Collection and Storage Effects for Telomere Length and DNA Methylation

April 2023

Daniel Notterman and Colter Mitchell

(with Jessica Faul, Lisa Schneper, and Jonah Fisher)

UNIVERSITY OF MICHIGAN

PRINCETON UNIVERSITY

Biological Aging And Weathering

Chronological age is a good, but imperfect, proxy of the concepts of biological aging and weathering (stress response)

- Provide an endophenotype for basic research/screens
- Facilitate evaluation of interventions aimed at delaying aging
- Help identify at-risk subpopulations

- Two recent biomarkers of these concepts:
  - Telomere length (TL)
  - DNA methylation (DNA)
- Both have also been used for other purposes as well
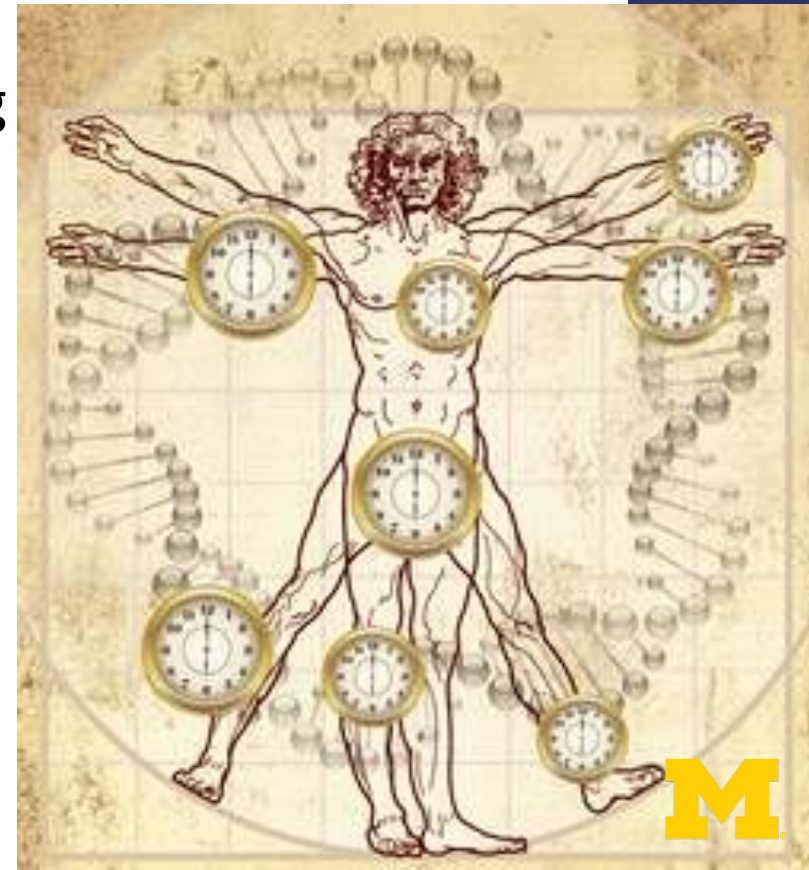
Concerns about collection

- Many studies have already begun assaying thousands of samples for TL and DNAm from their genetic collections

- However, little research has explored the effects of collection and storage conditions on TL and epigenetic data (see TRN for exception in TL research)

- Genomic samples are likely susceptible to normal variation in collection and storage and may be systematically biased even when current standard protocols are used

- 2018 Pilot—problematic batch

Examining Sources of Experimental Variability

- Sample
  - Source (saliva, whole blood, etc.)
  - Collection vessel (e.g. tube type)
  - Storage time and temperature
  - Cell distribution

- DNA
  - Extraction method
  - Suspension medium
  - Storage time and temperature
  - Freeze-thaw cycles
 - Quantification method

- Measurement
  - Reagents (brand and batch)
  - Analysis protocol
  - Operator
  - Other?

Our pilot examines the effects of sample type, collection, and storage on TL and DNAm measurement in saliva and venous blood samples

General Protocol

- SALIVA
  - Oragene kits collected at the same time as blood collection
  - Stored at room temperature for 0, 3, 6, 10 or 12 months before extraction and analysis
- VENOUS BLOOD
  - Collected using EDTA, Heparin, PAXgene tubes
  - Stored at 4°C for 0, 1, 2, 3, 7, or 28 days before DNA extraction, freezing, and complete blood count
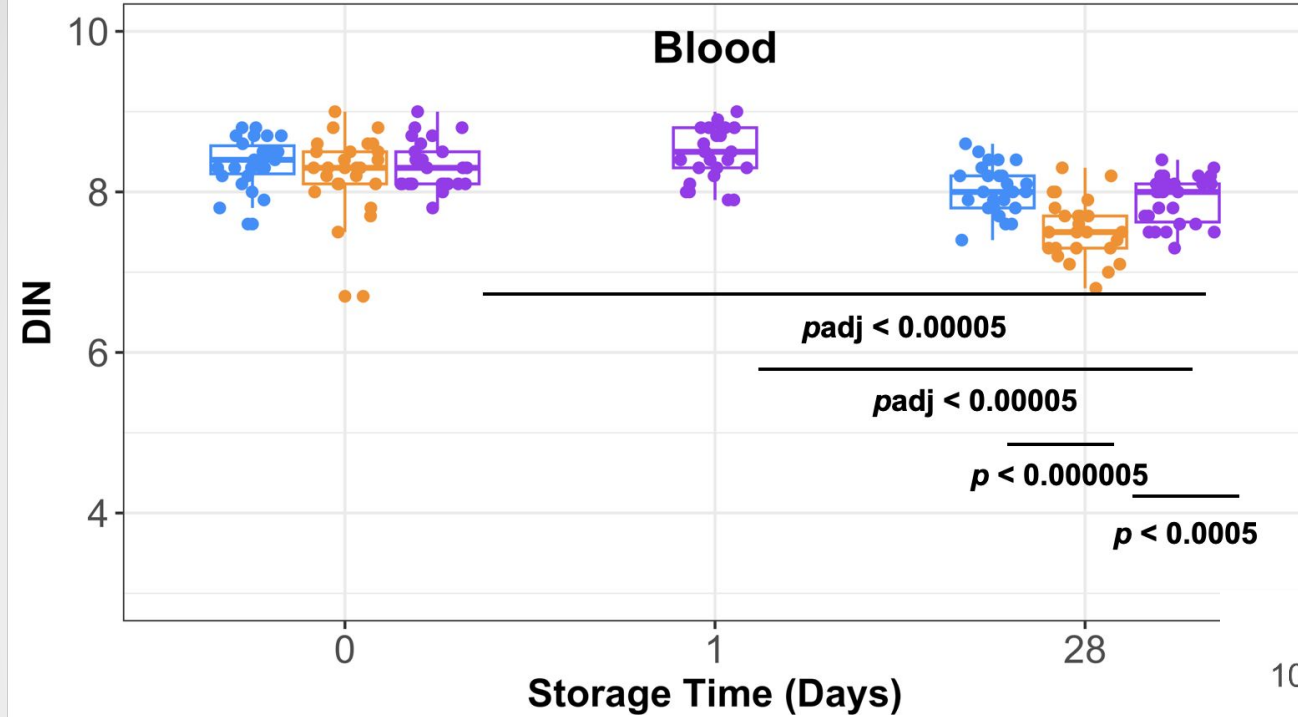
DNA Yield Over Time by Sample Type
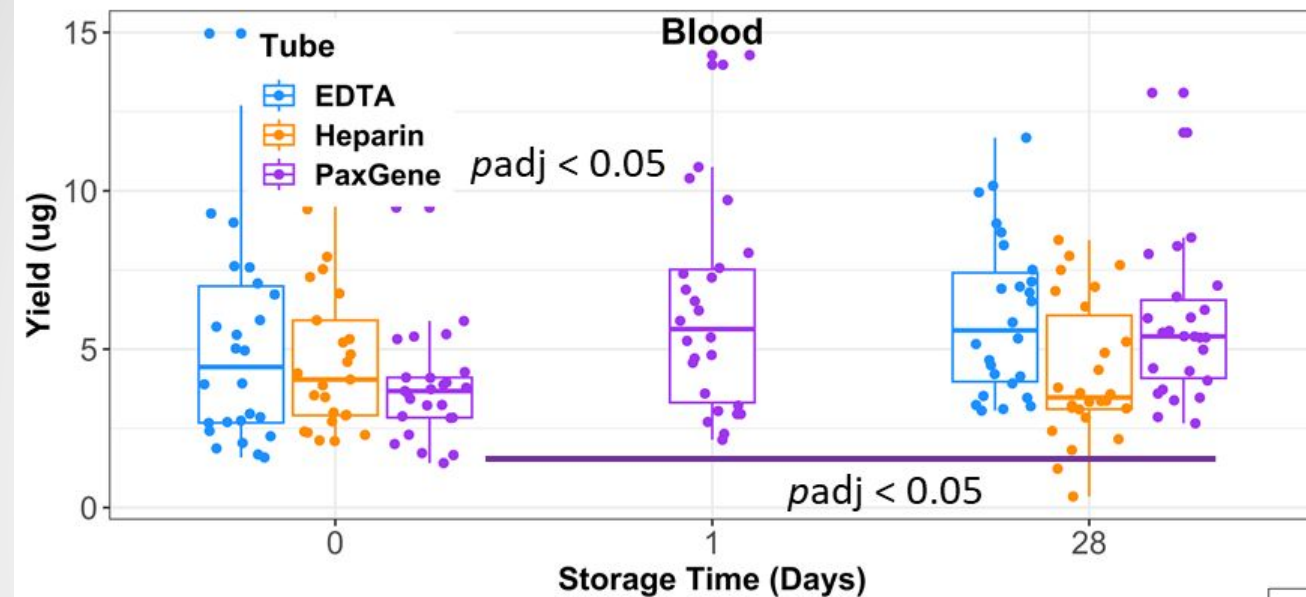
Significant differences in DNA yield between the day-of-collection time point and later points were observed for both Paxgene and saliva samples. This suggests delaying extraction to day 2 (blood).
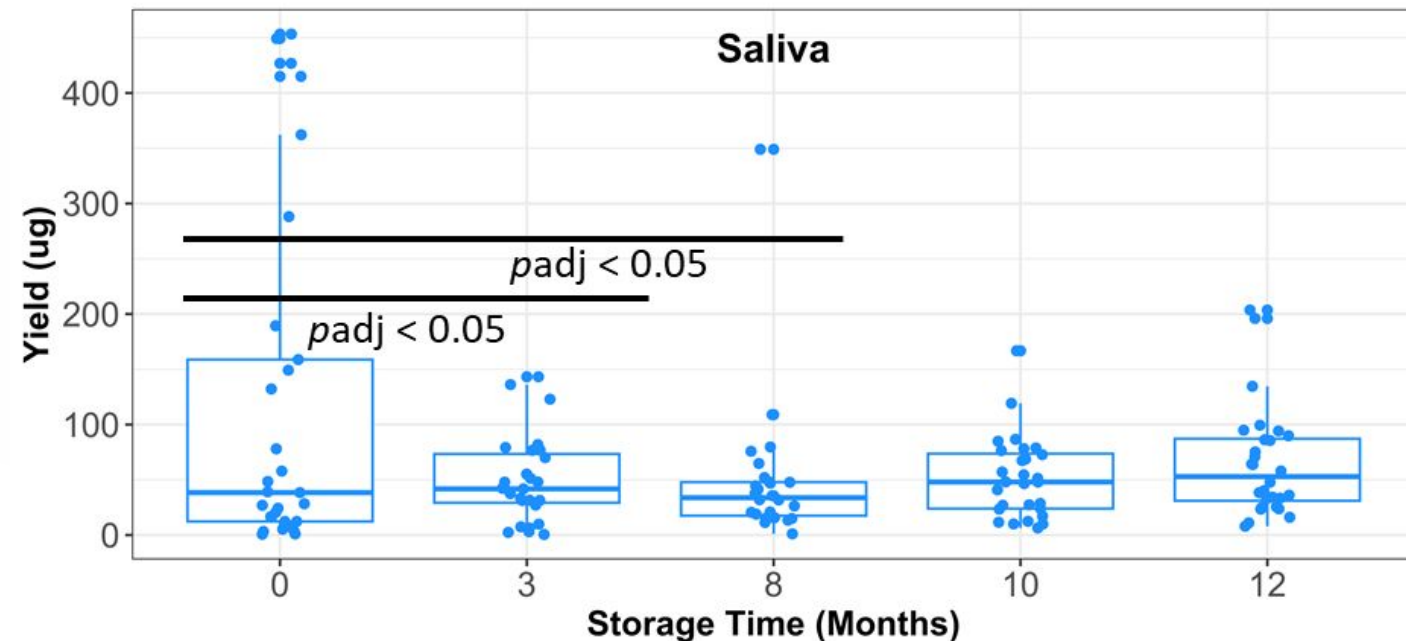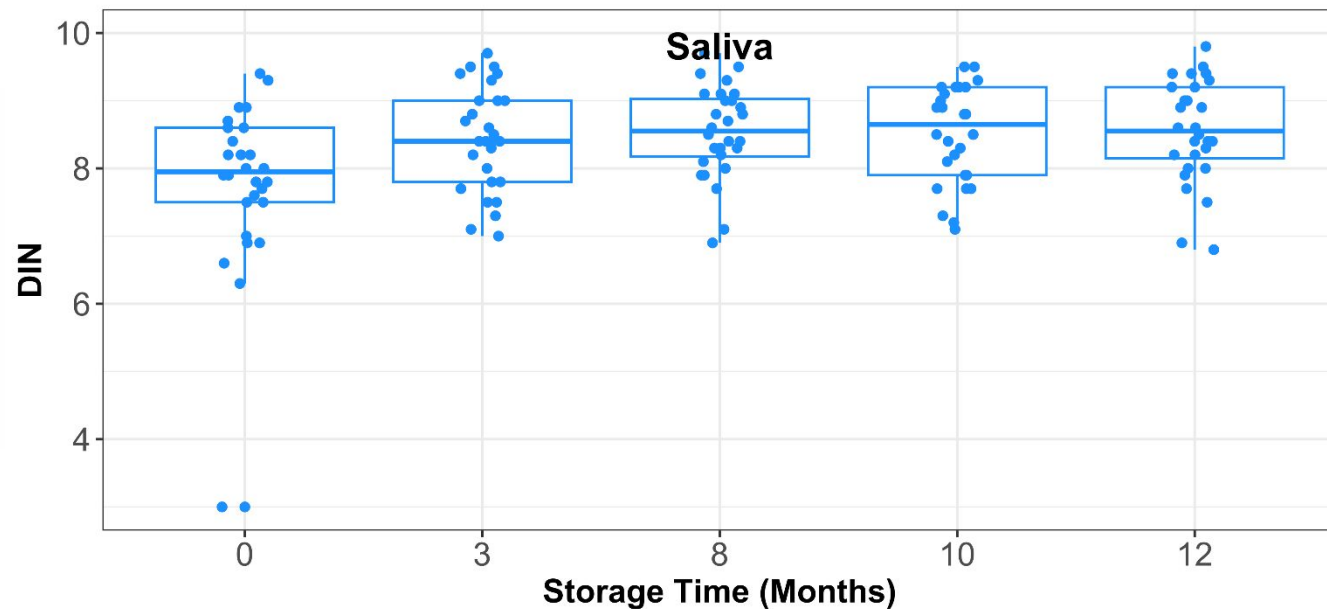
DNA Integrity Over Time by Sample Type

Most stable in EDTA, least stable in heparin.
Paxgene stability decreased between 1 and 28 days.

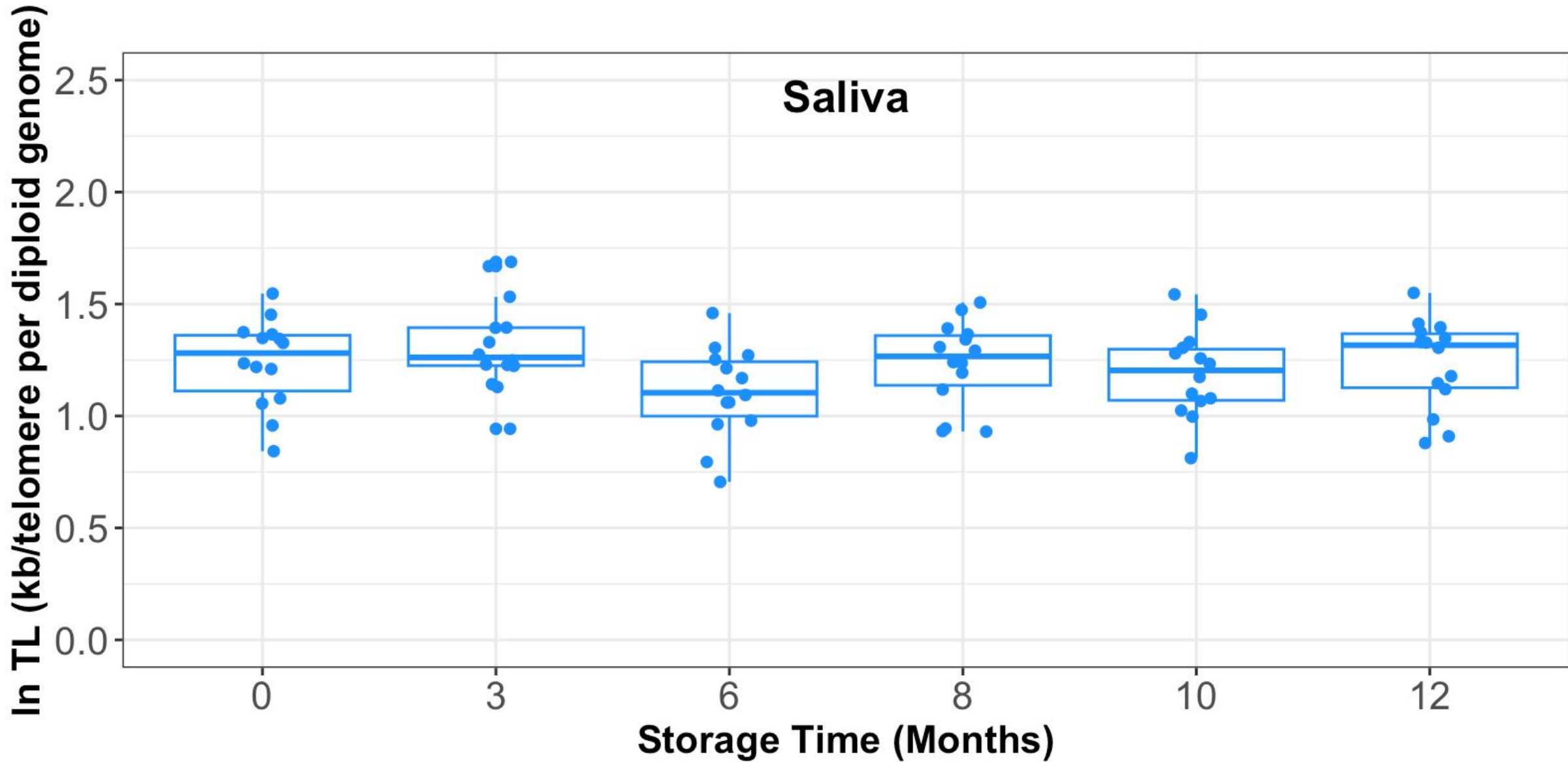Saliva held in the OG500 was stable for the 12-month duration of the study

Telomere Lenth Analysis

- N=28; adults
- At 1 year, all samples analyzed simultaneously using quantitative PCR (Geronimus et al. 2021, Mitchell et al., 2014; O'Callaghan 2011) in the Notterman Lab at Princeton
- CV assay 9% ICC: 0.8
- Significance tested by after logging.

TL Results Blood
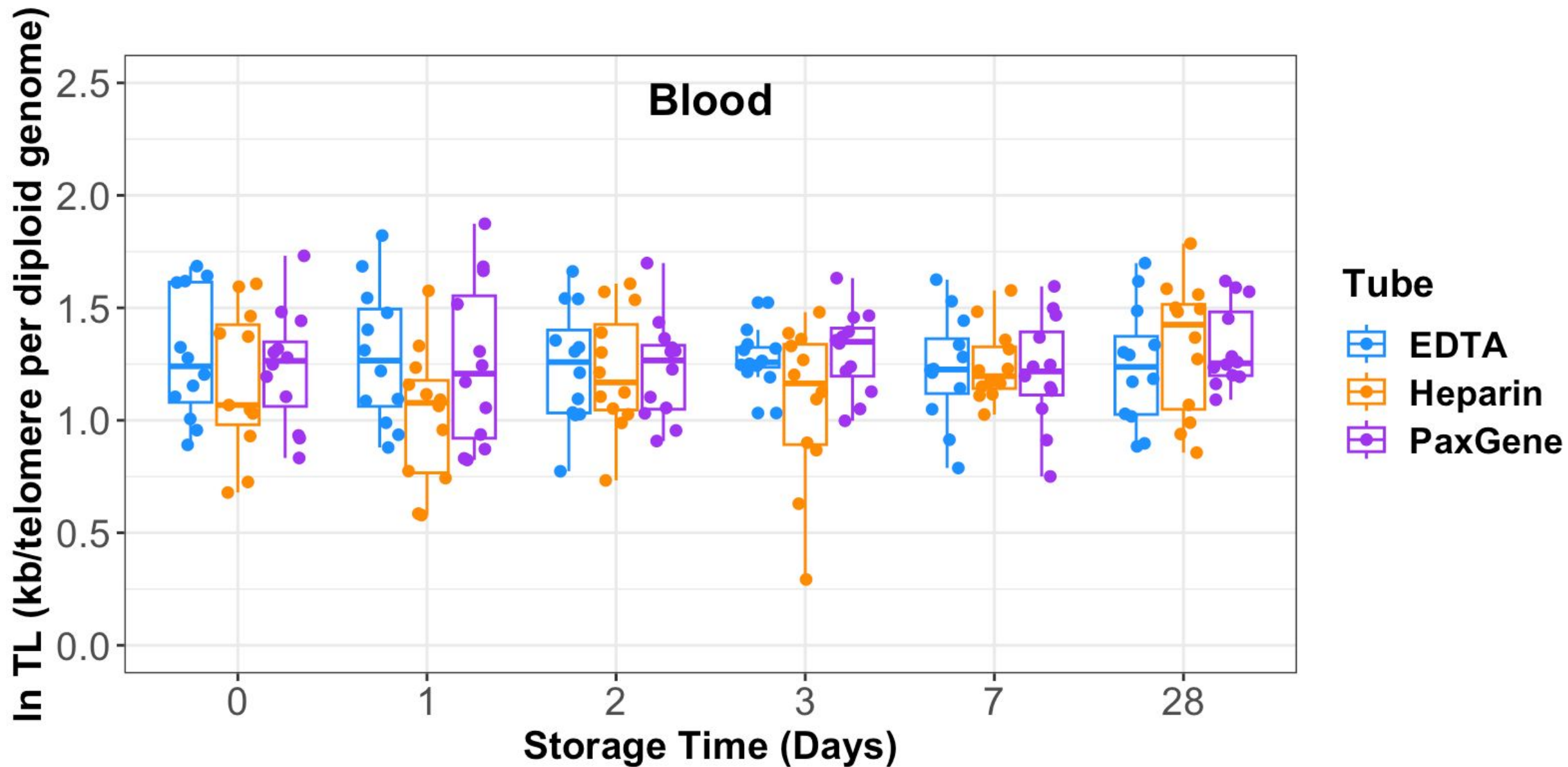
TL Results Saliva

DNA Methylation

- N=15

- Illumina Epic Array

- Re-ran tie 0 samples in another batch (i.e. 96-well plate run on a different day)

DNAm Quality Control

- All 288 samples are compared to 14 quality control metrics (4,032 total tests)

- 14 samples failed at least 1 metric and 7 samples were cut

- Saliva is more likely to fail but not to be cut

- Heparin and Saliva have lower intensity
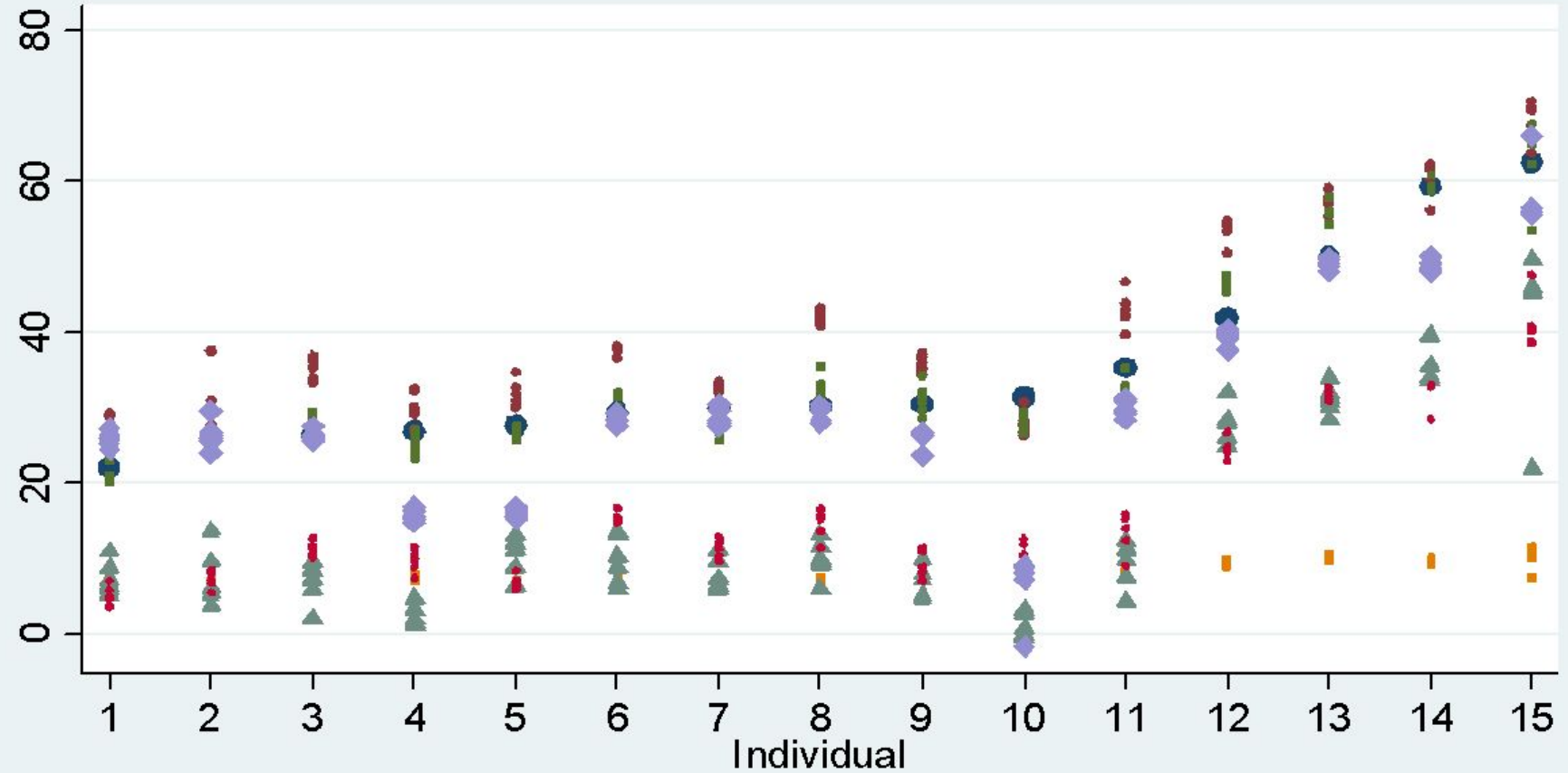
- **No effect of storage time**

Epigenetic Clock Variation

- Clock
- Tissue (sometimes)
- Batch
- Blood Collection
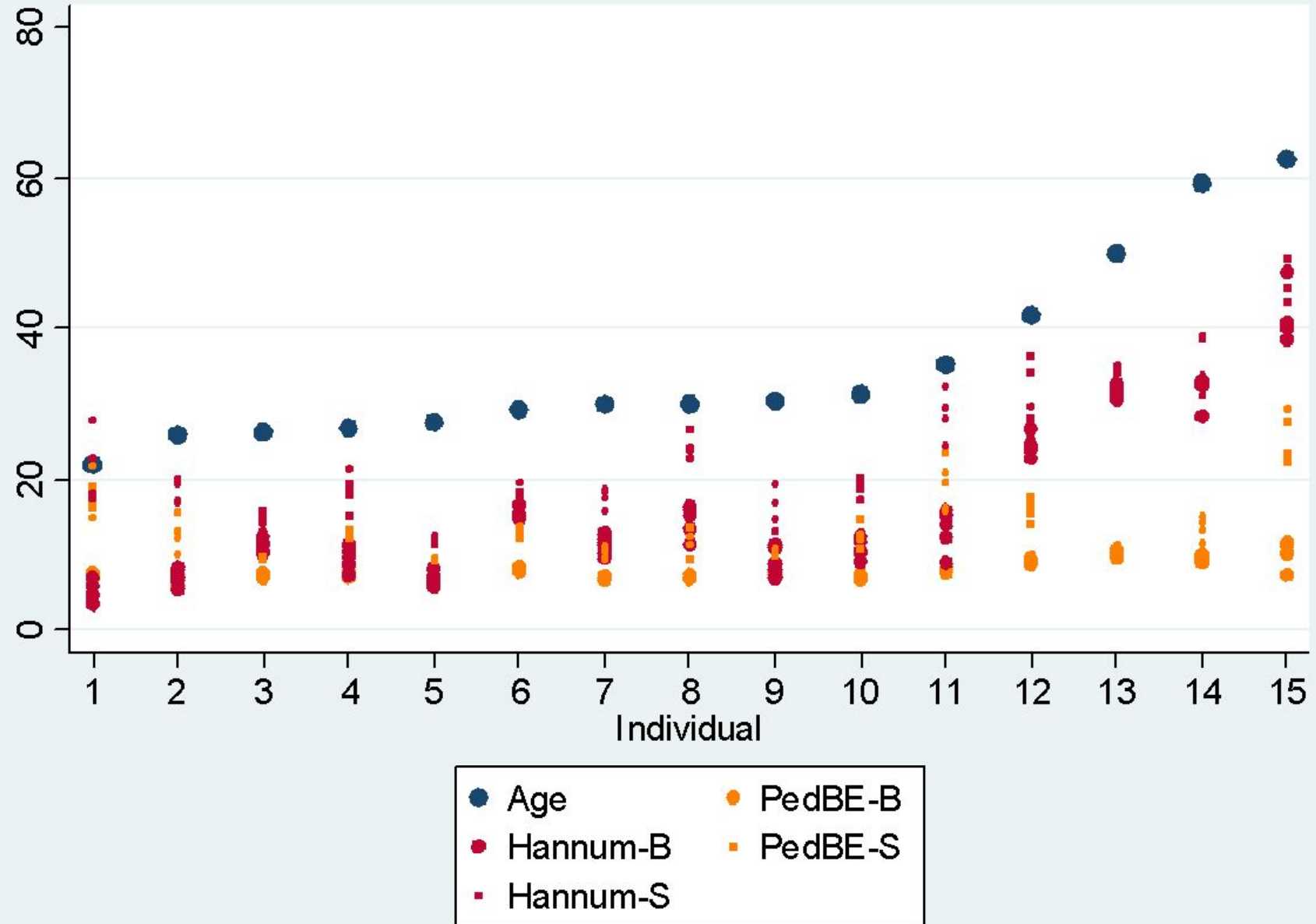- Storage time at < ideal temp
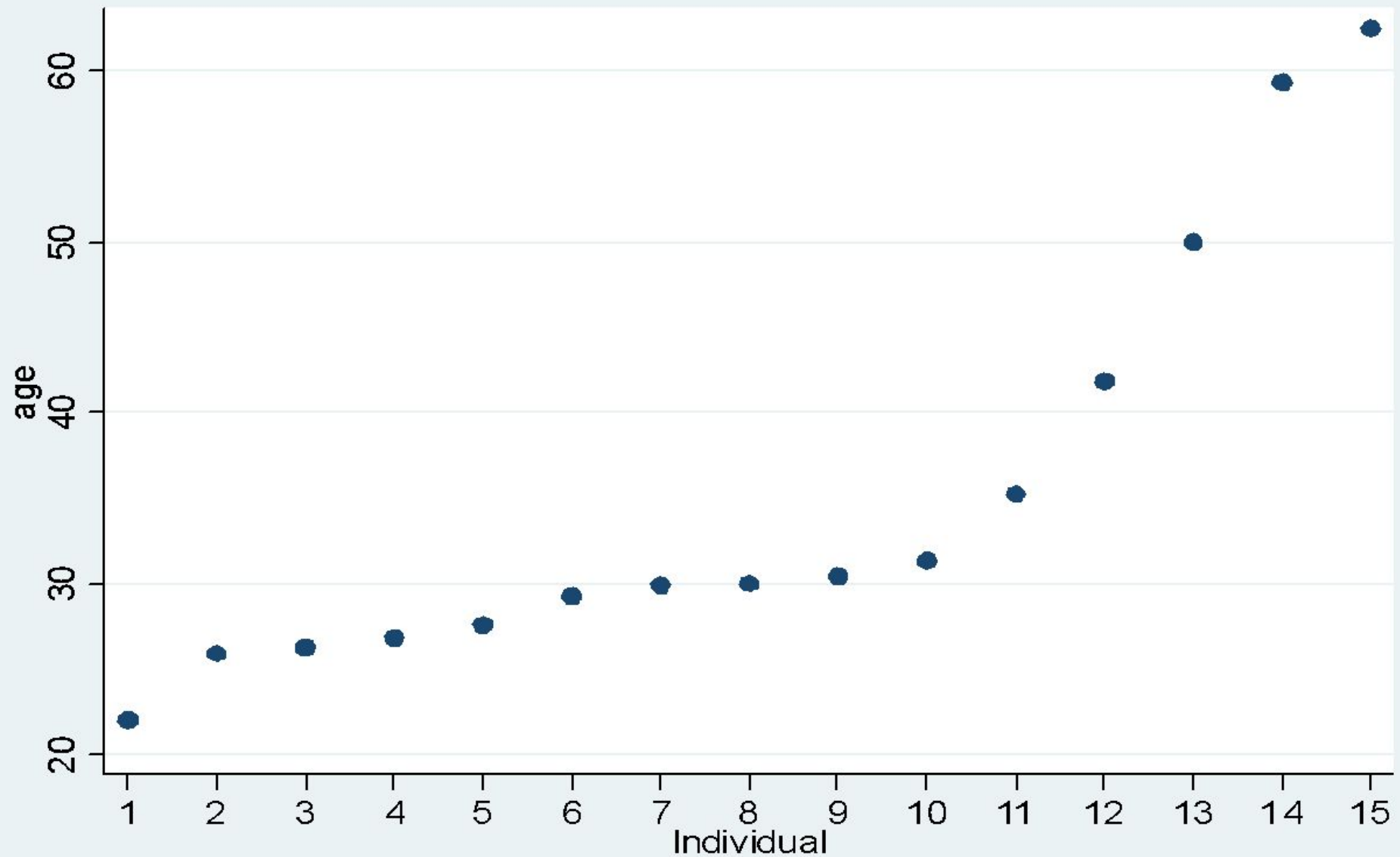
Differences in Clocks

- Blood
- PaxGene
- No batch effect
- All time points and clocks

- Within range of 2-5 years
- Between Clock 5-10 years
- How was clock developed?

Tissue

- Developed for multiple tissues
- 5-7 years between clocks
- 3-5 within
- No difference by tissue

- Developed for one tissue
- Within range of 3-5 years
- Between Clock 5-7
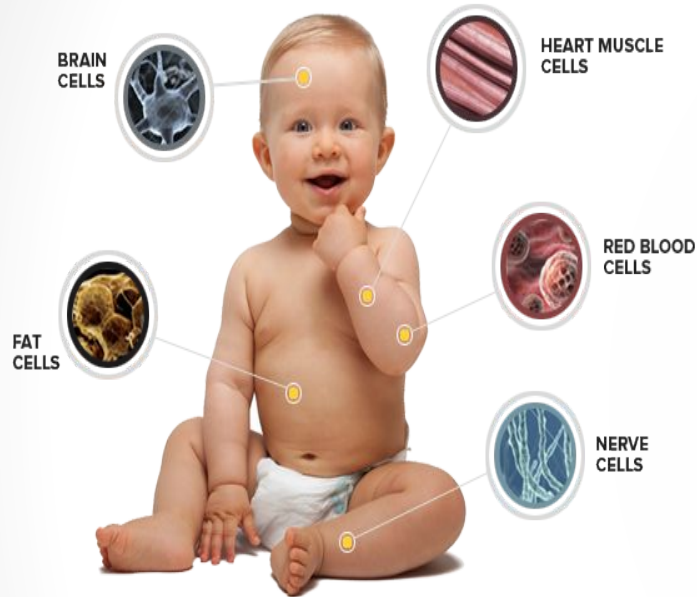- 4-5 year by tissue

Batch

- All time 0
- Colors are scores
- Shapes denote different batch
- Small but significant effect of batch for most clocks
- 1-2 year

- Some Clocks do have major batch effect problems
- Color difference is batch
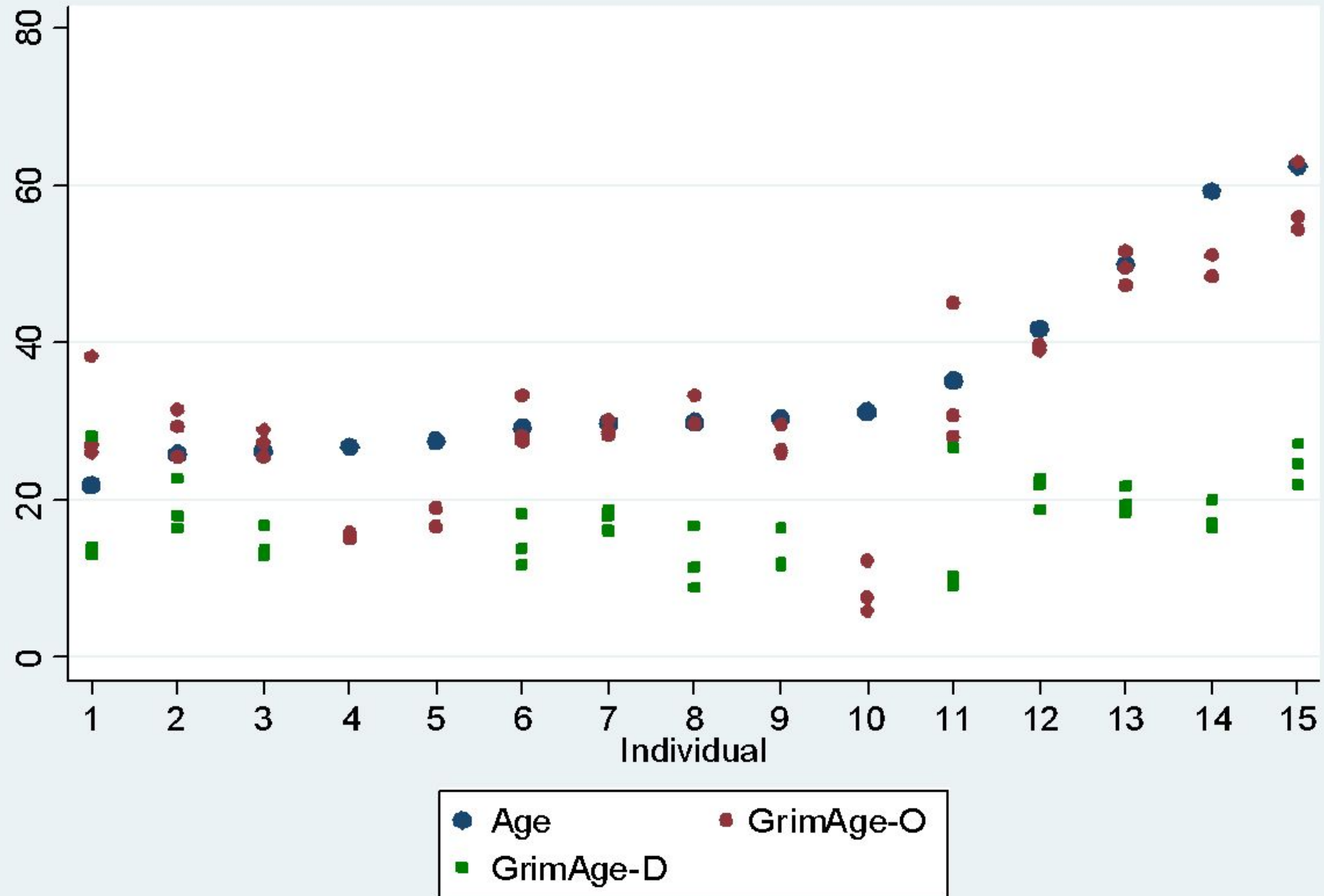
Cell Distribution

EPIGENETICS
2021, VOL. 17, NO. 2, 161–177
https://doi.org/10.1080/15592294.2021.1890874

Taylor & Francis
Taylor & Francis Group

Saliva cell type DNA methylation reference panel for epidemiological studies in children

Lauren Y. M. Middleton [a,b], John Dou[a], Jonah Fisher [c], Jonathan A. Heiss [d], Vy K. Nguyen [b,e], Allan C. Just [d], Jessica Faul[c], Erin B. Ware [c,f], Colter Mitchell[c,f], Justin A. Colacino [b,g,h,i], and Kelly M. Bakulski [a]

- Salivary collection kits had difficulty lysing epithethial cells
- Higher immune cell percentage in saliva methylation vs raw samples
- CBC Analyses Forthcoming

Summary

- Studies that plan to add TL or DNAm measures using stored DNA samples within reasonable protocols are unlikely to see collection effects

- DNA concentration in blood may not be accurately measured on the day of collection with the Paxgene tube or Oragene Kit.

- DNA Integrity (DIN) decreases in heparin following 28 days of storage

- Individual level variation is still high for most measures (3-5 years; not ready for clinical application)

- Differences between clocks is large 5-10 years (even for age clocks)

- Tissue—depends on application often comparable to batch (1-3 years)