examine several aspects of the accident, including the contribution of medical factors. Autopsies on aircraft pilots who die in the accident are performed by medical examiners or coroners with jurisdiction over the accident site. These autopsies culminate in a report detailing the injuries and observed medical conditions, and include the collection of tissue and fluid samples for forensic toxicological analyses conducted at the FAA Civil Aerospace Medical Institute (CAMI) Bioaeronautical Sciences Research Laboratory (BSRL). The autopsy report and toxicology results are provided to the NTSB to determine the findings and potential factors contributing to the accident. This work is performed under the authority of FAA Order 8020.11D, which directs accident investigators to coordinate provision of the ToxBox to the FAA Forensic Toxicology Research Team of the BSRL. This team “detects and measures drugs, alcohol, toxic gases, and toxic industrial chemicals in victims of fatal aircraft accidents as a contribution to the analysis of accident causation” (U.S. Federal Aviation Administration, 2021a). Additionally, FAA Order 1100.1C requires the FAA Office of Aviation Safety to investigate and support NTSB in investigation of aircraft accidents and incidents (U.S. Federal Aviation Administration, 2021b).

To this end, the BSRL’s Forensic Sciences section provides ToxBoxes to medical examiners and coroners so that supplies and instructions are immediately available to the collector. ToxBoxes are insulated shipping coolers containing collection supplies for whole blood, vitreous humor, spinal fluid, bile, urine, gastric contents, liver, kidney, heart, lung, spleen, brain, and muscle tissue samples. ToxBoxes are regionally pre-positioned at local FAA Flight Standards District Offices or larger medical examiner’s offices so that boxes are available with

minimal delay (Federal Aviation Administration, 2020). Under the medical privacy requirements of the Health Insurance Portability and Accountability Act of 1996, the FAA is considered a "public health authority", with authorization for receipt of protected health information (Federal Aviation Administration, 2006). The FAA Forensic Sciences section performs accessioning and toxicological analysis of forensic samples (Federal Aviation Administration, 2019). Depending on availability, forensic samples may also be used for civil aeromedical research in the interest of increasing aviation safety as authorized by 49 U.S.C. §44507(a)(1) (House of Representatives, Congress, 2021). Protections are taken to safeguard sensitive data and to de-identify specimens and data used in research.

Also within the BSRL, the Functional Genomics Research team conducts studies to identify molecular biomarkers, as described by the Biomarkers Definition Working Group (2001). While often applied to health monitoring, biomarkers can be used to understand the human response to potential aviation safety risk factors, such as pilot impairment due to fatigue, drug use, hypoxia, and other factors. These molecular biomarkers may be developed into useful accident investigation and prevention tools. However, optimal storage and preservation conditions for genetic molecules, such as ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), often differ from those used for forensic toxicological work. The Functional Genomics Research team has analyzed forensic specimens collected during the accident investigation process to examine the usefulness of postmortem samples for biomarker detection. In a study examining blood collected at the time of autopsy using collection tubes designed specifically to stabilize and preserve RNA, postmortem samples were capable of producing sufficient RNA for analysis (Burian et al., 2017). However, beyond limited-term research projects, no samples were routinely collected within the ToxBoxes for the express preservation and use in gene expression research.

The Functional Genomics Research team began placing whole blood research collection kits for DNA and RNA preservation in ToxBoxes in late 2022, initiating a long-term effort to bank specimens in a postmortem blood biorepository. Beyond DNA and RNA research, biorepository samples could be used for other functions, including metabolite and protein assessment or potentially pathogen genotyping when infections are expected. Based on the Functional Genomics Research team's historical work of identifying RNA biomarkers, it was known that PAXgene RNA blood tubes would be the highest priority to add to the ToxBoxes for gene expression research. Also, a secondary tube was of interest to preserve DNA for future use. Whereas RNA expression levels can identify transient conditions such as hypoxia or current cognitive impairment status, DNA can be a more stable indicator of long-standing health or performance safety risk factors. In this study, we compared two DNA extraction kits and protocols to determine which was best suited for extracting genomic DNA from clotted/coagulated whole blood as might be collected from accident victims. We also tested the performance of three whole blood collection tubes for downstream DNA extraction. The results were used to select appropriate kit components to place in ToxBoxes to establish a postmortem blood biorepository.

## Materials and Methods

### Institutional Review Board and Waiver of Consent

All research was conducted with the approval of the FAA Institutional Review Board (IRB). To perform research comparing sample collection tubes and methodologies, blood was collected from volunteers following informed consent. Additionally, an IRB waiver of consent was obtained to permit the collection and long-term storage of specimens from decedents, as a human subject is defined by 49 C.F.R. §11.102(f)(1-2) as “a living individual about whom an investigator conducting research obtains data through intervention or interaction with the individual, or identifiable private information” (Office of the Federal Register, National Archives and Records Administration, 2023). Because research specimens for the biorepository are only collected from deceased individuals based on the unforeseen circumstances of fatal accidents, individual or familial consent was not obtained for specimen inclusion. Readily identifiable personal information is protected and was not disclosed to non-BSRL personnel.

### Genomic DNA Extraction Kit Determination

To compare extraction methods and performance on potentially degraded samples, blood was collected from volunteer participants by venipuncture into Vacutainer No Additive tubes (BD Biosciences, 366408) by a trained phlebotomist at the CAMI Occupational Health Clinic. The blood tubes were allowed to coagulate overnight at room temperature (approximately 21 °C at the time of the experiment) before being transferred to a refrigerator at 4 °C for 24 hours. The coagulated blood was then poured into plastic PAXgene Blood DNA tubes (BD Biosciences, 761165).

All PAXgene Blood DNA tubes underwent a 24-hour step-down at -20 °C in wire racks to prevent thermal shock to the tubes and reduce the risk of cracking. They were then stored at -80 °C for an additional 24 hours. Tubes were allowed to thaw at room temperature for 2 hours prior to extraction. To compare extraction methods, genomic DNA was extracted from the coagulated blood using the QIAamp DNA Blood Mini kit (QIAGEN, 51104) following the “DNA Purification from Blood or Body Fluids (Spin Protocol)” protocol from the QIAamp DNA Mini and Blood Mini Handbook, and with the Gentra Puregene Blood kit (Qiagen, 158467) using Clotspin Baskets (Qiagen, 158932) following the Qiagen Supplementary Protocol PG03. Normal saline (sodium chloride 0.9%, Intermountain Life Sciences, Z1377) was used to bring the total input volume for each sample for the Gentra kit to the 5 mL minimum input volume described in the PG03 protocol.

### Genomic DNA Tube Determination

Additional blood samples were taken to compare DNA preservation among tubes with different chemical additives. Blood was collected by venipuncture into PAXgene Blood DNA tubes, plastic Vacutainer Ethylenediaminetetraacetic Acid Dipotassium (K2EDTA) 10.8 mg (6 mL) tubes (BD Biosciences, 367863), and glass Vacutainer Acid Citrate Dextrose (ACD) Solution B tubes (BD Biosciences, 364816) by a trained phlebotomist at the CAMI Occupational Health Clinic. Six biological replicates of each tube type were collected and underwent step-down freezing (as above). Tubes were thawed at room temperature for 2 hours, then extracted with the QIAamp DNA Blood Mini kit following the “DNA Purification from Blood or Body Fluids (Spin Protocol)” protocol from the QIAamp DNA Mini and Blood Mini Handbook.

## DNA Quality Control

DNA was analyzed for purity and yield using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, ND-2000c) and a Qubit 3.0 fluorometer (ThermoFisher Scientific, Q33216) using the dsDNA BR (Broad Range) Assay kit (ThermoFisher Scientific, Q32850). DNA Integrity Numbers were calculated using a 4200 TapeStation system (Agilent, G2991BA) with Genomic DNA ScreenTape tapes and reagents (Agilent, 5067-5365 and 5067-5366).

## Biorepository – Kit Assembly

After blood collection components were selected, whole blood research specimen collection kits were assembled and placed into ToxBoxes prior to shipment to medical examiners and coroners. The kits consisted of collection instructions, a PAXgene Blood RNA tube (Qiagen, 762165) and a K2EDTA tube for DNA: either K2EDTA 5.4 mg (3 mL) Vacutainer tubes (BD Biosciences, 367856), or K2EDTA (6 mL) Vacuette tubes (Greiner Bio-One, 456002). Although smaller 3 mL tubes were preferred in recognition that available blood can be limited in some accidents, the specific K2EDTA tube depended on the availability of components; 6 mL tubes were included in earlier kits, and 3 mL tubes were included in later kits. Tubes were enclosed in a labeled Styrofoam tube mailer (Sonoco, 364), and a 10 cc disposable syringe (Air-Tite Products, AL10), and a 16 g x 4” hypodermic needle (Air-Tite Products, N164) were added to the kit to permit standardized collections. All of the kit components were contained within a biohazard-labeled zip-lock bag (Fisherbrand, 01-800-08). Initial PAXgene tubes received from autopsies exhibited variable blood fill levels. Subsequently, kit assembly was modified to ensure PAXgene tubes were manually labeled with a fill line and the word “FILL” indicating the desired 2.5 mL blood volume, improving consistency.

## Biorepository – Sample Collection

PAXgene Blood RNA tubes and K2EDTA tubes were filled by medical examiners and coroners during the autopsy of aviation accident victims. Filled blood tubes were shipped to CAMI and received by the Forensic Sciences Quality Assurance team in ToxBoxes (consisting of an insulated Styrofoam shipping container and frozen gel packs to maintain temperature). The tubes were stored at -20 °C from receipt by Quality Assurance personnel until being provided to the Functional Genomics Research team. Each tube from each accident victim was assigned a unique identifier code. Any personally identifiable information was redacted, and the tube was labeled, then frozen by step-down at -20 °C for 24 hours before long-term storage at -80 °C.

## Biorepository – Data Collection

The following data were recorded for each tube: basic sample characteristics, including purpose (biorepository), condition (autopsy), nuanced IRB considerations when applicable (e.g., the potential for victims to be non-U.S. citizens); whether the blood tube was frozen upon receipt by the Quality Assurance team; the date the ToxBox was received; the date the sample was taken into possession by the Functional Genomics Research team; the preservative/tube type; the Forensic Toxicology case number; the sample intake notebook reference; whether sample intake deviated from the normal receipt protocol and, if so, how; the date and time the sample began the step-down at -20 °C; and the date and time the sample was stored at -80 °C. Additional information noted related to the quality of the sample (the visual appearance of the sample, whether the tube was filled correctly, the blood source if indicated), information related

to the tube itself (the tube lot number, whether the tube was expired at the time of sample collection and if so by how many days), and general sample tracking (the versions of the forms included with the collection kits sent to the medical examiners/coroners and the images taken of the tubes). As a form of quality control, each sample page is checked by a Functional Genomics Research team member other than the individual who performed the initial data entry. Both samples and data are maintained indefinitely as part of the biorepository. Although it is preferred to use and fill tubes that are not expired, the timeframes between providing a ToxBox kit to medical examiners and coroners and their actual use can sometimes result in filling tubes past their expiration date. Based on previous experience at the BSRL with other types of blood collection tubes, expired tubes still provide useful biospecimens. The primary concern is the potential failure of the vacuum seal, which facilitates precise volume control of blood draws. However, sample quality is not anticipated to degrade much if at all with use of expired tubes.

# Results

## Genomic DNA Extraction Kit Determination

To determine a proper means of extracting DNA from clotted or coagulated blood (common during postmortem collection), two extraction kits/protocols (QIAamp DNA Blood Mini kit and Gentra Puregene Blood kit using Clotspin Baskets) were compared. As the sample input volume required by each kit differs (the Gentra protocol requires a minimum of 5 mL of clotted blood, while the Qiagen protocol requires 200 µL whole blood), both concentration and the total DNA yield of the samples were considered. The mean 260/280 nm absorbance ratios were evaluated as an indication of the purity of nucleic acid, with a ratio of approximately 1.8 generally accepted as pure for DNA. Samples extracted by the two methods were comparable, with both methods producing pure genomic DNA from clotted/coagulated whole blood (Table 1). Also, the mean DNA Integrity Number (DIN) values (an indicator of the fragmentation within a genomic DNA sample, with a value of greater than 8.5 indicating high quality) were similar. Both methods had low mean 260/230 nm absorbance ratios (260/230 ratios <2.0 are considered low and may indicate higher extraction component carryover or impurities).

**Table 1**

*Comparison of Mean ± 1 Standard Deviation of DNA Quality Metrics Between Extraction Methods (N=3).*

| Extraction Kit | Nucleic Acid Conc. (ng/µL) | 260/280 nm Ratio | 260/230 nm Ratio | dsDNA Conc. (ng/µL) | dsDNA Yield (µg) | DNA Integrity Number (DIN) |
|---|---|---|---|---|---|---|
| Gentra Puregene | 89.7 ± 9.1 | 1.87 ± 0.16 | 1.36 ± 0.07 | 76.7 ± 8.7 | 38.2 ± 3.7 | 9.4 ± 0.1 |
| Qiagen QIAamp | 12.8 ± 2.1 | 1.86 ± 0.12 | 0.70 ± 0.04 | 9.8 ± 1.7 | 1.9 ± 0.3 | 9.0 ± 0.1 |

*Note.* Nucleic acid concentrations and ratios were assessed with a Nanodrop 2000, whereas dsDNA concentration and yield were quantified with a Qubit 3.0.

## Genomic DNA Tube Determination

In comparing blood preserved with PAXgene Blood DNA tubes, K2EDTA Vacutainer tubes, and ACD Solution B Vacutainer tubes, the three tube types produced similar DNA quantity and quality metrics (Table 2). Following the freezer step-down procedure, all tubes were intact without cracking. The PAXgene Blood DNA tubes performed marginally better than the other two tube types for all metrics save DIN, for which the ACD Solution B tubes performed best. All three tube types had mean 260/280 nm absorbance ratios above 1.8, indicating pure DNA, and had mean DINs at or about 8.5, indicating that the genomic DNA was of high quality and intact.

**Table 2**

*Comparison of Mean ± 1 Standard Deviation of DNA Quality and Quantity Metrics Among Tube Collection Types (N=6)*

| Tube Type | Nucleic Acid Conc. (ng/µL) | 260/280 nm Ratio | 260/230 nm Ratio | dsDNA Conc. (ng/µL) | dsDNA Yield (µg) | DNA Integrity Number (DIN) |
|---|---|---|---|---|---|---|
| PAXgene Blood DNA | 33.0 ± 5.3 | 1.84 ± 0.03 | 1.68 ± 0.23 | 27.1 ± 5.9 | 5.4 ± 1.1 | 8.4 ± 0.2 |
| $K_2$EDTA 10.8 mg (6 mL) | 30.4 ± 8.0 | 1.84 ± 0.05 | 1.55 ± 0.12 | 24.0 ± 6.6 | 4.8 ± 1.2 | 8.5 ± 0.2 |
| ACD Solution B | 27.0 ± 7.0 | 1.84 ± 0.04 | 1.58 ± 0.20 | 21.3 ± 5.2 | 4.3 ± 0.9 | 8.6 ± 0.3 |

*Note.* Nucleic acid concentrations and ratios were assessed with a Nanodrop 2000, whereas dsDNA concentration and yield were quantified with a Qubit 3.0.

## Biorepository – Kit Assembly

Biorepository collection kits were assembled to be included in each ToxBox separately from Toxicology specimen collection supplies. To emphasize this distinction, the included instructions in the whole blood research specimen collection kits (Figure 1) are printed on bright green paper. The contents of the kit are also described in these instructions. Each kit contains a PAXgene Blood RNA tube for the stabilization of RNA, and a K2EDTA tube for the stabilization of genomic DNA, located within a Styrofoam tube holder/mailer affixed with a label (Figure 2, 3). Collection kits were sent to the field beginning in August of 2022, yielding samples from 48 decedents through early May 2024.

**Figure 1**

*Instructions for Collection of Whole Blood Research Specimens*

## INSTRUCTIONS FOR COLLECTING WHOLE BLOOD RESEARCH SPECIMENS

(FOR FATALITIES ONLY)

**Please ensure that toxicology blood tubes (gray and green top) are filled prior to filling these research tubes. If there is limited blood available, toxicology tubes take priority and these research tubes may not be filled.**

CONTENTS OF THIS KIT
1 ea. "Instructions for Collecting Whole Blood Research Specimens" (these instructions)
1 ea. PAXgene® and $K_2$EDTA tubes in Styrofoam holder with rubber band
1 ea. 10 cc disposable syringe
1 ea. 16 g x 4" Needle
1 ea. biohazard zip-lock bag

WHOLE BLOOD RESEARCH SPECIMENS (RED-TOPPED PAXgene® AND PURPLE $K_2$EDTA TUBES)
Collect these research specimens following collection of whole blood samples detailed in "Instructions for Collecting and Shipping Toxicology Specimens."

Using the provided 16 g x 4" needle and 10 cc syringe, fill the tubes to the specified volume:
Red-topped PAXgene®: 2.5 mL
Purple $K_2$EDTA: 3 mL
If blood is limited, fill the PAXgene® tube before the $K_2$EDTA tube.

Note: Immediately after the blood collection, ensure proper mixing of the preservative and anticoagulant by slowly inverting the blood tubes at least 10 times. Do not shake vigorously!

Return the filled tubes to their original Styrofoam container. Then, complete the requested information on the label of the container. Replace rubber band to secure container and return the container to the biohazard zip-lock bag.

**Note: These tubes do not replace, and are not to be used in lieu of, the toxicology tubes detailed in "Instructions for Collecting and Shipping Toxicology Specimens."**

Safety information: The latest SDS can be downloaded from https://regdocs.bd.com/regdocs/sdsSearch. For PAXGene® input catalog number 762165; for $K_2$EDTA use 367856.

Please direct any questions regarding the collection of research specimens in PAXgene® and $K_2$EDTA tubes to Genomics@faa.gov. All other ToxBox question should be directed to 9-AMC-AAM600-SPECIMENS@faa.gov.

FAA_GEN_form1_v3

*Note.* Instructions to medical examiners and coroners included in the whole blood research specimen collection kits detailing how to fill and handle the whole blood collection tubes. The version of the instructions shown here is current at the time of writing.

**Figure 2**

*Blood Tube Holder Label on Research Specimen Collection Kit*

# WHOLE BLOOD

(RED PAXgene® AND PURPLE $K_2$EDTA TUBES)

FILL ONLY AFTER TOXICOLOGY TUBES

RESEARCH USE ONLY, NOT FOR ID PURPOSES

*EXPIRATION DATES ARE NOT APPLICABLE TO POSTMORTEM SAMPLES*

Victim Name:_____

Date of Accident:_____

Autopsy By:_____

Date of Autopsy:_____

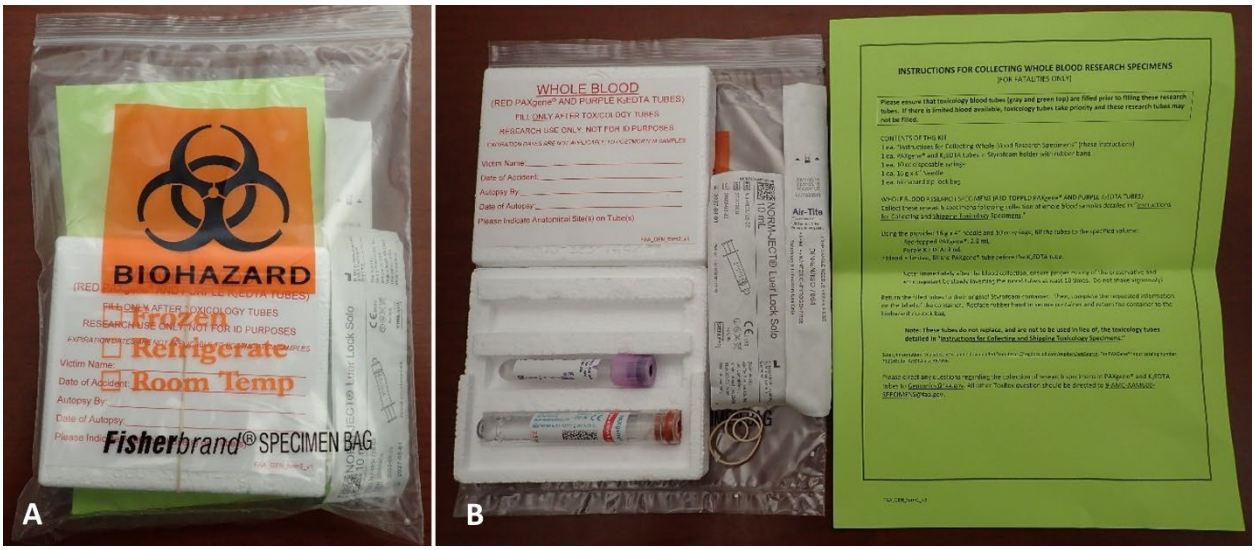
Please Indicate Anatomical Site(s) on Tube(s)

FAA_GEN_form2_v1

*Note.* The adhesive label affixed to the Styrofoam tube holder included in the specimen collection kits. As shown, the label includes fields for the victim's name, the date of accident, the name of the individual who conducted the autopsy, and the date of the autopsy. The version of the label shown here is current at time of writing.

**Figure 3**

*Research Specimen Collection Kit, Assembled and Individual Components*




*Note.* Panel A shows an assembled kit. Panel B shows individual kit components, with the tube holder opened and the PAXgene Blood RNA tube and $K_2$EDTA tube visible.

## Discussion

This report documents the establishment of a unique and long-term resource for civil aviation accident investigation. In general, sample banking has evolved to cover a diverse array of collection and storage approaches, allowing long-term accumulation of massive quantities of samples and data and providing unique opportunities for biomarker research and health care (Conroy et al., 2023; Coppola et al., 2019; Riegman et al., 2008). Long-term banking approaches can provide readily available specimens and data, and facilitate studies requiring large sample sizes for sufficient statistical power. For example, the UK Biobank has enabled population-based cancer research and accelerated identification of genetic variants for cancer risk (Conroy et al., 2023). Because of the relative infrequency of fatal civilian aviation accidents, advance planning with a biorepository approach is warranted to collect enough postmortem specimens for research. In 2017, the FAA BSRL received specimens from 272 individuals for aviation accident investigation, of which 130 tested positive for at least one drug (Cliburn et al., 2020). Depending on the accident factor of interest and how commonly it occurs, years of collection may be required to obtain an adequate number of cases for reliable statistical analysis.

With the establishment of the FAA's postmortem blood genomics biorepository, the agency will have the resources to test the application of novel molecular techniques to accident investigation. Technological advances and discoveries are constantly increasing the ability of molecular assays to deliver new insights and might expand the toolkit available for accident investigators. Analysis of DNA has long had a role in forensic criminal investigation and identification of persons (Budowle & van Daal, 2018; Jordan & Mills, 2021). Newer postmortem genetic research has revealed patterns associated with opioid use disorder and could improve the diagnosis of cardiomyopathy and sudden cardiac death (Liu et al., 2021; Christiansen et al., 2016; Tian et al., 2021).

### Genomic DNA Extraction Kit Determination

Both kits provided comparable performance in handling of clotted/coagulated blood with regards to genomic DNA purity and quality, cost, simplicity, and hands-on processing time. Additionally, the Qiagen protocol requires 200 µL whole blood, while the Gentra protocol requires a minimum of 5 mL of clotted blood. This led to the selection of the Qiagen QIAamp DNA Blood Mini kit in conjunction with the “DNA Purification from Blood or Body Fluids (Spin Protocol)” protocol for DNA extraction from whole blood samples.

### Genomic Tube Determination

Based on the current and previous use of PAXgene RNA Blood tubes by the Functional Genomics Research team, this tube type was a desired kit component to promote applications of the team’s gene expression research findings. PAXgene Blood RNA tubes are widely used to collect and preserve RNA from whole blood samples. They also have been used by the Functional Genomics Research team to successfully preserve RNA from aviation accident postmortem specimens and conduct biomarker research (Burian et al., 2017; Chai et al., 2005; Duale et al., 2012; Liu et al., 2015; Tang et al., 2019). However, DNA molecules also can yield insights that may one day be useful in accident investigation. Indeed, genetic mutations are currently used in limited circumstances when issuing medical certificates to pilot applicants. Following a stroke, the Factor V Leiden and Prothrobmin (Factor II) G20210A mutations may be

assessed for a pilot applicant as part of a comprehensive hypercoagulopathy panel on a case-by-case basis (Federal Aviation Administration, 2024).

While selecting a blood DNA collection tube, each of the tube types examined produced similar purity and quality of extracted genomic DNA, but other tube attributes were considered in tube selection. The PAXgene Blood DNA tubes and the K2EDTA tubes are plastic, whereas the ACD Solution B tubes are glass. To reduce the risk of shattering, the ACD tubes were removed from consideration. Further considerations of cost and historical and predicted availability led to the selection of K2EDTA tubes. Additionally, K2EDTA provides the added benefit of being a multi-purpose preservative. Samples collected in these tube types may also allow isolation of other molecules, such as metabolites or proteins, in case research needs expand in the future.

## Conclusion

This report documents the successful establishment of an FAA sample biorepository from initial protocol development and kit selection to implementation. We analyzed the performance of two DNA extraction methods and three tube types to arrive at a final determination that K2EDTA vacutainer tubes provided optimal DNA stabilization and storage characteristics, and this tube is provided in conjunction with the previously assessed PAXgene Blood RNA tube for blood RNA stabilization. These tubes are assembled into a biorepository sample collection kit, which includes applicable instructions and necessary collection supplies. This is packaged separately from the toxicology sample collection supplies, with the expectation that blood for the biorepository kit will be collected after all toxicological collections have been performed to ensure that the primary purpose of the ToxBox is fulfilled. Samples for this nascent biorepository will continue to be collected and stored, and may serve as a valuable resource for future aviation safety research to improve understanding of the causes of aviation accidents. Once enough samples are accumulated in the biorepository, they can be tested for the relative incidence of biomarker findings in cases associated with known or suspected aviation safety risk factors. Such research can be used to test and validate the application of biomarker metrics originally developed in living subject research to postmortem civil aviation accident victims. Ultimately, to transition from research to application in accident investigation, additional consideration for sample chain of custody and legal requirements will require review. Meanwhile, the biobank is expected to allow the FAA to directly test protocols on real-world cases to verify which gene sets perform best in the challenging field of forensic accident analysis.

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