
Federal Aviation Administration

DOT/FAA/AM-23/35
Office of Aerospace Medicine
Washington, DC 20591

# Utility of Capillary Blood for Gene Expression Studies

Christopher J. Tracy
David C. Hutchings
Vicky L. White
Scott J. Nicholson

Civil Aerospace Medical Institute
Federal Aviation Administration
6500 S. MacArthur Blvd
Oklahoma City, OK 73169

**February 2024**

**NOTICE**

This document is disseminated under the sponsorship of the U.S. Department of Transportation in the interest of information exchange. The United States Government assumes no liability for the contents thereof.

---

This publication and all Office of Aerospace Medicine technical reports are available in full-text from the National Transportation Library's Repository & Open Science Access Portal (https://rosap.ntl.bts.gov/)

**Technical Report Documentation Page**

| | | |
|---|---|---|
| 1. Report No.<br>DOT/FAA/AM-23/35 | 2. Government Accession No.<br>Click or tap here to enter text. | 3. Recipient's Catalog No.<br>Click or tap here to enter text. |
| 4. Title and Subtitle<br>Utility of Capillary Blood for Gene Expression Studies | | 5. Report Date<br>11/1/2023 |
| | | 6. Performing Organization Code<br>AAM-612 |
| 7. Author(s)<br>Christopher J. Tracy (https://orcid.org/0000-0002-4532-3327)<br>David C. Hutchings (https://orcid.org/0009-0008-4575-8444)<br>Vicky L. White (https://orcid.org/0009-0007-1164-5233)<br>Scott J. Nicholson (https://orcid.org/0000-0002-2201-744X) | | 8. Performing Organization Report No.<br>DOT/FAA/AM-23/35 |
| 9. Performing Organization Name and Address<br>Civil Aerospace Medical Institute<br>Federal Aviation Administration<br>6500 S. MacArthur Blvd<br>Oklahoma City, OK 73169 | | 10. Work Unit No. (TRAIS)<br>Click or tap here to enter text. |
| | | 11. Contract or Grant No.<br>Click or tap here to enter text. |
| 12. Sponsoring Agency Name and Address<br>Office of Aerospace Medicine<br>Federal Aviation Administration<br>800 Independence Ave., S.W.<br>Washington, DC 20591 | | 13. Type of Report and Period Covered<br>Technical Report |
| | | 14. Sponsoring Agency Code<br>Click or tap here to enter text. |

15. Supplementary Notes
Technical report DOI: https://doi.org/10.21949/1529628

16. Abstract
Blood collection method selection is critical when analyzing blood gene expression. Multiple blood collection methods and sites exist, each with advantages and disadvantages. For human blood gene expression analyses, blood is commonly collected by venipuncture at the antecubital fossa (interior elbow) using a range of vacutainer tubes. Several vacutainer tube types contain RNA-preserving additives, all adequately preserving blood RNA. Most human subjects accept venipuncture, which collects sufficient high-quality blood and RNA suitable for gene expression analysis by RNA sequencing (RNA-Seq). Venipuncture has drawbacks; it requires trained personnel, carries a risk of injury, is time-consuming, and is often unsuitable for in-the-field, at-home, or self-collection. Capillary blood collection by fingerstick is common and widely practiced but not often used for RNA-Seq analysis due to low and variable blood quantity, a lack of blood RNA preservation options, low RNA yields, and variable RNA quality. This study used total RNA-Seq to compare two different fingerstick blood collection, preservation, and RNA extraction methods with a commonly used venipuncture blood collection and RNA extraction method. We demonstrate that fingerstick blood collection produces RNA suitable for RNA-Seq, that each fingerstick method produces results more similar to one another than venipuncture-derived blood RNA, and that each fingerstick blood collection method can distinguish between experimental groups (male and female subjects). While the venipuncture method examined here is generally preferable to the fingerstick blood collection methods, particularly in well-controlled and resourced environments, capillary blood is suitable and useful for gene expression analysis.

| | | |
|---|---|---|
| 17. Key Word<br>Venous, capillary, blood draw, aviation accident investigation, RNA | 18. Distribution Statement<br>Document is available to the public through the National Transportation Library: https://rosap.ntl.bts.gov/ | |
| 19. Security Classification. (of this report)<br>Unclassified | 20. Security Classification. (of this page)<br>Unclassified | 21. No. of Pages<br>38 |

## Acknowledgements

We would like to thank Tracie Allison of the CAMI Occupational Clinic for her assistance and expertise in collecting samples used in this study.

## Table of Contents

## Tables and Figures

## List of Abbreviations

| Abbreviation | Definition |
|---|---|
| ANOVA | Analysis of Variance |
| CAMI | Civil Aerospace Medical Institute |
| cDNA | Complementary DNA |
| dbGaP | Database of Genotypes and Phenotypes |
| DEG | Differentially Expressed Gene(s) |
| DF | Degrees of Freedom |
| DOT | United States Department of Transportation |
| DRS | DNA/RNA Shield |
| EDTA | Ethylenediaminetetraacetic Acid |
| FAA | Federal Aviation Administration |
| FDR | False Discovery Rate |
| MEAN SQ | Mean Squares |
| MF | Middle Finger |
| NTSB | National Transportation Safety Board |
| PCA | Principal Component Analysis |
| PCR | Polymerase Chain Reaction |
| RIN | RNA Integrity Number |
| RNA | Ribonucleic Acid |
| RNA-Seq | RNA sequencing |
| RF | Ring Finger |
| SOV | Source of Variation |
| SUM SQ | Sum of Squares |
| WHO | World Health Organization |

## Abstract

Blood collection method selection is critical when analyzing blood gene expression. Multiple blood collection methods and sites exist, each with advantages and disadvantages. For human blood gene expression analyses, blood is commonly collected by venipuncture at the antecubital fossa (interior elbow) using a range of vacutainer tubes. Several vacutainer tube types contain RNA-preserving additives, all adequately preserving blood RNA. Most human subjects accept venipuncture, which collects sufficient high-quality blood and RNA suitable for gene expression analysis by RNA sequencing (RNA-Seq). Venipuncture has drawbacks; it requires trained personnel, carries a risk of injury, is time-consuming, and is often unsuitable for in-the-field, at-home, or self-collection. Capillary blood collection by fingerstick is common and widely practiced but not often used for RNA-Seq analysis due to low and variable blood quantity, a lack of blood RNA preservation options, low RNA yields, and variable RNA quality. This study used total RNA-Seq to compare two different fingerstick blood collection, preservation, and RNA extraction methods with a commonly used venipuncture blood collection and RNA extraction method. We demonstrate that fingerstick blood collection produces RNA suitable for RNA-Seq, that each fingerstick method produces results more similar to one another than to venipuncture-derived blood RNA, and that each fingerstick blood collection method can distinguish between experimental groups (male and female subjects). While the venipuncture method examined here is generally preferable to the fingerstick blood collection methods, particularly in well-controlled and resourced environments, capillary blood is suitable and useful for gene expression analysis.

# 1. Introduction

The Federal Aviation Administration's (FAA's) Functional Genomics team routinely collects venipuncture blood samples to analyze gene expression changes under aerospace-relevant conditions (Uyhelji et al., 2018; Nicholson et al., 2021; Nicholson et al., 2023; Munster et al., 2023a; Uyhelji et al., 2023). Venipuncture provides ample high-quality blood and allows collections to take place in a wide variety of collection tubes (Kim et al., 2014). However, venipuncture has several disadvantages. Venipunctures require trained personnel, inflict discomfort upon the subject (particularly when in-dwelling catheters or butterfly needles are used in long-term studies), require large collection volumes that may limit the number of collections possible in long-term studies, and present a low risk of adverse events including infection, syncope (fainting), bruising, and damage to the median and radial nerves (Galena, 1992; World Health Organization [WHO] Best Practices for Injections and Related Procedures Toolkit, 2010; Tsukuda et al., 2019; Voin et al., 2017).

Fingerstick blood sampling, wherein capillary blood is collected through a superficial skin puncture that projects no further than 2.4 mm below the epithelial surface, presents an attractive alternative to venipuncture (WHO Best Practices on Drawing Blood: Best Practices in Phlebotomy, 2010; Krieza et al., 2015). Using commercial puncture devices, such punctures may be performed successfully by minimally trained laypersons, with a low chance of harm to the subject. Capillary blood collection is commonly performed at the fingertip, and less commonly at the heel, arm, and palm (Jungheim & Koschinsky, 2002; WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy, 2010).

Blood from fingerstick punctures is typically collected in glass or plastic microcapillary tubes, absorptive cards or devices, or by dripping directly into sample tubes. Fingerstick sampling collects much lower sample volumes than venipuncture; therefore, subsequent processing steps must compensate for smaller blood volumes and lower amounts of extracted biological molecules available for analysis (Robison et al., 2009; Pirritano et al., 2018; Speake et al., 2017; Rinchai et al., 2016; Kabeer et al., 2018). The variety of commercial microsampling tubes and vials nearly matches that of conventional blood tubes, but as of this writing there are no microsampling devices equivalent to the RNA-preserving venipuncture tubes (PAXgene RNA blood tubes) currently used by the FAA Functional Genomics team. However, previous studies have adapted and optimized methods of preserving fingerstick capillary blood for RNA extraction (Robison et al., 2009; Kabeer et al., 2018), and one published method of capillary blood preservation and mRNA profiling by RNA sequencing (RNA-Seq) exists that demonstrates RNA preservation and correspondence between capillary and venous blood gene expression (Toma et al., 2020).

To assess the usefulness of fingerstick blood in determining gene expression profiles using total RNA-Seq, we compared global gene expression between RNA obtained from two separate fingerstick blood collection and RNA extraction methods with RNA obtained from venipuncture blood collections using PAXgene tubes. All RNA-Seq results were analyzed to identify gene expression profiles for each sample, and those profiles were compared to assess gene expression differences between collection and extraction methods.

As little evidence exists detailing variance in gene expression according to capillary blood collection site, gene expression profiles obtained from the middle and ring fingers using blood RNA collected and extracted using the same method were compared to assess gene expression variance between fingerstick sampling locations. To determine the relative sensitivity of each collection method, male and female subjects were compared directly within each sample type to detect gene expression differences between experimental groups (Tian et al., 2012; Jansen et al., 2014; Bybjerg-Grauholm, 2017). The main objective of this study was to determine if fingerstick blood sampling is a suitable replacement for venipuncture blood sampling, to identify the optimum methods and locations for capillary blood collection, and to examine the usefulness of capillary blood RNA collected by fingerstick to distinguish between experimental groups (i.e., between male and female subjects).

## 2. Methods

*Institutional Review Board and Consent*

All research was conducted with the approval of the FAA Institutional Review Board. All subjects signed an informed consent form before being included in the study, including permission to release RNA sequence data to the National Institutes of Health Database of Genotypes and Phenotypes (dbGaP). No personally identifying information was appended to or associated with experimental samples, and subjects were randomly assigned a numeric identifier used to uniquely label and identify samples. All analyses were conducted blinded to subject demographics except for subject sex. To assess the subjective level of pain felt for each blood collection method, a survey was provided to each subject to rate their pain from 0 (no pain) to 10 (greatest pain), the pain felt immediately after each collection and then again after the session.

*Sample Collection*

Subjects appeared at the collection location without participating in any study-specific preparation, such as fasting, but were advised to be adequately hydrated before reporting for collection. To prepare for blood collection, each subject washed their hands in warm water (as warm as tolerable) for two minutes to warm the hands. Hand-warming was determined to dramatically increase the amount of capillary blood collected by fingerstick during method development (not shown). Following handwashing, subjects dried their hands and progressed immediately to fingerstick blood collection. Fingertips and venipuncture sites were sanitized with alcohol wipes prior to collection. Fingerstick sampling was performed on the middle and ring fingers (MF and RF, respectively). During method development, MF and RF locations were determined to produce the most reliable and consistent blood flow; various palm and upper arm/shoulder locations were unproductive. Venipuncture collection proceeded following fingersticks.

Blood samples were collected at four separate sites. Venipuncture blood was collected using standard butterfly needles (Becton, Dickinson and Company [BD], Franklin Lakes, NJ) and

PAXgene Blood RNA Tubes (BD Biosciences, 762165). Two PAXgene tubes were collected for each subject, typically from the right arm. All fingersticks were performed using BD Microtainer Contact-Activated Lancets (Becton Dickinson, 366594). Two separate fingerstick blood collection and RNA extraction methods were used. Capillary blood was collected using two 100-µL Microvette tubes containing ethylenediaminetetraacetic acid (EDTA) as the anticoagulant (Sarstedt Microvette 100 K3E, 20.1278.100, Nümbrecht, Germany). Each participant provided 200 µL of blood from the MF of the hand opposite the venipuncture arm. Microvettes were tapped gently against a hard surface to expel all blood from the plastic microcapillary into the Microvette tube. The tubes were capped using the attached closure, and the filled Microvette was gently tapped horizontally from the side 10 times to ensure thorough mixing. From the MF and RF of the hand adjacent to the venipuncture site, 200 µL of blood per finger was collected using two 100 µL untreated Minivette tubes per puncture site (Sarstedt Minivette POCT 200, 17.211.100). Each Minivette was filled to capacity, and the collected blood was immediately expelled gently into a cryovial containing 600 µL of 1x Zymo DNA/RNA Shield (Zymo Research, R1200-125, Irvine, CA), then the tubes were capped and inverted 10 times to mix.

All tubes were stored at 4 °C following collection. Microvette-collected blood was stored at 4 °C for 2-5 hours and was then used immediately for total RNA extraction using the Norgen Total RNA Purification Plus Micro kit (Norgen Biotek Corp., 48500, Ontario, Canada). Microvette-collected samples were not frozen to avoid RNA degradation due to cellular damage upon freezing. Minivette-collected blood was kept at 4 °C for 2-5 hours following collection, then placed at -20 °C for 24 hours and stored at -80 °C until extraction.

*RNA Extraction*

RNA was extracted from EDTA Microvette-collected fingerstick blood using the Total RNA Purification Plus Micro kit (Norgen Biotek Corp., 48500), with the following modifications: 700 µL RL buffer with β-mercaptoethanol (for sample stabilization per kit recommendation) was mixed with each 100-µL EDTA-preserved blood sample in the Microvette tube and mixed by vortexing for 30 seconds. The resulting solution, amounting to 600 µL, was spun through a gDNA removal column, followed by spinning the flow-through through a new gDNA removal column. Flow-through volume was assessed by drawing the entire flow-through into a pipette set to a high volume and then reducing the volume until no air remained in the pipette tip. Based on that volume, 0.6 volumes of ethanol were then added to each sample, mixed, and 600 µL of those samples was spun through the purification column in successive spins until the entire sample passed through the column. Samples were eluted in 25 µL of RNAse-free water. Total RNA was isolated from Minivette-collected fingerstick blood samples using the Quick-RNA Whole Blood kit (Zymo Research, R1201), including on-column DNAse digestion. The entire 200 µL of fingerstick blood in 600 µL 1x Zymo DNA/RNA Shield (DRS) was used for each extraction. DRS-preserved samples were removed from -80 °C storage and thawed on ice. Following thawing, samples were extracted according to the manufacturer's RNA Purification (Whole Blood) protocol with the following modifications: 16 µL proteinase K was added to the thawed

preserved blood solution, 800 µL isopropanol was added to the resulting mixture and spun through the purification column in 800-µL increments. The remainder of the protocol proceeded per the manufacturer's instructions, but 21 µL of Nuclease-free water was used for final elution. Total RNA from PAXgene blood tubes was extracted using the PAXgene miRNA kit (performed in a QiaConnect robotic liquid handling device) using the manufacturer's protocol with one modification; samples were eluted in 80-µL nuclease-free water in place of EB buffer.

For each Microvette-Norgen RNA extraction (referred to hereon as Norgen), 100 µL of blood was used. Conversely, 200 µL of blood was used for each Minivette-Zymo RNA extraction (referred to hereon as Zymo MF or RF, depending on the fingerstick site). All 2.5 mL of blood collected in each PAXgene tube was processed using the PAXgene miRNA kit extraction (referred to hereon as PAXgene). After extraction, all PAXgene, Microvette, and Minivette RNA samples were assessed for purity and yield using a NanoDrop 2000c spectrophotometer (ThermoFisher Scientific, ND-2000c, Waltham, MA) and a Qubit 3.0 fluorometer (ThermoFisher Scientific, Q33216) using the Broad Range Assay kit (ThermoFisher Scientific, Q10210). RNA integrity numbers were calculated using a 4200 TapeStation system (Agilent, G2991BA, Santa Clara, CA) and RNA ScreenTape and reagents (Agilent, 5067-5576, 5067-5578, 5067-5577).

*RNA-Seq cDNA Synthesis, Library Preparation, and Sequencing*

Samples were diluted to 11 ng/µL in RNase-free water (ThermoFisher, AM9938), stored at -80 °C, and then shipped on dry ice to the Baylor College of Medicine Human Genome Sequencing Center for library preparation and sequencing. Samples were randomly re-arrayed to minimize potential batch effects and spiked with ERCC synthetic RNA (ThermoFisher, 4456740). From each sample, 9 µL (99 ng) of RNA was input to construct libraries using the TruSeq Stranded Total RNA with Ribo-Zero Globin kit (Illumina Inc., 20020612, San Diego, CA) following the manufacturer's protocol (Illumina Inc., RS-122-9007DOC, Part # 15031048 Rev. E, October 2013). Ribosomal RNA/Globin depleted RNA samples were purified using Agencourt RNAClean XP beads (Beckman Coulter, A63987, Brea, CA) and then used for first and second-strand complementary DNA (cDNA) synthesis. The cDNA was A-tailed and ligated with the TruSeq UD Indexes V2 (Illumina Inc., 20042113), polymerase chain reaction (PCR) was amplified using the Illumina Primer Cocktail Mix included in the library kit, and then purified using AMPure XP beads (Beckman Coulter, A63882). Libraries were quantified using the Fragment Analyzer 5300, pooled in equimolar ratios, and then the pools were quantified using qPCR. Libraries were sequenced on the Illumina NovaSeq 6000 platform using 2x150 bp paired-end reads to generate a minimum of 60 million reads per library. RNA-Seq output files were deposited in the National Center for Biotechnological Information's dbGaP under accession number phs003496.v1.p1.

*RNA-Seq Analysis Pipeline*

Full alignment and quality control pipeline code, including individual software parameters and flags, is described in Supplementary File 1. The pipeline was performed in an Amazon Web

Service cloud instance developed and maintained by the Department of Transportation's Secure Data Commons group. MultiQC (v1.14; Ewels et al., 2016) was used to generate .html summary reports after each step of the pipeline, summarizing the results from each program. Quality control of the raw fastq files was performed using FASTQC (v0.12.1; Andrews, 2010). The raw reads were trimmed of adapters and quality filtered using CutAdapt (v4.3; Martin, 2011). Quality control of the trimmed and filtered reads via FASTQC was performed again to assess the trimming and filtering. The trimmed and filtered reads were aligned to the ENCODE human reference genome (GRCh38.p13.48, indexed with a 149 bp sjdb overhang) and GTF annotation using STAR (v2.7.10.b; Dobin et al., 2013), generating Aligned.sortedByCoord.out.bam files and paired Unmapped.out.mate files consisting of all unmapped reads. BAM index files were generated using samtools (v1.17; Li et al., 2009). Post-alignment quality control on the Aligned.sortedByCoord.out.bam files was performed using Qualimap (v2.2.2d; García-Alcalde et al., 2012). Feature count matrices were generated using the featureCounts function of Subread (v2.0.4; Liao et al., 2014).

*Statistical Analysis and Differential Gene Expression Analysis*

Statistical and differential gene expression analyses were performed using R Statistical Software (v4.3.0; R Core Team, 2023) in conjunction with RStudio (build 446; Posit Team, 2023). We used two-way ANOVA with interaction to assess differences within various metrics. First, we examined mean levels of immediate and residual pain, considering both the blood collection method and participant sex. Second, we evaluated mean levels of RNA extraction metrics. These metrics included concentration, total yield, absorbance ratios at 260/280 and 260/230 nm, and the RNA integrity number (RIN), again analyzing the impact of RNA extraction method and sex. Tukey's honest significant difference test was used post-hoc to compute the significantly different means. The analysis code was adapted from training material from the Harvard Chan Bioinformatics Core Differential Gene Expression workshop (Mistry et al., 2021). The differential gene expression analysis code is described in Supplementary File 2. The following packages in R were called directly to perform these analyses and prepare figures and tables for publication: DESeq2 (v.1.40.1; Love et al., 2014), tidyverse (v2.0.0; Wickham et al., 2019), GeneStructureTools (v1.20.0; Signal, 2023), limma (v3.56.1; Ritchie et al., 2015), ggplot2 (v3.4.2; Wickham, 2016), pheatmap (v1.0.12; Kolde, 2019); apeglm (v1.22.1; Zhu et al., 2018), ggrepel (v0.9.3; Slowikowski, 2023), DEGreport (v1.36.0; Pantano, 2023), AnnotationHub (v3.8.0; Morgan & Shepherd, 2023), ensembldb (v2.21.0; Rainer et al., 2019), annotables (v0.2.0; Turner, 2023), pals (v1.7; Wright, 2021), patchwork (v1.1.2; Pedersen, 2022), ggpubr (v0.6.0; Kassambara, 2023), scales (v1.2.1; Wickham & Seidel, 2022), ggiraphExtra (v0.3.0; Moon, 2020), flextable (v0.9.2; Gohel & Skintzos, 2023a), ggiraph (v0.8.7; Gohel & Skintzos, 2023b), officer (v0.6.2; Gohel, 2023), magrittr (v2.0.3; Bache & Wickham, 2022), pals (v1.7; Wright, 2021), UpSetR (v1.4.0; Gehlenborg, 2019), ggsignif (Ahlmann-Eltze & Patil, 2021), and biomaRt (v2.56.1; Durinck et al., 2009).

## 3. Results and Discussion

### *Pain Felt During and After Blood Collection*

Whole blood samples were collected from 41 adult subjects (20 males and 21 females) via a single venipuncture (PAXgene), fingersticks on the MF and RF of the venipuncture-adjacent hand, and a fingerstick on the MF of the venipuncture-opposite hand (referred to as Zymo MF, Zymo RF, and Norgen MF, respectively). One subject experienced blood collection difficulty and was excluded from the study, leaving 40 subjects (20 males and 20 females). No significant difference in means was observed for sex, collection method, or the interaction between sex and collection method for either immediate (Fig. 1, Table 1) or residual (Table 2) pain, although pain according to collection method approached significance (P=0.055).

In general, more immediate and residual pain was felt with fingerstick collection, and several subjects reported fingertip soreness or sensitivity one day following blood collection. Males experienced a greater range of pain during fingerstick collections, while females experienced greater pain levels during venipuncture collections (Figure 1A and B). Residual pain assessments demonstrated that pain receded from initial levels in all collection methods, with fingersticks generating slightly less residual pain than venipunctures and females reporting slightly less residual pain than males in all cases. However, none of these differences were significant. Occasional difficulty was experienced in both venipuncture and fingerstick collections; venipuncture collections sometimes required more than one puncture to find a productive vein, and some fingerstick collections required additional punctures to collect sufficient blood.

**Table 1. Two-way ANOVA with interaction results for immediate pain.** No significant difference for means was observed for the sources of variation sex, collection method, or the interaction for sex and collection method.

*Pain Felt Immediately Following Each Collection*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 4.858 | 4.858 | 1.890 | 0.171 |
| Collection | 3 | 4.074 | 1.358 | 0.528 | 0.664 |
| Sex:Collection | 3 | 4.044 | 1.348 | 0.524 | 0.666 |
| Residuals | 155 | 398.471 | 2.571 | | |

*Note.* SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

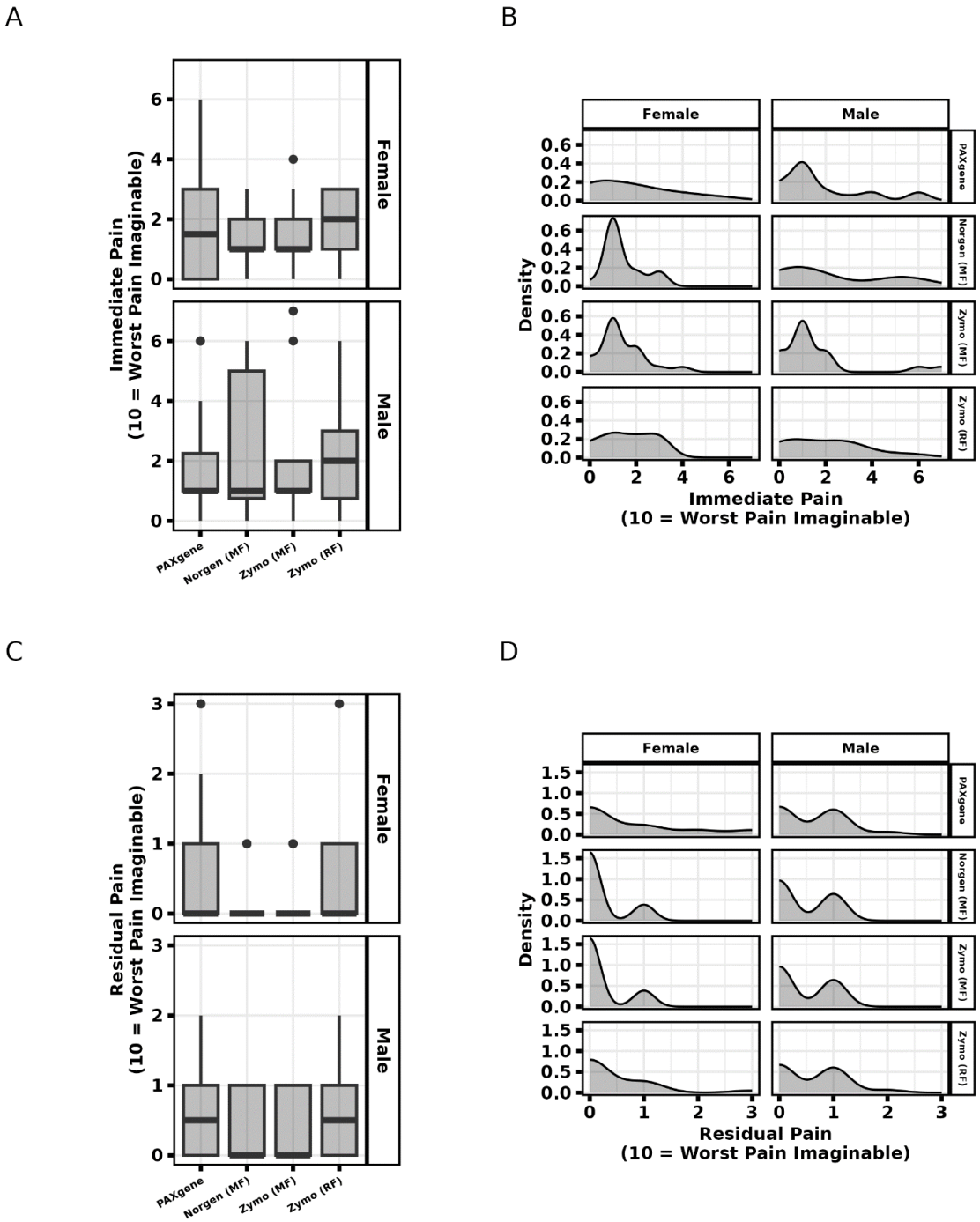


**Figure 1. Self-reported subject immediate and residual pain.** (**A** and **B**) Boxplots (A) and density plots (B) of subject self-reported immediate pain on a scale of 1 to 10 (with 10 representing the worst pain imaginable) between male and female subjects across the four collection methods. No significant differences were observed (*N*=20 each for males and females for each collection method). (**C** and **D**) Boxplots (C) and density plots (D) of subject self-reported residual pain on the same pain scale and for the same comparisons. No significant differences were observed (*N*=20 each for males and females for each collection method).

**Table 2. Two-way ANOVA with interaction results for residual pain.** No significant difference for means was observed for the sources of variation sex, collection method, or the interaction for sex and collection method.

*Residual Pain Felt Following Completion of the Blood Draw Session*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 0.525 | 0.525 | 1.332 | 0.250 |
| Collection | 3 | 3.063 | 1.021 | 2.589 | 0.055 |
| Sex:Collection | 3 | 0.915 | 0.305 | 0.773 | 0.511 |
| Residuals | 155 | 61.129 | 0.394 | | |

*Note.* SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

Following sample collection, total RNA was isolated from each sample and quality and quantity were assessed (Fig. 2). One extraction was performed from each PAXgene tube collected from the venous blood draw (PAXgene 1 and 2), one extraction from each 100-µL fingerstick EDTA-Norgen MF collection (Norgen MF1 and MF2), and one extraction each from the fingerstick Zymo MF and RF sticks (200 µL blood pooled for each location). A significant difference ($P < 0.001$) in means was observed among collection methods for RNA concentration (Table 3), total RNA yield (Table 4), 260/280 nm absorbance ratio (Table 5), 260/230 nm absorbance ratio (Table 6), and RIN (Table 7). In all, PAXgene-collected blood yielded the highest RNA quantity (>10 µg per tube, Fig. 2A and B) and spectrophotometric quality (excepting a lower 260/230 ratio for PAXgene tube 2 collections, due to a processing error), but produced lower-RIN than either fingerstick blood collection method (Fig. 2C-E). RNA quantity and quality metrics varied significantly between fingerstick collection methods, with Norgen samples producing higher 260/280 ratios and RIN, but lower and more variable 260/230 ratios than Zymo MF or RF samples (Fig. 2C-E). However, Zymo MF and RF samples yielded more RNA than Norgen samples (Fig. 2A and B). No significant difference was observed by sex for any RNA metric except RIN ($P$ value <0.05), with RNA from female RIN averaging 0.2 units higher than males. No significant difference was observed for the interaction between sex and collection method for any RNA metric.

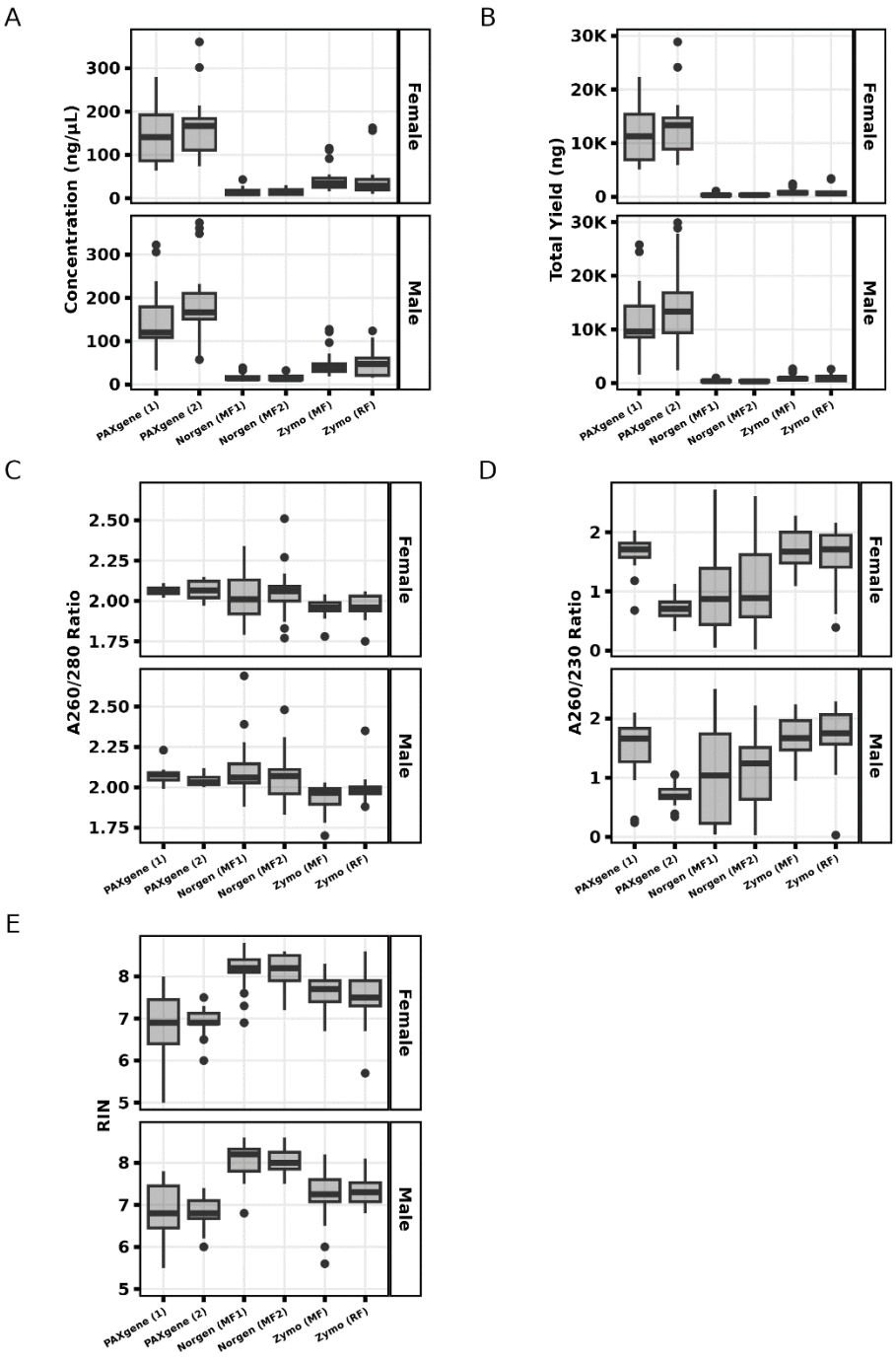


**Figure 2. RNA extraction metrics.** (**A** to **E**) Boxplots of extracted RNA sample concentration faceted by sex (A), total RNA yield (B), 260/280 nm absorbance ratio (C), 260/230 nm absorbance ratio (D), and RIN values (E) for each of the extractions (*N*=20 each for males and females for each extraction). Significant differences exist between individual extractions for each of the metrics and are shown in Figure 3.

**Table 3. Two-way ANOVA with interaction results for RNA concentration.** A significant difference for means was observed for the source of variation collection method ($P < 0.001$) but not for sex or the interaction of sex and collection method.

*Analysis of Significant Differences Between Collection and Extraction Combinations by Sample RNA Concentration*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 4.01e+03 | 4.01e+03 | 1.627 | 0.203 |
| Collection | 5 | 1.00e+06 | 2.01e+05 | 81.401 | 0.000 |
| Sex:Collection | 5 | 3.80e+03 | 7.59e+02 | 0.308 | 0.908 |
| Residuals | 232 | 5.72e+05 | 2.46e+03 | | |
| ns P > .05, * P < .05, ** P < .01, *** P < 0.001 | | | | | |

*Note.* SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

**Table 4. Two-way ANOVA with interaction results for total yield.** A significant difference for means was observed for the source of variation collection method ($P < 0.001$), but not for sex or the interaction of sex and collection method.

*Analysis of Significant Differences Between Collection and Extraction Combinations by Total RNA Yield*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 7.97e+06 | 7.97e+06 | 0.552 | 0.458 |
| Collection | 5 | 8.10e+09 | 1.62e+09 | 112.228 | 0.000 |
| Sex:Collection | 5 | 1.57e+07 | 3.14e+06 | 0.217 | 0.955 |
| Residuals | 232 | 3.35e+09 | 1.44e+07 | | |

*Note.* Ns P > .05, * P < .05, ** P < .01, *** P < 0.001. SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

**Table 5. Two-way ANOVA with interaction results for 260/280 nm absorbance ratio.** A significant difference for means was observed for the source of variation collection method ($P < 0.001$) but not for sex or the interaction of sex and collection method.

*Analysis of Significant Differences Between Collection and Extraction Combinations by 260/280 nm Ratio*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 0.013 | 0.013 | 1.241 | 0.266 |
| Collection | 5 | 0.564 | 0.113 | 10.437 | 0.000 |
| Sex:Collection | 5 | 0.094 | 0.019 | 1.744 | 0.125 |
| Residuals | 232 | 2.506 | 0.011 | | |

*Note.* Ns P > .05, * P < .05, ** P < .01, *** P < 0.001. SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

**Table 6. Two-way ANOVA with interaction results for 260/230 nm absorbance ratio.** A significant difference for means was observed for the source of variation collection method ($P < 0.001$) but not for sex or the interaction of sex and collection method.

*Analysis of Significant Differences Between Collection and Extraction Combinations by 260/230 nm Ratio*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 0.012 | 0.012 | 0.040 | 0.842 |
| Collection | 5 | 35.341 | 7.068 | 24.281 | 0.000 |
| Sex:Collection | 5 | 0.486 | 0.097 | 0.334 | 0.892 |
| Residuals | 232 | 67.535 | 0.291 | | |

*Note.* Ns P > .05, * P < .05, ** P < .01, *** P < 0.001. SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

**Table 7. Two-way ANOVA with interaction results for RNA integrity number.** A significant difference for means was observed for the source of variations sex ($P < 0.05$) and collection method ($P < 0.001$) but not for the interaction of sex and collection method.

*Analysis of Significant Differences Between Collection and Extraction Combinations by RNA Integrity Number*

| SOV | DF | Sum Sq | Mean Sq | F-value | P-value |
|---|---|---|---|---|---|
| Sex | 1 | 1.725 | 1.725 | 6.463 | 0.012 |
| Collection | 5 | 59.959 | 11.992 | 44.938 | 0.000 |
| Sex:Collection | 5 | 0.946 | 0.189 | 0.709 | 0.617 |
| Residuals | 229 | 61.109 | 0.267 | | |

*Note.* Ns P > .05, * P < .05, ** P < .01, *** P < 0.001. SOV = source of variation, DF = degrees of freedom, Sum Sq = sum of squares, Mean Sq = Mean Squares. P > .05 is insignificant.

Post-hoc testing of the significantly different means showed that for RNA concentration and total RNA yield, significant differences at the 95% family-wise confidence level existed between each fingerstick method and the PAXgene collection ($P < 0.01$) and between the Zymo MF and Norgen MF2 collections ($P < 0.05$; Fig. 3A and 3B). Significant differences in 260/280 nm and 260/230 nm absorbance ratios ($P < 0.01$) demonstrated that Zymo collections produced significantly lower 260/280 ratios than any other method, while Zymo and Paxgene methods were equivalent. Zymo samples produced significantly higher 260/230 ratios than Norgen samples but did not differ from PAXgene samples (disregarding the improperly prepared PAXgene V2 samples). Norgen samples produced significantly lower 260/230 ratios than other methods (Fig. 3C and 3D). Finally, for RIN, the significant comparisons were between the Norgen samples and all other collection/extraction methods ($P < 0.01$) and between the Zymo MF and RF collections and the PAXgene collections ($P < 0.01$), with Norgen>Zymo>PAXgene in order of greatest RIN (Fig. 3E). There were no significant differences in any RNA quality metric within methods. For the purposes of this study, sex is considered a binary between male and female; in keeping with this binary, a significant difference also existed for RIN between male and female ($P$ value < 0.05) (Fig. 3F).

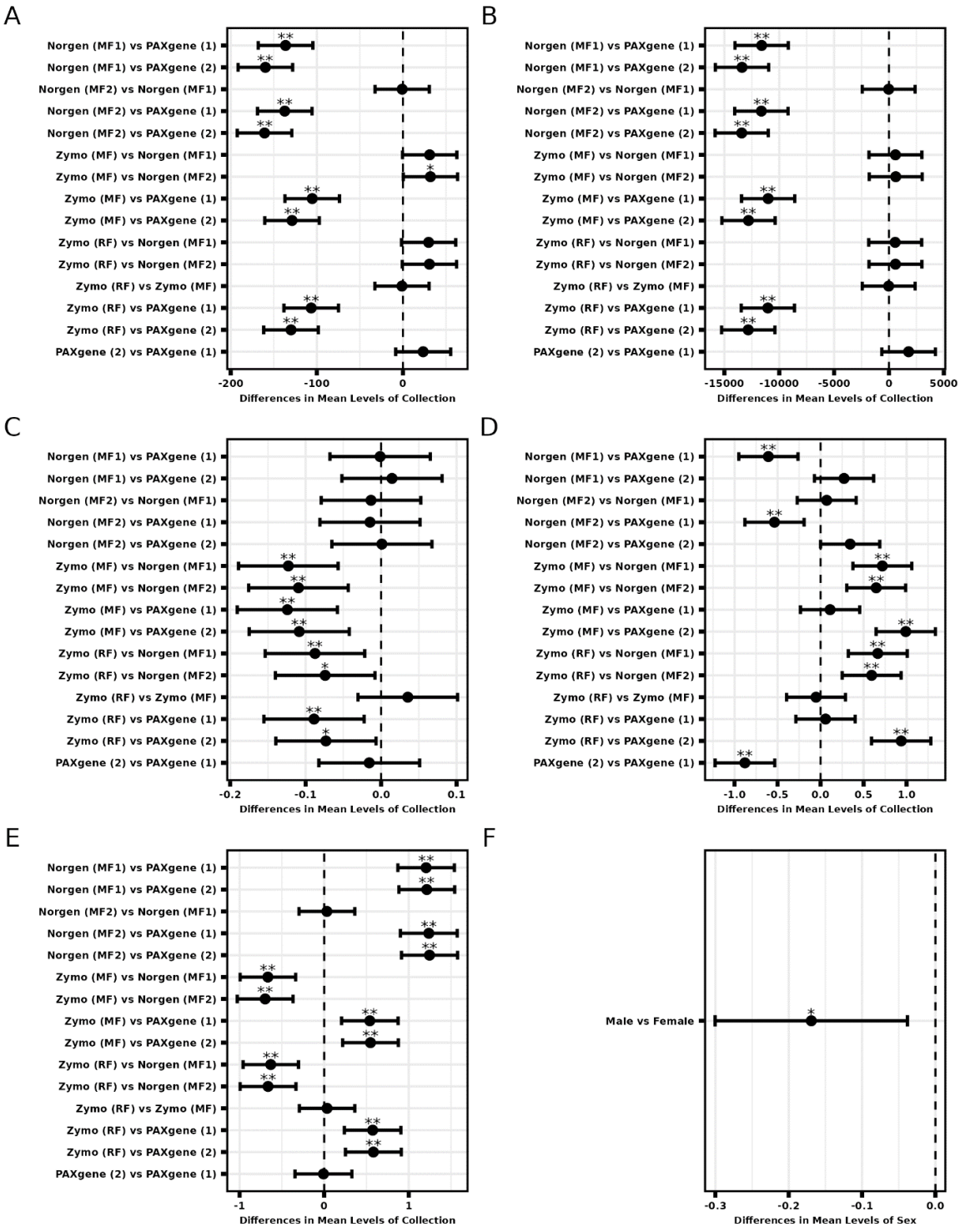


**Figure 3. Tukey's honestly significant difference results for RNA extraction metrics.** Ninety-five percent family-wise confidence level plots for each RNA extraction metric shown in Figure 2. (**A** to **E**) Extracted RNA sample concentration (A), total RNA yield (B), 260/280 nm absorbance ratio (C), 260/230 nm absorbance ratio (D), and RIN values (E) significance comparisons based on collection method ($N$=40; * $P < 0.05$; ** $P < 0.01$). (**F**) RIN values significance comparison on the basis of sex ($N$=20 each for males and females; * $P < 0.05$).

### *Differential Gene Expression between Collection/Extraction Methods*

Total RNA sequencing data were generated from each sample collected from all subjects. Principal components analysis (PCA) demonstrated clear segregation of samples by collection/extraction method along principal component 1 (explaining 36% of sample difference; Fig. 4A), with the fingerstick samples clustered away from the venous samples, and by sex, with the samples collected from male and female subjects clearly delineated along principal component 2 (explaining 15% of sample difference; Fig. 4B). When the data were subset by individual collection/extraction method, samples continued to cleanly segregate based on sex (Fig. 4C-F).

Differential gene expression analysis was performed by DESeq2 with apeglm log fold shrinkage using a false discovery rate (FDR)-adjusted *P*-value significance threshold of 0.05 and a $\log_2$ fold change threshold of |0.58|, corresponding to a fold change of approximately |1.5|. Comparing Norgen to PAXgene samples, 3,233 genes were significantly upregulated, and 16,899 genes were significantly down-regulated, for a total of 20,132 differentially expressed genes (DEGs; Supplementary Table 1; Fig. 5A). A similar pattern was observed for the other two fingerstick versus venous collection/extraction methods: comparing Zymo MF to PAXgene showed 2,633 genes were significantly upregulated, and 17,743 genes were significantly down-regulated, for a total of 20,376 DEGs (Supplementary Table 2; Fig. 5B), and comparing Zymo RF to PAXgene showed 2,830 genes were significantly upregulated, and 16,412 genes were significantly down-regulated, for a total of 19,242 DEGs (Supplementary Table 3; Fig. 5C). These results demonstrate that PAXgene-derived blood RNA differs widely in terms of expression profile from each of the examined fingerstick methods.

In comparing the two fingerstick collection/extraction methods, Zymo MF to Norgen MF and Zymo RF to Norgen (Supplementary Tables 4 and 5, respectively), 1,667 and 1,912 genes were significantly upregulated, 719 and 759 genes were significantly down-regulated, for a total of 2,386 and 2,671 DEGs, respectively (Fig. 5D-E). Finally, comparing the same fingerstick collection/extraction method across two different fingers, Zymo MF to Zymo RF showed no significant DEGs (Fig 5F).

The top 20 significant DEGs sorted by FDR-adjusted *P*-value were plotted for the five comparisons with significant DEGs (Fig. 6), displaying the clearest expression differences between fingerstick and venous collections. A large degree of overlap exists across and among the five comparisons, with 15,356 DEGs in common among the three venous versus fingerstick collection/extraction methods and 994 DEGs in common across all five comparisons (Fig. 7). Directly comparing Zymo MF and RF did not detect significant differences in gene expression, and only 1,174 genes were unique to the intersection of comparisons of each of these methods to PAXgene collection. One hundred thirty-eight DEGs were unique to the Zymo MF and RF vs. Norgen comparison. These findings further suggest that the MF and RF expression profiles are largely similar.

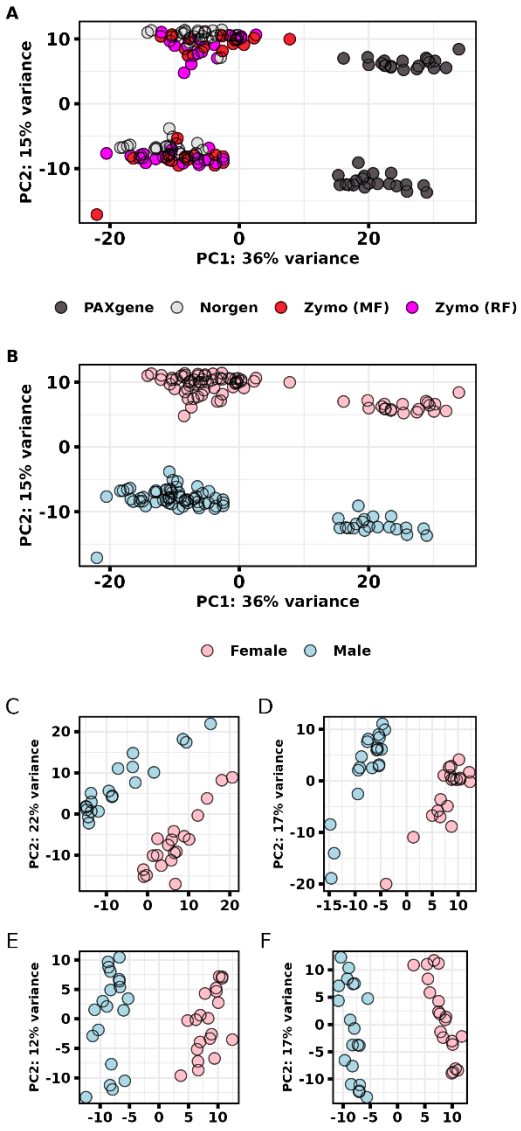


**Figure 4. Principal component analysis.** PCA was performed using DESeq2's plotPCA function on normalized, log-transformed counts. (**A**) Capillary fingerstick Norgen, Zymo MF, and Zymo RF samples are tightly clustered in two groups, while the venous blood draw PAXgene samples also tightly cluster in two groups alone. (**B**) The cause of the grouping pattern seen in (A) is explained by subject sex, with male and female distinctly separate regardless of extraction method. (**C** to **F**) Male and female subjects remain distinctly clustered in PAXgene (C), Norgen (D), Zymo MF (E), and Zymo RF (F) subsets.

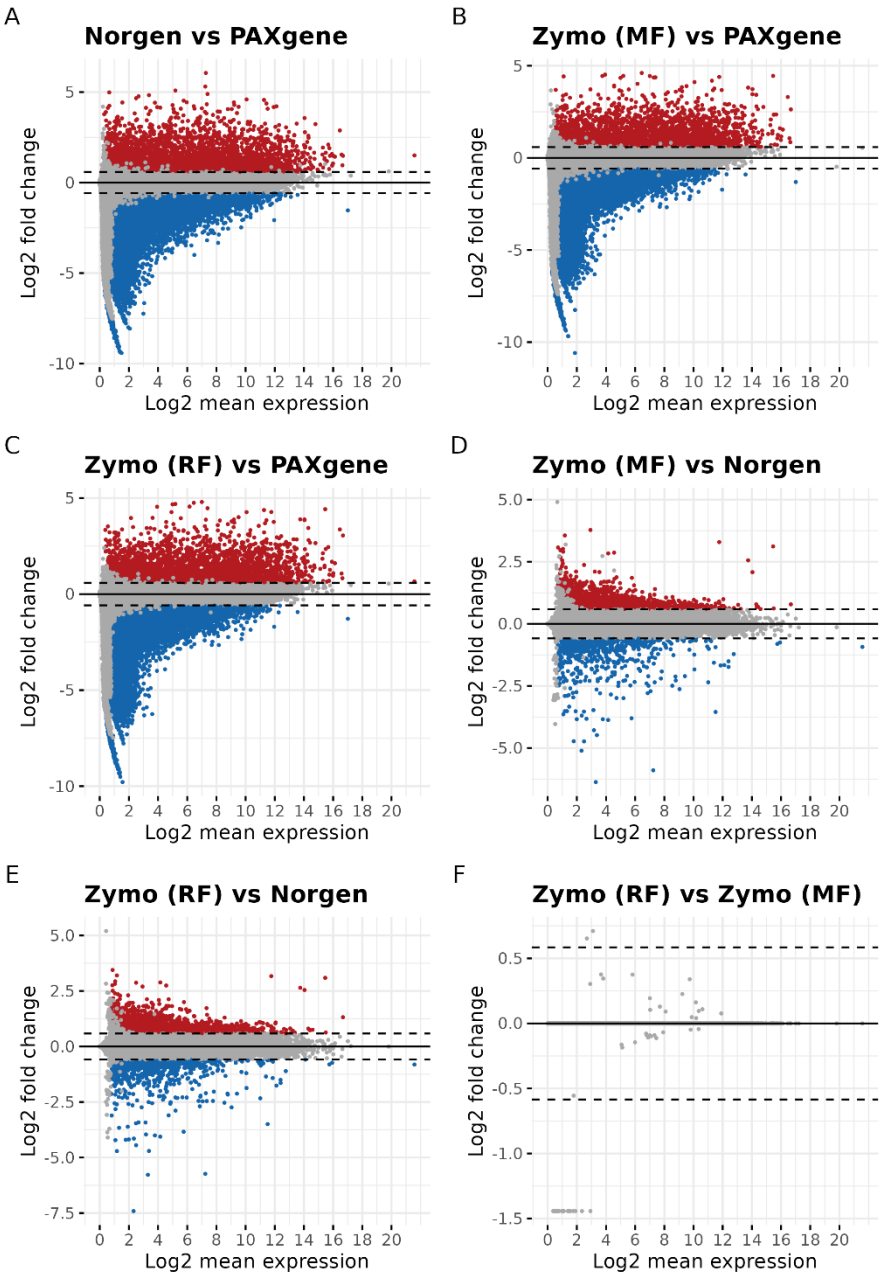


**Figure 5. MA plots of significant collection/extraction method DEGS.** DEGs identified in each comparison. (**A** to **F**) MA plots for Norgen (MF) versus PAXgene (A), Zymo MF versus PAXgene (B), Zymo RF versus PAXgene (C), Zymo MF versus Norgen (D), Zymo RF versus Norgen (E), and Zymo RF versus Zymo MF (F) comparisons. The horizontal dashed lines represent the positive and negative 0.58 $\log_2$ fold change thresholds and the red dots and blue dots represent significant differentially expressed upregulated and down-regulated genes, respectively, with an FDR-adjusted *P* value threshold of 0.05. No significant DEGs were identified in the Zymo RF versus Zymo MF comparison. Expression is measured in Counts Per Million (CPM).

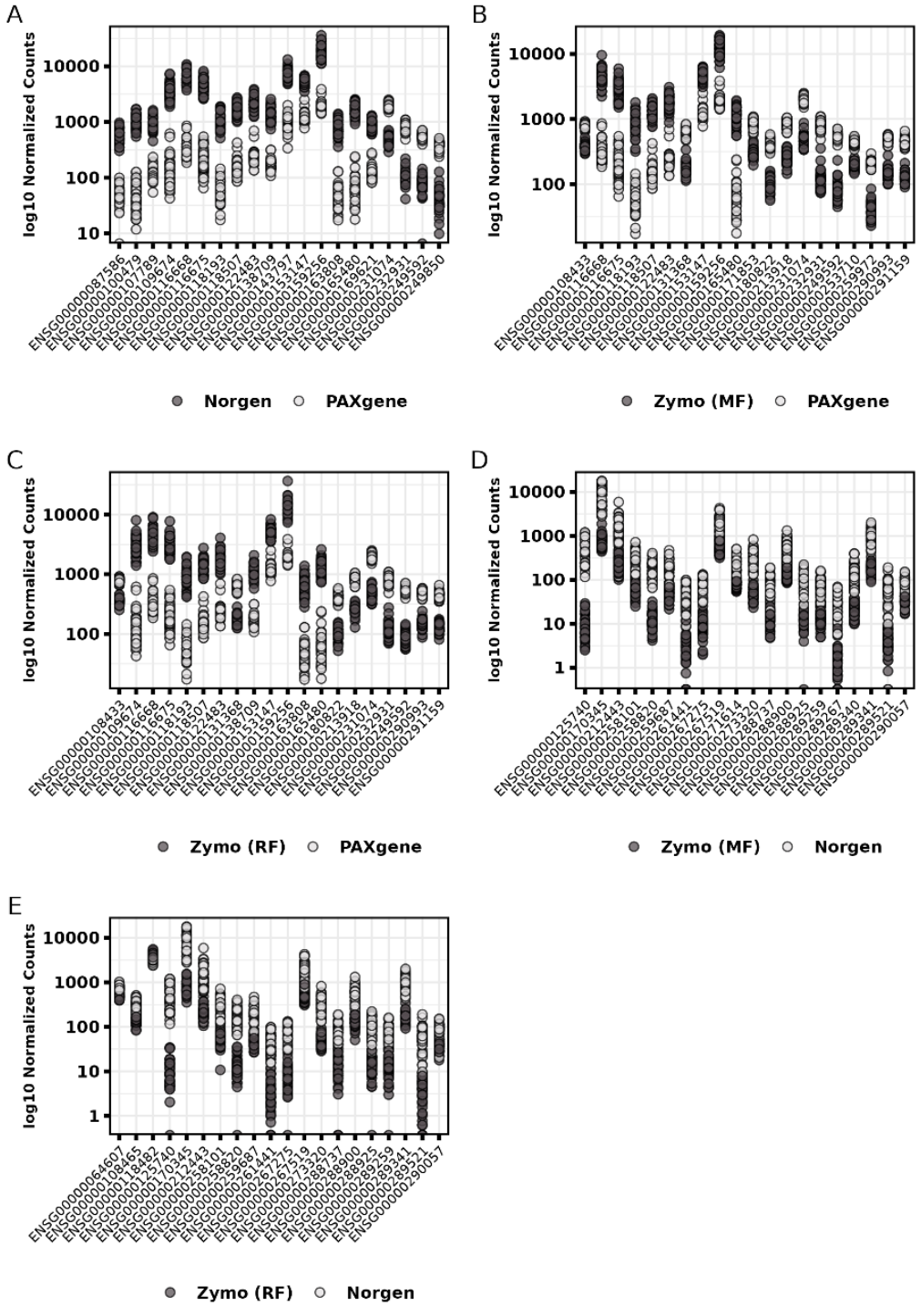


**Figure 6. Top 20 significant collection/extraction method DEGs.** (**A** to **E**) The normalized counts of the top 20 DEGs with the lowest FDR-adjusted *P* values for each collection/extraction method arranged from left to right by Ensembl ID number: Norgen versus PAXgene (A), Zymo MF versus PAXgene (B), Zymo RF versus PAXgene (C), Zymo MF versus Norgen (D), and Zymo RF versus Norgen (E).

A much larger degree of difference exists between the RNA collected from fingerstick and venous blood than between fingerstick collection and extraction methods, likely due to the difference in composition between the two types of blood. The magnitude of differential expression between venous and fingerstick blood seen here exceeds that previously reported (4,490 DEGs; Stein et al., 2016). Capillary blood collected by fingerstick is known to contain a large proportion of non-blood cells derived from interstitial fluid and from cellular damage resulting from the nature of the draw itself and to exhibit differing cellular composition and metabolic characteristics than venous blood (Dobrodeeva et al., 2020, Bongen et al., 2019, Becker et al., 2022). Fingerstick methods were more similar to one another than to the Paxgene method, and no significant differences in gene expression profiles were noted between fingerstick locations on the same hand. This finding is important in determining which sample types to use when comparing results between studies. If the desire is to compare expression profile results with previous studies, one should attempt to match blood collection methods between studies, with RNA extraction method matching also of importance.

***Distinction Between Male and Female Subgroups Within Each Collection/Extraction Method***

When comparing male and female subjects across all four collection/extraction methods, 112 genes were significantly upregulated, and 110 genes were significantly down-regulated, for a total of 222 DEGs (Supplementary Table 6; Fig. 8A). Comparing male versus female samples within collection/extraction methods showed 38 upregulated genes and 20 down-regulated genes (for a total of 58 DEGs) for PAXgene (Supplementary Table 7; Fig. 8B), 44 upregulated genes and 12 down-regulated genes (for a total of 56 DEGs) for Norgen (Supplementary Table 8; Fig. 8C), 43 upregulated genes and 15 down-regulated genes (for a total of 58 DEGs) for Zymo MF (Supplementary Table 9; Fig. 8D), and 41 upregulated genes and 37 down-regulated genes (for a total of 78 DEGs) for Zymo RF (Supplementary Table 10; Fig. 8E).

The top 20 significant DEGs sorted by adjusted *P*-value (FDR) were plotted for male versus female across all collection/extraction methods and for each collection/extraction method individually (Fig. 9), demonstrating a clear distinction of transcript abundance between male and female subgroups, or absent transcription in female subjects, in all comparisons. Across all and between the individual collection/extraction methods when comparing male versus female, 16 DEGs were shared, with 146 DEGs unique to the all-collection/extraction methods comparison, 9 DEGs to the PAXgene comparison, 5 DEGs to the Norgen comparison, 7 DEGs to the Zymo MF comparison, and 40 DEGs to the Zymo RF comparison (Fig. 10). No DEGs were unique solely to the Zymo MF-Zymo RF intersection.

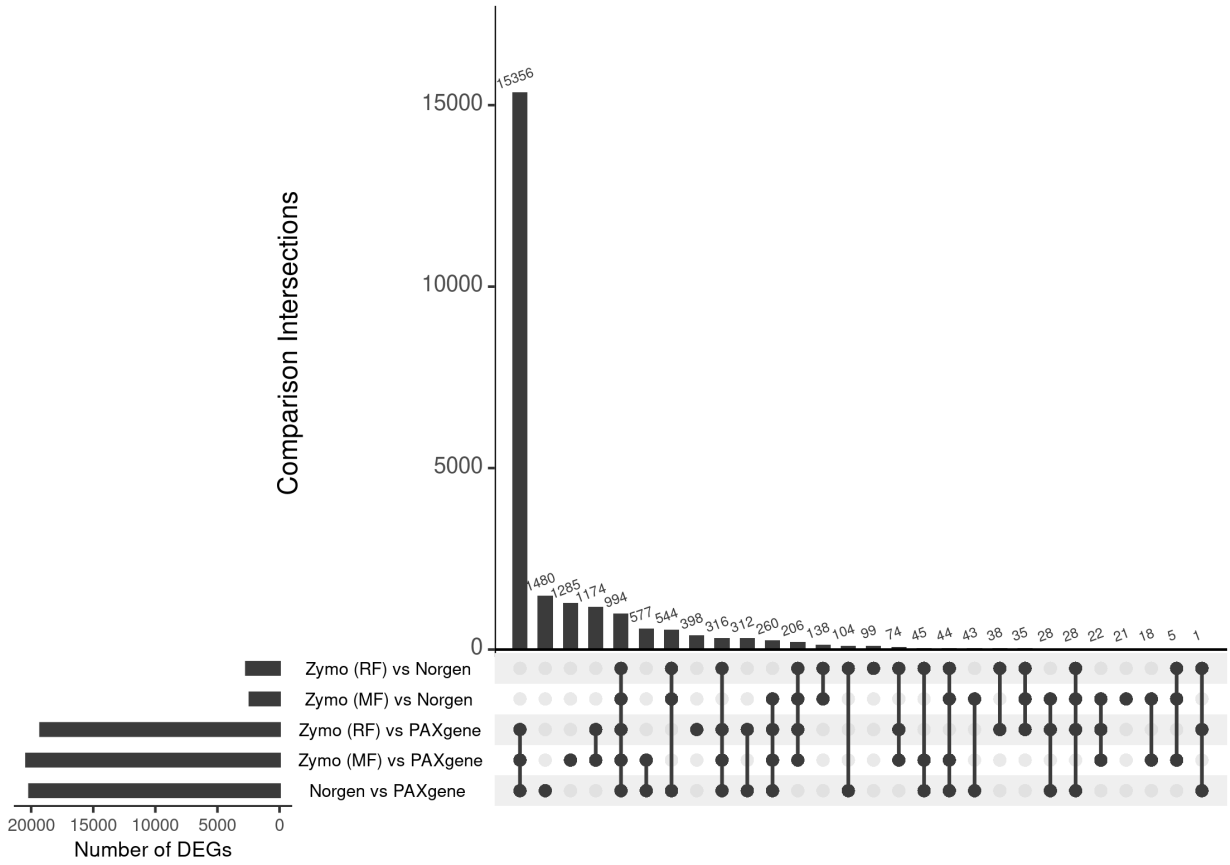


**Figure 7. Overlap of collection/extraction method DEGs.** Intersection of all collection/extraction method differentially expressed gene sets. Of the three capillary fingerstick versus venous blood draw comparisons, the Norgen versus PAXgene gene set consists of 20,132 DEGs, the Zymo MF versus PAXgene gene set 20,376 DEGs, the Zymo RF versus PAXgene gene set 19,242 DEGs. The gene sets of the two capillary fingerstick versus capillary fingerstick comparisons consist of 2,386 DEGs for Zymo MF versus Norgen and 2,671 DEGs for Zymo RF versus Norgen. The column plot represents intersection sizes between the five comparisons, while the matrix plot shows each of the 28 comparison intersections. The single dots within the matrix plot represent DEGs belonging to only that comparison not found in any of the other comparisons.

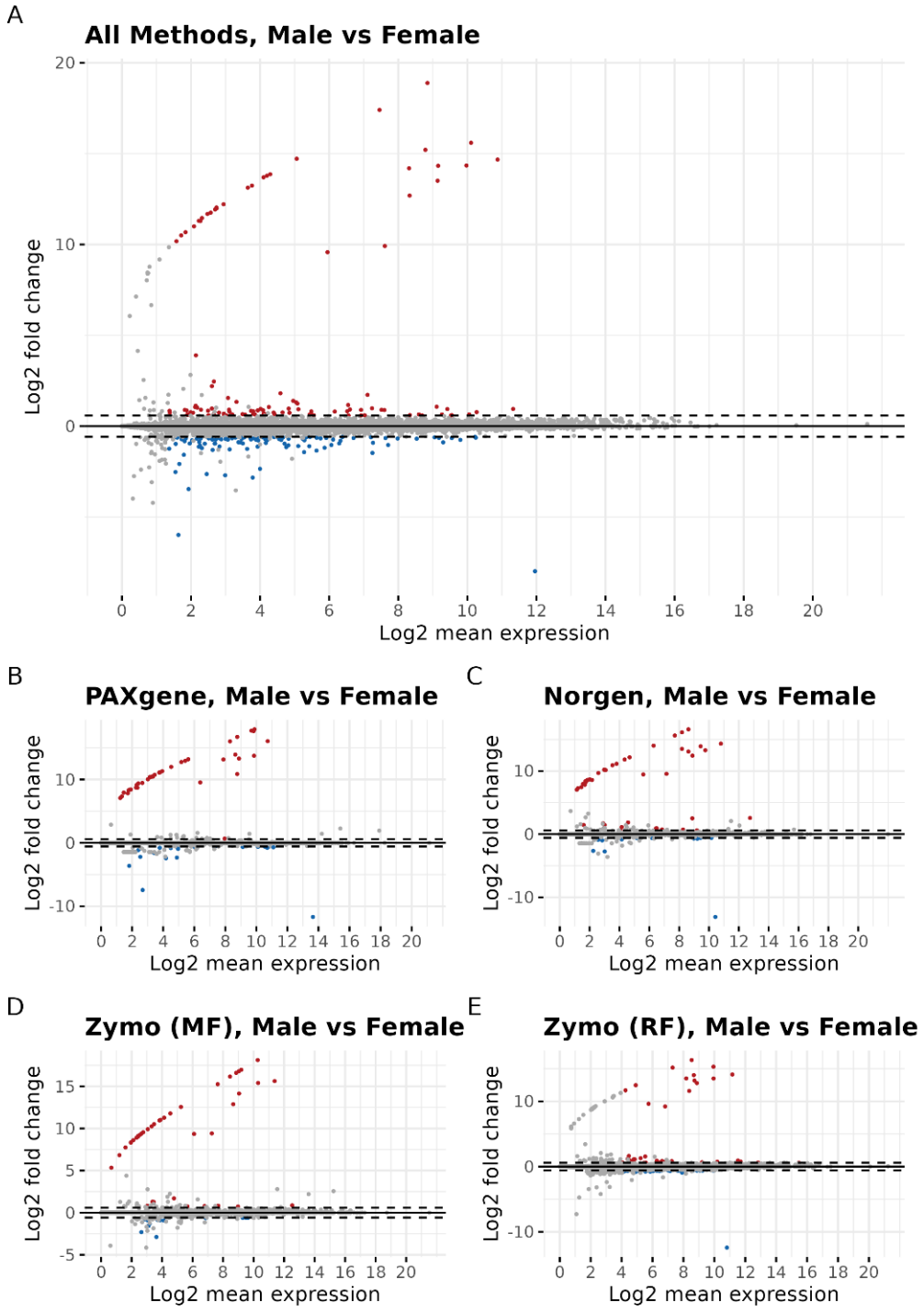


**Figure 8. MA plots of significant sex comparison DEGs.** DEGs. (**A** to **E**) MA plots for male versus female comparisons for all methods (A), PAXgene (B), Norgen (C), Zymo MF (D), and Zymo RF (E). The horizontal dashed lines represent the positive and negative 0.58 $\log_2$ fold change thresholds, and the red dots and blue dots represent significant differentially expressed upregulated and down-regulated genes, respectively, with an FDR-adjusted *P* value threshold of 0.05.

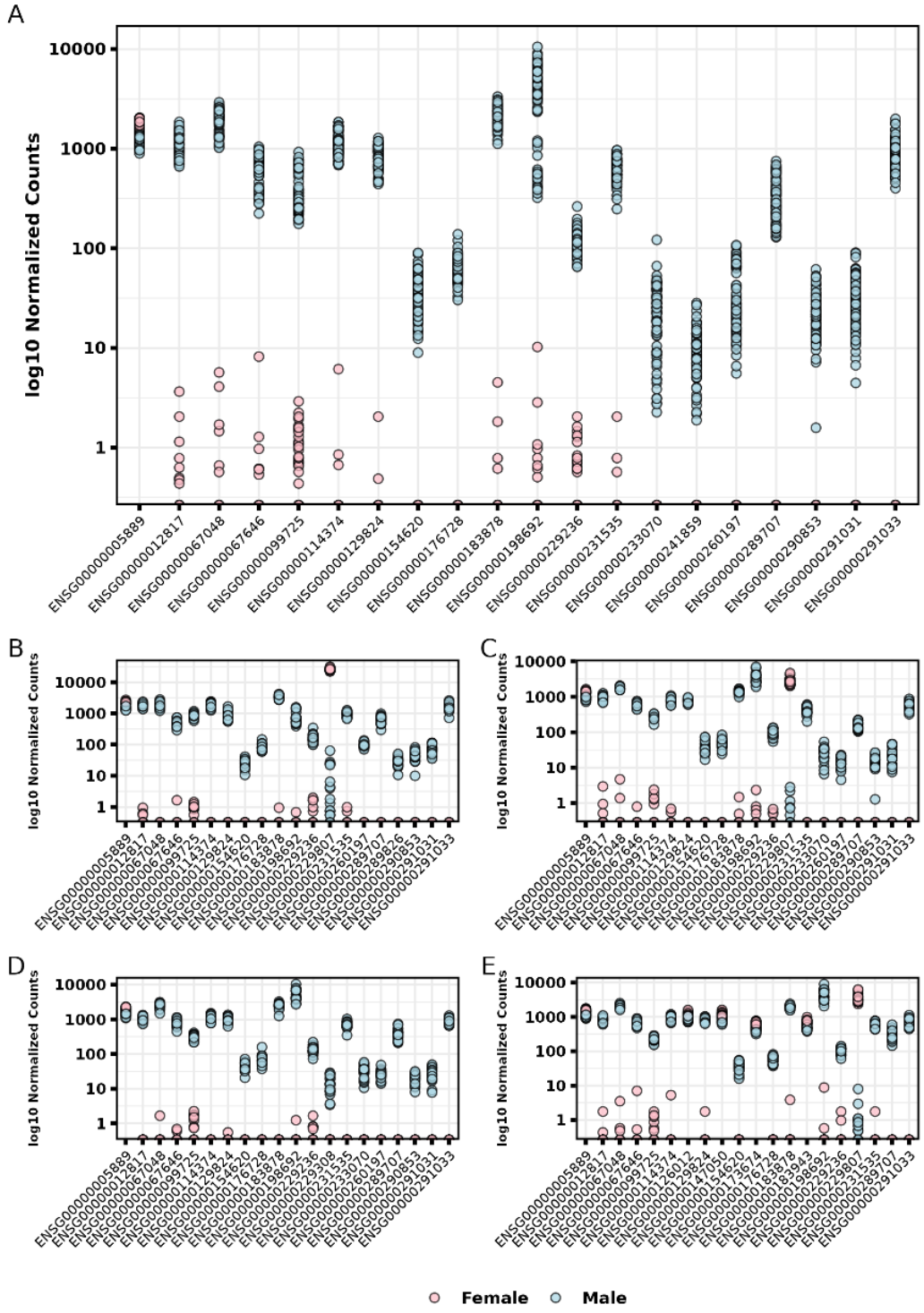


**Figure 9. Top 20 significant sex comparison DEGs.** (**A** to **E**) The normalized counts of the top 20 DEGs with the lowest FDR-adjusted *P* values for male versus female comparisons arranged from left to right by Ensembl ID number: all methods (A), PAXgene (B), Norgen (C), Zymo MF (D), and Zymo RF (E).

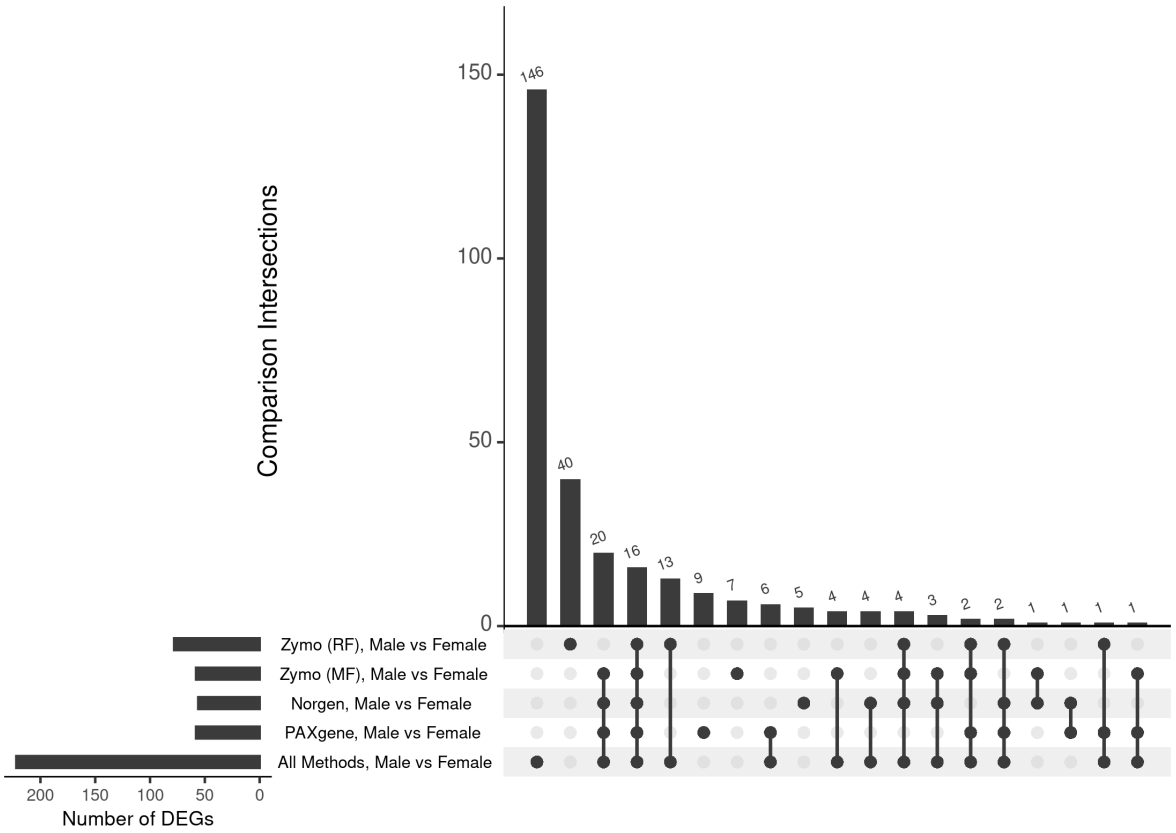


**Figure 10. Overlap of significant sex comparison DEGs.** Intersection of all male versus female differentially expressed gene sets. The all methods comparison gene set consists of 222 DEGs, while the individual PAXgene, Norgen, Zymo MF, and Zymo RF gene sets consist of 58, 56, 58, and 78 DEGs, respectively.

In comparing male transcript abundance minus female transcript abundance, transcripts that were more abundant in males (such as Y-chromosome transcripts) were expected to be upregulated, and those more abundant in females (such as female-specific X-chromosome transcripts) were expected to be downregulated. This pattern was observed in all transcripts attributed to X or Y chromosomes in all comparisons (Supplementary Tables 6-10). The percentage of DEGs attributed to X or Y chromosomes varied according to comparison: All-methods combined comparison (14.4% Y chromosome and 10.8% X), Zymo MF (55.2% Y and 19% X), Zymo RF (17.9% Y and 12.8% X), Norgen MF (55.4% Y and 16.1% X), Paxgene (63.8% Y and 32.8% X). The identity of X and Y transcripts identified in each comparison varied, with a core of 16 X or Y transcripts common to all comparisons and 20 X or Y genes common specifically to the PAXgene, Norgen MF, and Zymo MF methods. The all-methods comparison showed 9 unique X/Y transcripts, 8 X/Y transcripts unique to the PAXgene method, 0 to Norgen MF, 3 to Zymo MF, and 1 to Zymo RF (Supplemental Figure 1). Although there was incomplete overlap between DEGs identified in each comparison, the DEGs that were identified conformed to the expected pattern, and each of the examined methods are useful in identifying differences in

transcript abundance according to sex, including those with log fold changes near the minimum threshold of |0.58|.

Thus, while each method produced RNA that could be distinguished between male and female, the identity of the genes that permitted that determination varied between methods. Further, the number of unique DEGs in each method identified between the Zymo-MF and Zymo-RF when comparing males and females within the method indicates that, regardless of the lack of DEGs in the direct comparison of those datasets, fingerstick sampling site should be consistent to optimize any comparison.

## 4. Conclusion

The series of comparisons made here determined that fingerstick blood collections and each method of RNA extraction performed on those blood collections produces RNA that is useful for gene expression analysis using total RNA-Seq. While blood drawn into PAXgene tubes by venipuncture is more plentiful, and thus produces higher total yield and concentration than from fingerstick collections, in each case, capillary blood RNA produced sufficient RNA of adequate quality to sequence and to conduct reliable gene expression comparisons using those RNA-Seq results. The differing profiles observed between fingerstick and venous blood were expected due to differences in sample composition and RNA extraction method.

When deciding which collection and extraction method is most suitable for a given study, one must consider the study aims. If subjects are to be sampled repeatedly over time, if large quantities of RNA are desired, and if trained personnel are available to perform blood draws, then venipuncture blood drawn into PAXgene tubes is likely preferable for most gene expression studies. Blood drawn through venipuncture yielded RNA of more consistent quality and quantity compared to the fingerstick method. Given the significant variance in individual gene expression observed between these two methods, it is advisable to use PAXgene-drawn venous blood when correlating results with previous studies that employed this biosample collection method. For any future research, prioritizing the collection of PAXgene-drawn venous blood would ensure better comparability. However, if trained personnel are unavailable, such as large-scale field or at-home collections, or if storage space, costs, or permitted sample collection volumes are limited, fingerstick collections are sufficient to produce quality results. Within fingerstick collection and extraction methods, we also observed the respective benefits of the two different applications tested in this study.

The Norgen method, drawn into EDTA-Norgen sampling devices, gave the most variable quantity and quality RNA, but produced RNA-Seq results equivalent to the Zymo method (Minivette-collected) despite a lower initial blood volume (100 µL vs 200 µL). The Microvette devices used in the Norgen method were convenient, rapid, and simple to collect blood with, and sufficiently preserved blood for up to 5 hours at 4 °C. This method would be most useful if blood were to be collected in a clinic or other controlled location with available refrigerated storage and if processing were to proceed the day of collection.

The Zymo method (collected using untreated Minivette devices and stabilized in an RNA preservative) produced a higher RNA yield with slightly lower RIN than the Norgen samples. However, this yield advantage is questionable, considering that 200 µL of blood was used for each extraction, compared with 100 µL per extraction for the Norgen method; the RNA yield of the sum of those two separate collections typically exceeded that of the single Zymo RNA extraction. Regardless, the Zymo method served to preserve the blood for RNA extraction adequately, and this method would be more useful in instances where immediate processing and extraction is not possible, where blood needs to be preserved at temperatures higher than -80 °C for some time, or for at-home or field collections. The sampling site location should also be made as consistent as possible; although no significant differences were noted between Zymo MF or RF samples, there was incomplete overlap between DEGs identified when comparing male and female samples within each sample type. In summary, each collection-extraction method tested here is suitable for use in gene expression analyses, but that study's analytical goal should guide the use of any method.

**Funding**: This research was funded by the Federal Aviation Administration. **Conflicts of interest**: The authors declare that they have no competing interests. **Peer review**: Internal review was accomplished in accordance with AAM-600-0002-WI. **Data accessibility**: The alignment pipeline script and R code necessary for independent replication of the gene expression analysis is included as a supplement to this publication. **Author Contributions:** CT performed computational analyses of sequencing data, VW performed sample collections and assisted with experimental design, DH performed sample and RNA processing, SN performed experimental design, sample collection, and data analysis.

## 5. References


Ahlmann-Eltze, C., & Patil, I. (2021, March 31). ggsignif: R Package for Displaying Significance Brackets for 'ggplot2'. https://doi.org/10.31234/osf.io/7awm6

Andrews, S. (2010). FastQC: A quality control tool for high throughput sequence data [Online]. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Bache, S., & Wickam, H. (2022). magrittr: A forward-pipe operator for R. Retrieved from https://CRAN.R-project.org/package=magrittr

Becker, M., Gscheidmeier, T., Groß, H. J., Cario, H., Woelfle, J., Rauh, M., Metzler, M., & Zierk, J. (2022). Differences between capillary and venous blood counts in children—A data mining approach. *International Journal of Laboratory Hematology*, *44*(4), 729-737. https://doi.org/10.1111/ijlh.13846

Bongen, E., Lucian, H., Khatri, A., Fragiadakis, G. K., Bjornson, Z. B., Nolan, G. P., Utz, P. J., & Khatri, P. (2019). Sex differences in the blood transcriptome identify robust changes in immune cell proportions with aging and influenza infection. *Cell Reports*, *29*(7), 1961-1973. https://doi.org/10.1016/j.celrep.2019.10.019

Bybjerg-Grauholm, J., Hagen, C. M., Khoo, S. K., Johannesen, M. L., Hansen, C. S., Bækvad-Hansen, M., Christiansen, M., Hougaard, D. M., & Hollegaard, M. V. (2016). RNA sequencing of archived neonatal dried blood spots. *Molecular Genetics and Metabolism Reports*, *10*, 33-37. https://doi.org/10.1016/j.ymgmr.2016.12.004

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *1*(29), 15-21. https://doi.org/10.1093/bioinformatics/bts635

Dobrodeeva, L. K., Samodova, A. V., & Balashova, S. N. (2020). Cellular reactions in capillary and venous blood in northerners to a short-term period in a climatic chamber. *Immunity, Inflammation and Disease*, *8*(3), 408-414. https://doi.org/10.1002/iid3.322

Durinck, S., Spellman, P. T., Birney, E., & Huber, W. (2009). Mapping identifiers for the integration of genomic datasets with the T/Bioconductor package biomaRt. *Nature Protocols*, *4*, 1184-1191. https://doi.org/10.1038/nprot.2009.97

Ewels, P., Magnusson, M., Lundin, S., & Käller, M. (2016). MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*, *32*(19), 3047-3048. https://doi.org/10.1093/bioinformatics/btw354

Galena, H. J. (1992). Complications occurring from diagnostic venipuncture. *Journal of Family Practice*, *34*(5), 582-584.

García-Alcalde, F., Okonechnikov, K., Carbonell, J., Cruz, L. M., Götz, S., Tarazona, S., Dopazo, J., Meyer, T. F., & Conesa, A. (2012). Qualimap: evaluating next-generation sequencing alignment data. *Bioinformatics*, *28*(20), 2678-2679. https://doi.org/10.1093/bioinformatics/bts503

Gehlenborg, N. (2019). UpSetR: A more scalable alternative to Venn and Euler diagrams for visualizing intersecting sets. Retrieved from https://CRAN.R-project.org/package=UpSetR

Gohel, D. (2023). officer: Manipulation of Microsoft Word and PowerPoint documents. Retrieved from https://CRAN.R-project.org/package=officer

Gohel, D., & Skintzos, P. (2023a). flextable: Functions for tabular reporting. Retrieved from https://CRAN.R-project.org/package=flextable

Gohel, D., & Skintzos, P. (2023b). ggiraph: Make 'ggplot2' graphics interactive. Retrieved from https://CRAN.R-project.org/package=ggiraph

Jansen, R., Batista, S., Brooks, A. I., Tischfield, A., Willemsen, G., van Grootheest, G., Hottenga, J.-J., Milaneschi, Y., Mbarek, H., Madar, V., Peyrot, W., Vink, J. M., Verweij, C. L., de Geus, E. J. C., Smit, H., Wright, F. A., Sullivan, P. F., Boomsma, D. I., & Penninx, B. W. J. H. (2014). Sex differences in the human peripheral blood transcriptome. *BMC Genomics*, *15*, 33. https://doi.org/10.1186/1471-2164-15-33

Jungheim, K., & Koschinsky, T. (2002). Glucose monitoring at the thenar: Evaluation of upper dermal blood glucose kinetics during rapid systemic blood glucose changes. *Hormone and Metabolic Research*, *34*(6), 325-329. https://doi.org/10.1055/s-2002-33261

Kabeer, B. S. A., Tomei, S., Mattei, Brummaier, T., McGready, R., Nosten, F., & Chaussabel, D. (2018). A protocol for extraction of total RNA from finger stick whole blood samples preserved with TempusTM solution. *F1000 Research*, *7*:1739. https://doi.org/10.12688/f1000research.16622.1

Kassambara, A. (2023). ggpubr: 'ggplot2' based publication ready plots. Retrieved from https://CRAN.R-project.org/package=ggpubr

Kim, J. H., Jin, J. O., Park, J. A., Chang, Y. H., Hong, Y. J., & Lee, J. K. (2014). Comparison of three different kits for extraction of high-quality RNA from frozen blood. *SpringerPlus*, *3*, 76. https://doi.org/10.1186/2193-1801-3-76

Kolde, R. (2019). pheatmap: Pretty heatmaps. Retrieved from https://CRAN.R-project.org/package=pheatmap

Krieza, J. L., Dorotic, A., Grzunov, A., & Maradin, M. (2015). Capillary blood sampling: National recommendations on behalf of the Croatian Society of Medical Biochemistry

and Laboratory Medicine. *Biochemia Medica (Zagreb)*, *25*(3), 335-358. https://doi.org/10.11613/bm.2015.034

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078-2079. https://doi.org/10.1093/bioinformatics/btp352

Liao, Y., Smyth, G. K., & Shi, W. (2014). featureCounts: An efficient general-purpose program for assigning sequence reads to genomic features. *Bioinformatics*, *30*(7), 923-930. https://doi.org/10.1093/bioinformatics/btt656

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, *17*(1), 10-12. https://doi.org/10.14806/ej.17.1.200

Mistry, M., Piper, M., Liu, J., & Khetani, R. (2021). hbctraining/DGE_workshop_salmon_online: Differential Gene Expression Workshop Lessons from HCBC (first release). *Zenodo*. https://doi.org/10.5281/zenodo.4783481

Moon, K. (2020). ggiraphExtra: Make interactive 'ggplot'. Extension to 'ggplot2' and 'ggiraph'. Retrieved from https://CRAN.R-project.org/package=ggiraphExtra

Morgan, M., & Shepherd, L. (2023). AnnotationHub: Client to access AnnotationHub resources. Retrieved from https://doi.org/10.18129/B9.bioc.AnnotationHub

Munster, S. K., Uyhelji, H. A., & Nicholson, S. J. (2023). *An evaluation of the downstream effects of purification methods on RNA-Seq differential expression* (No. DOT/FAA/AM-23/01). United States Department of Transportation, Federal Aviation Administration, Office of Aviation, Civil Aerospace Medical Institute. https://doi.org/10.21949/1524442

Munster, S. K., Nicholson, S. J., & Uyhelji, H. A. (2023). *RNA-Seq Alignment and Differential Expression Software Comparison* (No. DOT/FAA/AM-23/02). United States Department of Transportation, Federal Aviation Administration, Office of Aviation, Civil Aerospace Medical Institute. https://doi.org/10.21949/15244423

Nicholson, S. J., Munster, S. K., Uyhelji, H. A., Hutchings, D., White, V. L., Booth, J. L., Burian, D. M., & Metcalf, J. P. (2021). *Transcriptional responses to altitude-induced hypoxia in bronchial epithelium, broncho-alveolar cells, and blood differ between smokers and nonsmokers* (No. DOT/FAA/AM-21/25). United States Department of Transportation, Federal Aviation Administration, Office of Aviation, Civil Aerospace Medical Institute.

Nicholson, S. J., Munster, S. K., White, V. L., Burian, D. M., Bryant, D. N., & Wade, M. E. (2023). *Comparison of the human transcriptional response to three hypoxic environments* (No. DOT/FAA/AM-23/03). United States Department of Transportation, Federal Aviation Administration, Office of Aviation, Civil Aerospace Medical Institute. https://doi.org/10.21949/1524444

Pantano, L. (2023). DEGreport: Report of DEG analysis. Retrieved from https://doi.org/10.18129/B9.bioc.DEGreport

Pedersen, T. (2022). patchwork: The composer of plots. Retrieved from https://CRAN.R-project.org/package=patchwork

Pirritano, M., Fehlmann, T., Laufer, T., Ludwig, N., Gasparoni, G., Li, Y., Meese, E., Keller, A., & Simon, M. (2018). Next generation sequencing analysis of total small noncoding RNAs from low input RNA from dried blood sampling. *Analytical Chemistry*, *90*(20), 11791-11796. https://doi.org/10.1021/acs.analchem.8b03557

Posit Team. (2023). RStudio: Integrated development environment for R. Posit Software, PBC, Boston, MA. Retrieved from http://www.posit.co/

R Core Team. (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Retrieved from https://www.R-project.org/

Rainer, J., Gatto, L., & Weichenberger, C. X. (2019). ensembldb: An R package to create and use Ensembl-based annotation resources. *Bioinformatics*, *35*(19), 3151-3153. https://doi.org/10.1093/bioinformatics/btz031

Rinchai, D., Anguiano, D. E., Nguyen, P., & Chaussabel, D. (2016). Finger stick blood collection for gene expression profiling and storage of tempus blood RNA tubes. *F1000 Research*, *5*, 1385. https://doi.org/10.12688/f1000research.8841.2

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7), e47. https://doi.org/10.1093/nar/gkv007

Robison, E. H., Mondala, T. S., Williams, A. R., Head, S. R., Salomon, D. R., & Kurian, S. M. (2009). Whole genome transcript profiling from fingerstick blood samples: A comparison and feasibility study. *BMC Genomics*, *10*, 617. https://doi.org/10.1186/1471-2164-10-617

Signal, B. (2023). GeneStructureTools: Tools for spliced gene structure manipulation and analysis. Retrieved from https://doi.org/10.18129/B9.bioc.GeneStructureTools

Slowikowski, K. (2023). ggrepel: Automatically position non-overlapping text labels with 'ggplot2'. Retrieved from https://CRAN.R-project.org/package=ggrepel

Speake, C., Whalen, E., Gersuk, V. H., Chaussabel, D., Odegard, J. M., & Greenbaum, C. J. (2017). Longitudinal monitoring of gene expression in ultra-low-volume blood samples self-collected at home. *Clinical & Experimental Immunology*, *188*(2), 226-233. https://doi.org/10.1111/cei.12916

Stein, D. F., O'Connor, D., Blohmke, C. J., Sadarangani, M., & Pollard, A. J. (2016). Gene expression profiles are different in venous and capillary blood: Implications for vaccine studies. *Vaccine*, *34*(44), 5306-5313. https://doi.org/10.1016/j.vaccine.2016.09.007

Tian, Y., Stamova, B., Jickling, G. C., Liu, D., Ander, B. P., Bushnell, C., Zhan, X., Davis, R. R., Verro, P., Pevec, W. C., Hedayati, N., Dawson, D. L., Khoury, J., Jauch, E. C., Pancioli, A., Broderick, J. P., & Sharp, F. R. (2012). Effects of gender on gene expression in the blood of ischemic stroke patients. *Journal of Cerebral Blood Flow and Metabolism*, *32*(5), 780-791. https://doi.org/10.1038/jcbfm.2011.179

Toma, R., Duval, N., Pelle, B., Parks, M. M., Gopu, V., Torres, P. J., Camacho, F. R., Shen, N., Krishnan, S., Hatch, A., & Tily, H. (2020). A clinically validated human capillary blood transcriptome test for global systems biology studies. *BioTechniques*, *69*(4), 289-301. https://doi.org/10.2144/btn-2020-0088

Tsukuda, Y., Funakoshi, T., Nasuhara, Y., Nagano, Y., Shimizu, C., & Iwasaki, I. (2019). Venipuncture nerve injuries in the upper extremity from more than 1 million procedures. *Journal of Patient Safety*, *15*(4), 299-301. https://doi.org/10.1097/pts.0000000000000264

Turner, S. (2023). annotables: Ensembl annotation tables. Retrieved from https://github.com/stephenturner/annotables

Uyhelji, H. A., Kupfer, D. M., White, V. L., Jackson, M. L., Van Dongen, H. P. A., & Burian, D. M. (2018). Exploring gene expression biomarker candidates for neurobehavioral impairment from total sleep deprivation. *BMC Genomics*, *19*(1), 341. https://doi.org/10.1186/s12864-018-4664-3

Uyhelji, H. A., White, V. L., Munster, S. K., & Nicholson, S. J. (2023). *Gene expression biomarkers of the response to sleep loss with and without modafinil* (No. DOT/FAA/AM-23/35). United States Department of Transportation, Federal Aviation Administration, Office of Aviation, Civil Aerospace Medical Institute. https://doi.org/10.21949/1529628

Voin, V., Iwanaga, J., Sardi, J. P., Fisahn, C., Loukas, M., Oskouian, R. J., & Tubbs, R. S. (2017). Relationship of the median and radial nerves at the elbow: Application to avoiding injury during venipuncture or other invasive procedures of the cubital fossa. *Cureus*, *9*(3), e1094. https://doi.org/10.7759/cureus.1094

World Health Organization. (2010). *Best practices for injections and related procedures toolkit*. Geneva, Switzerland: World Health Organization.

World Health Organization. (2010). *Best practices on drawing blood: Best practices in phlebotomy*. Geneva, Switzerland: World Health Organization.

Wickham, H. (2016). ggplot2: Elegant graphics for data analysis. Springer-Verlag New York.

Wickham, H., & Seidel, D. (2022). scales: Scale functions for visualization. Retrieved from https://CRAN.R-project.org/package=scales

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., Kuhn, M., Pedersen, T. L., Miller, E., Bache, S. M., Müller, K., Ooms, J., Robinson, D., Seidel, D. P., Spinu, V., Takahashi, K., Vaughan, D., Wilke, C., Woo, K., & Yutani, H. (2019). Welcome to the tidyverse. *Journal of Open Source Software*, *4*(43), 1986. https://doi.org/10.21105/joss.01686

Wright, K. (2021). pals: Color palettes, colormaps, and tools to evaluate them. Retrieved from https://CRAN.R-project.org/package=pals

Zhu, A., Ibrahim, J. G., & Love, M. I. (2018). Heavy-tailed prior distributions for sequence count data: Removing the noise and preserving large differences. *Bioinformatics*, *35*(12), 2084-2092. https://doi.org/10.1093/bioinformatics/bty895