Introduction

Longitudinal prospective cohorts allow the study of the onset and progression of conditions such as aging,1 cancer,2 and cardiovascular disease.3 When paired with biobanking initiatives, the biological material and associated data (BMA D) collected from these cohorts can be used not only for prospective basic research, but also retrospective studies.4 The combination of longitudinal studies and biobanking has promoted the advancement of research in numerous fields, as exemplified by the UK Biobank, a large population-based prospective study that permits the investigation of genetic and non-genetic determinants of diseases of aging,5 and the Framingham Heart Study, which has been collecting and distributing BMAD related to cardiovascular disease since 1948.6 The BMAD collected and distributed by these 2 longitudinal studies has resulted in over 6000 publications to date.7,8
Biobanks can improve the traceability, authenticity, and fitness-for-purpose of BMaD by providing standardized services for the acquisition, transport, processing, storage, and distribution of biospecimens. Compliance with biobanking standards and implementation of a Quality Management System (QMS) can further improve the quality of these materials by ensuring that biobanking operations are performed according to established procedures and by promoting continuous improvements of processes. Moreover, standardization of biobanking processes is paramount to ensuring uniform, reliable, and predictable biospecimen quality in multisite and long-term studies. As such, it is critical for biobanks to be involved in the early stages of study planning to ensure they can support the needs of the project.

Biobanks also have a responsibility toward their stakeholders, broadly summarized into 3 categories: biospecimen donors; funders; and BMaD users/researchers. Sustainability in biobanking consists of operational, financial, and social dimensions, and has a different impact for each of these 3 stakeholder groups. When involved in the early planning of projects, biobanks can address all 3 sustainability dimensions for their stakeholders, which in turn allows them to support long-term studies.

The Dog Aging Project (DAP) is a longitudinal study of aging in the domestic dog (Canis familiaris), funded by the National Institute on Aging (NIA), that follows tens of thousands of companion dogs across the United States to identify the biological and environmental factors that affect aging. In addition to supporting specific DAP research goals, all BMaD collected by the DAP are made accessible to external researchers. As such, the DAP Biobank has been created to support both the immediate goals of the DAP, as well as future research by the broader scientific community, by ensuring the integrity and fitness-for-purpose of its BMaD.

The Cornell Veterinary Biobank (CVB), a core resource at the Cornell University College of Veterinary Medicine (CUCVM), has supported the biomedical and translational research community at Cornell University and internationally since 2006 by collecting, acquiring, processing, quality testing, storing, and distributing BMaD. It is the first biobank in the world to receive accreditation to the International Organization for Standardization (ISO) normative reference ISO 20387:2018 General Requirements for Biobanking, having successfully implemented a quality management system and demonstrated compliance to the standard for all of its listed processes. Competency gained by biobank personnel working in compliance with ISO 20387 increased the CVB’s ability to develop and implement new procedures in a more efficient manner, thereby minimizing the impact of COVID-19 pandemic-related challenges on biobanking operations. In an industry where specimen underutilization is a widespread concern, implementation of quality practices that increase the integrity and value of biological materials can be highly beneficial.

In this manuscript, we describe how the CVB developed the DAP Biobank through the implementation of a QMS and by utilizing a dynamic strategy that allows for real-time adjustments of processes in response to risk-assessment data, preliminary findings from process implementation, and user needs.

Materials and Methods

DAP study plan and specimen collection

The Dog Aging Project (DAP) aims to identify the biological and environmental determinants of aging in dogs through 4 unique research activities: (1) a clinical phenotype project, to develop novel clinical and physical metrics for the analysis of comorbidities, frailty, and inflammaging to define an “aged dog” phenotype; (2) a genetics project, to identify genetic variation associated with aging-related traits, and with exceptional longevity, by whole genome sequencing; (3) a systems biology project, to develop predictors of aging and age-related traits based on the peripheral blood mononuclear cell (PBMC) epigenome, fecal microbiome, and plasma metabolome; and (4) the Test of Rapamycin In Aged Dogs, or TRIAD, a double-blind, placebo controlled trial designed to test the effect of low-dose rapamycin on healthspan and lifespan in large-breed, middle-aged dogs. Dog owners, recruited through mainstream and social media, are invited to nominate their dogs through the DAP website, provide informed consent, and complete a comprehensive Health and Life Experience Survey to be enrolled in the DAP Pack. Out of the >50,000 dogs projected to be enrolled in the DAP Pack, 10,000 are being selected to participate in 1 of 3 sampled cohorts based on qualifying electronic veterinary medical records: the Foundation cohort dogs provide cheek swabs as a source of DNA for genotyping by sequencing to identify the genetic determinant of age-related traits; the Precision cohort dogs provide biospecimens annually, in addition to cheek swab specimens, to support the study of the molecular mechanisms and -omics of aging; and the TRIAD cohort dogs provides the same specimens as the Precision cohort, but twice a year with additional clinical examinations, to determine the impact of intervention through rapamycin on aging. All de-identified data collected by DAP, including survey results, analysis of biospecimens, and environmental data, are made available on DAP’s research data repository to support open science.

Through the use of a custom-designed collection kit (Supplemental Methods 1), Precision and TRIAD cohort participants provide whole blood, feces, urine, and hair specimens, which are sent to the DAP laboratory at Texas A&M University (TAMU) for processing, aliquoting, temporary storage, and through-shipping to downstream laboratories and the DAP...
Establishing the DAP Biobank

The DAP was funded through a U19 grant from the NIA in 2018. In May 2019, the DAP team together with the CVB submitted a second proposal to the NIA to support the establishment and operations of the DAP Biobank. With approved funding, the CVB created the DAP Biobank as part of its operations. The DAP Biobank utilizes the quality management system established for general CVB processes, but has separate standard operating procedures for the reception, processing, quality testing, storage, and distribution of DAP specimens. These procedures and associated quality assurance activities were developed in compliance with ISO 20387:2018.

The DAP Biobank personnel include the CVB management team, consisting of the director, the assistant director of clinical services, and the assistant director of laboratory services, 2 technical biobanking specialists, a quality assurance manager, and a marketing and communication specialist. The CVB management team participates in weekly sample logistics meetings with the DAP to provide guidance for the collection, transport, processing, and storage of biological materials to be done in compliance with normative requirements. The DAP Biobank quality assurance manager, along with the CVB management team, participates in bi-monthly quality assurance/quality control (QA/QC) committee meetings to coordinate educational efforts and implement quality assurance practices throughout the DAP. The DAP Biobank marketing and communication specialist participates in weekly meetings with the DAP external communications team to align marketing objectives and coordinate content creation and dissemination, and weekly meetings for recruitment and retention efforts of DAP participants.

A pilot phase (February to May 2021) was created and implemented by the DAP research team to develop, test, and optimize the workflow and processes associated with the collection of specimens from the Precision and TRIAD cohorts, including the reception, processing, quality testing, and storage of biological material at the DAP Biobank. Following the completion of the pilot phase and implementation of identified improvements, the early phase (July to October 2021) of collection, processing, and banking of specimens from DAP participants began.

Banking specimens from DAP participants

Initial stabilization and transportation to DAP Biobank. Following processing by the DAP laboratory at TAMU, PBMCs, serum, and plasma specimens were temporarily stored at −80°C in 2″ storage boxes by specimen type until overnight shipment on dry ice to the DAP Biobank at CUCVM. Shipping containers consisted of an outer cardboard box with an inner polystyrene insulating container. Date and time of arrival at the DAP Biobank were recorded, along with the presence of remaining dry ice and whether the specimens were in a frozen state.

Whole blood specimens preserved in EDTA and temporarily stored at 4°C at the DAP laboratory at TAMU were shipped overnight to the DAP Biobank at CUCVM on a weekly basis with wet ice packs. Blood vials were placed in absorbent sleeves and resealable plastic bags, and shipped in containers as described above. Date and time of arrival at the DAP Biobank were recorded, along with a temperature reading of one of the specimens using an infrared thermometer; this was informed by previous pilot data showing minimal temperature variation among blood tubes within a shipping container (data not shown).

Reception. Each animal and its corresponding specimens were accessioned into the CVB biobank information management system (BIMS) and given unique CVB identifiers, linked to the previously assigned DAP identifiers, to allow for traceability throughout the biospecimen life cycle. Accession data was transferred automatically through an application programing

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Table 1. Whole blood specimens collected from Precision and TRIAD cohort participants and intended for banking at the DAP Biobank at CUCVM, following processing and/or temporary storage at the DAP Laboratory at TAMU.

| BLOOD TUBE TYPE | TAMU PROCESSING | TEMPORARY STORAGE | CUCVM PROCESSING | LONG-TERM STORAGE | TARGET ALIQUOT NUMBER AND SIZE |
|-----------------|----------------|-------------------|------------------|------------------|-------------------------------|
| EDTA            | -              | 4°C               | DNA extraction   | −80°C            | 1 × 0.5 mL                    |
| EDTA            | -              | 4°C               | Aliquoting       | −80°C            | 2 × 0.5 mL                    |
| EDTA            | PBMC isolation | −80°C             | -                | LN₂ vapor        | 4 × 500000                    |
| Serum separator | Serum isolation | −80°C          | -                | −80°C            | 2 × 500 µL                    |
| Heparin         | Plasma isolation | −80°C          | -                | −80°C            | 2 × 250 µL                    |

Abbreviations: CUCVM, Cornell University College of Veterinary Medicine; PBMC, peripheral blood mononuclear cell; TAMU, Texas A&M University.
interface (API) designed to collate critical information from the DAP electronic data capture system (Research Electronic Data Capture [REDCap], hosted in Microsoft’s Azure cloud platform) and electronic laboratory notebook system (eLabJournal, eLabNext) and submitted to the CVB BIMS. As the DAP retains demographic, clinical, and environmental information about the banked participants, to be made available to requesting researchers,15 the CVB only accessioned information required for its processes and to maintain traceability (Table 2). This avoids unnecessary redundancy in data storage and facilitates the distribution workflow. A manual accessioning process was also developed as a backup in case of API failure. A photograph of each whole blood tube showing both DAP and CVB identifiers was taken to demonstrate specimen traceability.

**Table 2.** Data recorded in DAP Biobank biobanking information management system.

| LEVEL       | DATA PROVIDED BY DAP                                      | DATA PROVIDED BY CVB                                          |
|-------------|----------------------------------------------------------|--------------------------------------------------------------|
| Animal      | DAP animal ID                                           | CVB animal ID                                               |
|             | Animal name                                             |                                                              |
|             | Owner name                                              |                                                              |
|             | Breed                                                   |                                                              |
|             | Sex                                                     |                                                              |
|             | Date of birth                                           |                                                              |
|             | Cohort                                                  |                                                              |
| Specimen    | DAP specimen ID                                         | CVB specimen ID                                              |
|             | Specimen type                                           | DNA concentration, purity, integrity*                        |
|             | Specimen collection date                                | DNA extraction quality assurance data*†                      |
|             | Animal weight at time of collection                     |                                                              |
| Aliquot     | DAP aliquot barcode                                     | CVB aliquot barcode                                          |
|             | Preservation method                                     | Preservation method*                                         |
|             | Preservation date and time                              | Preservation date and time*                                  |
|             | Preservation method*                                    | Storage method                                              |
|             | Preservation date and time                              | Storage date and time                                        |
|             | Preservation date and time                              | Storage location                                            |
|             | Preservation date and time                              |                                                              |
|             | Storage method                                          |                                                              |
|             | Storage date and time                                   |                                                              |
|             | Storage location                                         |                                                              |

Abbreviations: DAP, Dog Aging Project; CVB, Cornell Veterinary Biobank.
*For whole blood specimens processed at the CVB
†Processing dates, technician ID, reagent, and equipment ID

For each DAP participant, high molecular weight genomic DNA was extracted from one tube of EDTA whole blood using a standard salt precipitation method: red blood cells were lysed using 3 times the original blood volume of ammonium chloride lysis solution (1 mM EDTA, 10 mM NaHCO₃, 155 mM ammonium chloride); white blood cells were pelleted through centrifugation and lysed by incubation in 1x original blood volume of lysis solution (10 mM TrisCl, 1 mM EDTA, 0.5% SDS) with 0.2 mg/mL proteinase K at 55°C for 1 hour followed by room temperature incubation for >24 hours; protein was precipitated from the lysate using 0.4x original blood volume of 10 M ammonium acetate solution and centrifugation; DNA was precipitated from the supernatant using 100% isopropanol and purified using 70% ethanol, before being reconstituted in TE buffer (10 mM Tris, 0.2 mM EDTA). Total DNA concentration and purity were determined by spectrophotometry on a NanoDrop ND1000 (Thermo Scientific). DNA integrity was determined by electrophoresis using an Agilent 4200 TapeStation system with Genomic DNA ScreenTapes and reagents (Agilent), which provided a DNA integrity number (DIN) ranging from 1 for completely degraded DNA to 10 for completely intact DNA.

The remaining tube of EDTA whole blood was split into 0.5 to 1.0 mL aliquots and transferred to cryotubes using 18 G needles and syringes for precise measurements and ease of transfer. No further processing was performed on plasma, serum, or PBMC specimens, once received by the biobank.

**Long-term storage.** DNA, whole blood, plasma, and serum aliquots were stored at −80°C in an automated storage unit (SAM HD, Hamilton). PBMC aliquots were stored in liquid nitrogen vapor phase in a Biостore III Cryo automated freezer (Azenta
All specimens were stored in barcoded cryovials (0.5-1.9 mL dual-coded or tri-coded externally threaded storage vials, Azenta Life Sciences) labeled (1” × 0.5” cryogenic barcode labels, GA International LabTag) with de-identified information for traceability, including DAP animal ID, CVB animal ID, DAP specimen ID and CVB specimen ID.

**Distribution.** As part of its commitment to open science, the DAP welcomes withdrawal requests by any researcher. All requests are evaluated by the DAP Ancillary Study committee to avoid duplicate efforts and to ensure ethical and scientifically rigorous use. Once approved, the DAP Biobank removes the specimens from storage and processes them, as needed. Material certificates are created to provide the researchers with information pertinent to the fitness for the intended purpose of the specimens. A template material transfer agreement was created for distribution of DAP biobanked specimens. A pilot grant program was developed to fund small innovative research projects and promote the distribution and use of the banked specimens.

**Statistical analysis**

Results are presented as mean ± standard deviation (SD). Processing interval was calculated as the interval from collection of the whole blood specimen to its processing for DNA extraction at the CVB. Preservation interval for unprocessed whole blood specimens was calculated as the interval from collection of the biospecimen to its aliquoting and storage at the CVB; preservation interval for blood derivatives was calculated as the interval from collection of the biospecimen to its processing and/or aliquoting and storage at TAMU. Adjusted DNA amount was calculated as total extracted DNA amount/original blood volume. Comparison of mean adjusted DNA amount and DIN by shipment status (on time or delayed) was done using a 2-sample T-test. Correlation analysis of mean adjusted DNA amount and DIN with processing interval and arrival temperature was done by linear regression analysis. To improve the normality of distribution, and to reduce heteroscedasticity of residuals, the variables were transformed prior to regression analysis (Supplemental Figure 1). We used the square-root transformation for arrival temperature and adjusted DNA amount, a logarithmic transformation for processing interval, and the function 1-log(10-x) for DIN. Differences and correlations were considered significant at $P < .05$ (Minitab 20.1 Statistical Software, Minitab).

**Results**

**Documentation of processes**

By being involved in early planning stages of the project, the biobank research team was able to contribute to the development of appropriate workflows and processes that culminated in the biobanking of fit-for-purpose biospecimens. The processes were documented, and forms were created to standardize capture of critical data. Using historical distribution data, the biobank research team recommended the type and volume of biospecimens to store for future distribution to researchers. This was reflected in the DAP biospecimen collection plan and the design of the custom-built collection kit sent to Precision and TRIAD cohort participants. Additionally, the biobank research team recommended that blood derivatives processed at the DAP laboratory at TAMU be stored in cryotubes compatible with the automated storage systems, thereby ensuring that the biospecimens could be efficiently stored without having to be thawed and transferred to appropriate cryotubes.

The biobank research team determined preanalytical data points to be captured and recorded, including specimen collection time, temperature at arrival at the DAP laboratory at TAMU and DAP Biobank at CUCVM, and processing, preservation, and storage times.

**Biospecimen transportation**

During the pilot phase of the DAP (February to May 2021), biospecimens were collected from 18 dogs to test the transportation, processing, and storage procedures. Whole blood specimens in EDTA were shipped from TAMU to CUCVM in 6 batches (Figure 1), with each batch containing biospecimens from 2 to 5 dogs; 4 batches arrived within 24 hours of shipping with a mean temperature of 9.25 ± 4.28°C, and 2 were delayed overnight, arriving approximately 48 hours after shipping with a mean temperature of 16.0 ± 0.28°C.

During the early phase of the DAP (July to October 2021), biospecimens were collected from 91 dogs. Whole blood specimens in EDTA were shipped from TAMU to CUCVM in 16 batches (Figure 1), with each batch containing biospecimens from 2 to 11 dogs; 14 batches arrived within 24 hours of shipping with a mean temperature of 12.65 ± 3.59°C, one was delayed overnight, arriving approximately 48 hours after shipping with a temperature of 17.6°C, and one was delayed for 2 days, arriving approximately 72 hours after shipping with a temperature of 22.3°C.

Following the arrival of the third delayed shipment in July, the shipping method was switched from "standard overnight" to "priority overnight" to decrease the risk of transit delays. The following 14 shipments arrived within the expected timeframe; only the last shipment was delayed overnight despite the updated shipping method.

The shipment preparation procedure was modified following the first 9 shipments to maintain appropriate temperature throughout shipping while allowing for variability in the number of specimens shipped. For the first 9 shipments, one ice pack was included with the biospecimens; for the last 13 shipments, one ice pack was included for every 10 specimens. For one shipment in September, the ice pack was accidentally omitted from the shipment.
Blood derivatives that were processed and preserved at TAMU were shipped overnight to CUCVM on dry ice. Dry ice was present and the aliquots were still frozen on arrival.

**Biospecimen aliquots**

In addition to the 109 tubes of whole blood used for DNA extraction, the biobank received an additional 108 tubes of whole blood preserved in EDTA which were split into 164 aliquots for banking (Table 3). The mean preservation interval for whole blood specimens was 8.0 ± 2.8 days.

The biobank received blood derivatives from 106 dogs; 101 of these dogs had matching banked whole blood specimens, while 5 had not had whole blood collected for biobanking (Supplemental Table 1). In total, 273 aliquots from 76 PBMC specimens, 130 aliquots from 78 plasma specimens, and 70 aliquots from 46 serum specimens were banked (Table 3). The mean preservation interval for PBMCs, plasma, and serum specimens was 1.3 ± 0.8 days, 1.3 ± 0.9 days, and 1.5 ± 1.1 days, respectively.

**Biospecimen quality control**

The quality metrics of the whole blood specimens preserved in EDTA for DNA extraction are shown in Table 4, grouped by project phase and shipment status. Whole blood specimens arrived at CUCVM and were processed for DNA extraction 9.1 ± 3.8 days following collection. Total volume of whole blood available for DNA extraction varied from 0.1 to 3.3 mL, resulting in a wide range of total DNA amount extracted for each specimen. When normalized to the original blood volume, the adjusted DNA amount also varied among cases, with no evidence of being associated with shipment status (on time: 35.1 ± 16.0 µg/mL; delayed: 50.1 ± 31.3 µg/mL; T-value = 1.64, DF = 11, P = .129) or arrival temperature (Figure 2A and B); it had a weak, but significant negative correlation with the processing interval (Figure 2C and D). DNA integrity was high, with 98 out of 109 specimens having a DNA integrity number (DIN) ≥ 9.0 and the remaining specimens having a DIN > 7.0. There was no evidence of DIN being associated with shipment status (on time: 9.46 ± 0.45; delayed: 9.30 ± 1.1; T-value = -0.21, DF = 11, P = .835) or arrival temperature (Figure 3A and B); it had a weak significant negative correlation with processing interval (Figure 3C and D).

**Discussion**

The DAP Biobank was created to biobank and distribute biospecimens collected as part of a large-scale longitudinal study. The development of its operations by the research team at the CVB, a biobank accredited to ISO 20387:2018, was critical to ensuring adequate collection and processing of the specimens for biobanking and downstream use. This included general considerations like specimen type, volumes/amounts, and quantities, but also more specific details such as cryotube types, specialized labels for ultra-low temperature storage, and pre-analytical data captured throughout the specimen life cycle. To the general scientific community, biobanking is often an
afterthought during the design of an experiment, whether as a source of specimen acquisition or as storage of specimen remnants.\(^{23,24}\) By involving the CVB from the early stage of the project, the DAP was able to design a workflow to support its research goals efficiently and effectively.

Preanalytical processes, defined as the procedures occurring between collection and experimental analysis of the specimens,\(^{25,26}\) can have a critical impact on the fitness and variability of specimens. For example, preanalytical conditions such as the collection to processing interval and temperature are known to have an impact on the stability of biomarkers in plasma\(^{27-29}\) and serum \(^{30-32}\) and can affect gene expression analysis in PBMCs.\(^{33,34}\) It is therefore important to capture as much data as possible regarding these preanalytical processes to make informed decisions about the use of, and to correctly interpret results of research derived from these specimens.\(^{25,26,35}\)

The transport of specimens from the collection sites to the DAP laboratory at TAMU and to their final storage location at the DAP Biobank at CUCVM has been a considerable challenge. The DAP has been designed as a geographically widespread project to enable the study of dogs living in racially and socio-economically diverse households throughout the United States. Given the wide geographic range of DAP participants and to ensure standardized protocols for processing the specimens, the DAP utilized commercially available shipping solutions with daily pick-up schedules. In general, this made it possible to process and preserve blood derivatives and to stabilize EDTA whole blood specimens approximately 24 hours after collection; when shipping delays outside of the control of the DAP occurred, this time would increase to 48 to 72 hours. Similarly, the decision was made to do weekly shipments of refrigerated EDTA blood specimens from TAMU to the DAP Biobank at CUCVM as a balance between a short collection to processing interval and frequency of shipment which would determine cost of shipping per specimen. This is less ideal than many clinic-based human studies, where specimen collection,

### Table 3. Summary of banked whole blood and blood derivative specimens collected from DAP participants (mean ± SD (range)), by biobank phase.

| SPECIMEN TYPE | PHASE  | N     | ALIQUOTS PER SPECIMEN | PRESERVATION INTERVAL (DAYS) |
|---------------|--------|-------|------------------------|------------------------------|
| Whole blood   | Pilot  | 18    | 1.0 ± 0.0              | (1-1)                        |
|               | Early  | 90    | 1.6 ± 0.5              | (1-2)                        |
| PBMC          | Pilot  | 8     | 1.0 ± 0.0              | (1-1)                        |
|               | Early  | 68    | 3.9 ± 0.4              | (2-4)                        |
| Plasma        | Pilot  | 12    | 1.3 ± 0.6              | (1-3)                        |
|               | Early  | 66    | 1.7 ± 0.9              | (1-3)                        |
| Serum         | Pilot  | 3     | 1.3 ± 0.6              | (1-2)                        |
|               | Early  | 43    | 1.5 ± 0.5              | (1-2)                        |

Abbreviation: PBMC, peripheral blood mononuclear cells.

### Table 4. Quality metrics of DNA extracted from whole blood collected from DAP participants (mean ± SD (range)), by biobank phase and shipment timeliness.

| PHASE | SHIPMENT | N  | PROCESSING INTERVAL (DAYS) | BLOOD VOLUME (ml) | TOTAL DNA (μg) | ADJUSTED DNA (μg/ml) | DIN |
|-------|----------|----|---------------------------|-------------------|----------------|---------------------|-----|
| Pilot | On time  | 14 | 15.9 ± 5.74 (9-24)        | 0.77 ± 0.16 (0.5-1.0) | 18.7 ± 12.5 (1-40) | 26.59 ± 20.10 (1.1-76.0) | 9.54 ± 0.20 (8.9-9.7) |
|       | Delayed  | 4  | 12.8 ± 2.2 (10-15)        | 0.73 ± 0.38 (0.4-1.1) | 35.0 ± 17.0 (15-56) | 60.90 ± 52.9 (28.2-140.0) | 9.65 ± 0.24 (9.4-9.9) |
| Early | On time  | 83 | 7.9 ± 1.9 (4-13)          | 1.01 ± 0.46 (0.1-3.3) | 36.9 ± 22.3 (2-131) | 36.50 ± 14.88 (2.0-94.3) | 9.46 ± 0.45 (7.2-9.9) |
|       | Delayed  | 8  | 8.3 ± 2.3 (3-10)          | 1.10 ± 0.26 (0.9-1.7) | 47.0 ± 13.1 (31-72) | 44.70 ± 15.39 (20.6-72.0) | 9.30 ± 1.08 (7.1-9.9) |
| Total |          | 109| 9.1 ± 3.8 (3-24)          | 0.98 ± 0.42 (0.1-3.3)| 35.2 ± 21.5 (1-131)| 36.73 ± 18.70 (1.1-140.0)| 9.47 ± 0.49 (7.1-9.9) |

Abbreviation: DIN, DNA integrity number.
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processing, and storage are performed at the same location or institute and can be done within minutes or hours of each other. However, limitations must be accepted when working with a project of this scale. With the guidance of the biobank research team, the DAP implemented procedures for the capture of critical preanalytical data, including collection, processing, preservation, and storage times, processing conditions, and temperature at critical steps. These data are made available to downstream users, who will ultimately determine if those specimens are fit for their intended purpose.

During the pilot and early phases of the DAP Biobank implementation, we investigated the impact of preanalytical conditions on the quality and quantity of DNA extracted from EDTA whole blood. Temperature of specimens upon arrival to the DAP Biobank at CUCVM from the central DAP laboratory at TAMU did not have an effect on the adjusted DNA amount or DIN, even following delays in shipments resulting in progressively increased ambient temperatures. This is consistent with previous findings that storage of EDTA whole blood specimens at 4°C for several weeks does not significantly

Figure 2. Relationships between adjusted DNA amount extracted from EDTA whole blood specimens and (A) temperature of blood on arrival to the DAP Biobank and (C) processing interval (N = 109). Regression analysis (B) does not find evidence that arrival temperature is associated with adjusted DNA, but (D) finds evidence that longer processing intervals are associated with lower adjusted DNA.

Figure 3. Relationships between DIN of DNA extracted from EDTA whole blood specimens and (A) temperature of blood on arrival to the DAP Biobank and (C) processing interval (N = 109). Regression analysis (B) does not find evidence that arrival temperature is associated with DIN, but (D) finds evidence that longer processing intervals are associated with lower DIN.
impact DNA quantity or quality. Although a weak inverse correlation was seen between adjusted DNA amount and processing interval, the mean amount of DNA extracted from specimens is sufficient for most downstream applications, such as genotyping or whole genome sequencing. As the CVB generally distributes 1 to 5 µg aliquots of DNA to its users, it is anticipated that sufficient DNA amounts have been banked for each animal for at least one distribution, with multiple aliquots available for the majority of the animals.

The exact blood volume received by the DAP Biobank depends on multiple factors, such as dog size, behavior at collection, and prescribed priority order of draw based on the blood tube type and the various downstream uses within the DAP (Supplemental Methods 1). Based on the total volume that is possible to collect on the individual participant at the appointment time, blood volume available for DNA extraction is variable. As part of biobanking best practices, the blood volume is documented at reception at the DAP Biobank at CUCVM. The variability in adjusted DNA amount extracted from each EDTA whole blood specimen is also in part due to the health status of the donor. Since DNA is extracted from nucleated white blood cells, donors with leukocytosis (an elevated white blood cell count) will have a higher amount of DNA available per mL of whole blood. It is therefore expected that adjusted DNA amount will vary among individuals, even when processing intervals and shipping conditions are identical.

DNA integrity is critical for certain downstream applications requiring high molecular weight DNA, such as sequencing. The DIN calculated by the TapeStation is a useful tool for quantitative analysis of DNA integrity and determining the fitness of a DNA specimen for sequencing. There has been variation in prior studies as to the acceptable cutoff, ranging from 4.0 to 7.0 or 8.0, when analyzing DNA specimens for next generation sequencing, though sequencing data can still be obtained from DNA specimens with DIN < 4.0. Though a weak inverse correlation was found between DIN and processing interval, all DNA specimens processed during the pilot and early phases of the DAP, had a DIN ≥ 7.2 and 98 out of the 109 (89.9%) had a DIN ≥ 9.0. Therefore, it is highly likely that all DNA specimens banked during the pilot and early phase of the DAP are fit for sequencing.

The implementation and operation of a biobank on this scale requires substantial financial support. The NIA awarded funding to the DAP biobank for the reception, preparation, preservation, quality testing, storage, and distribution of DAP biospecimens by the CVB. The successful funding was due in part to the inclusion of 3 components not always found in academic projects.

Firstly, the proposal included a quality assurance manager to oversee the DAP Biobank quality management system, including establishing quality procedures and guidelines, performing internal audits, and developing a preventative and corrective action process. The quality assurance manager also created an opportunity for dynamic evaluation and improvement of processes based on the results of QA/QC activities and user feedback, including updating the biospecimen shipping method to reduce transit time, photographing whole blood tube labels to demonstrate specimen traceability, and performing data audits to ensure data integrity and authenticity. This promotes sustainable biobanking by ensuring fit-for-purpose biospecimens that meet the needs of the users, while protecting the interests of the biospecimen donors and funders.

Secondly, the proposal included a marketing and communication specialist to develop and implement a marketing and communication strategy for the DAP Biobank. This enabled the DAP Biobank to explore different methods of communication with its stakeholders, particularly the participants’ owners and the researchers utilizing the banked biospecimens. Communication with participating dog owners and the public at large not only aids in the DAP’s recruitment efforts, but also provides education and transparency in biobank operations to the DAP participants. Communication with the scientific community also allows the DAP Biobank to highlight the biospecimens available for research. Many researchers are not aware of the biospecimens available at a biobank, or that such biospecimens with extensive associated data even exist. By advertising the DAP Biobank collection, utilization of the DAP specimens is expected to be increased.

Further communication with researchers who have withdrawn biospecimens from the DAP Biobank in the form of feedback surveys will also enable the biobank to adjust its processes to ensure the fitness for the intended purpose of the biospecimens through a dynamic quality management system.

Lastly, the proposal included a pilot grant structure with 2 grants assigned each year to fund small innovative research projects, along with providing the biospecimens and biobank services to accomplish these research goals. These small projects can result in preliminary data used in grant applications for larger projects and promote the use of biobanked specimens. To support DAP’s Diversity, Equity, and Inclusion commitment and to increase the diversity of biobank users, we are prioritizing the announcement of this pilot grant program at Historically Black Colleges and Universities (HBCUs). This will support the success of underrepresented minority trainees and early-career investigators.

**Conclusion**

We have found that it is advantageous for researchers to involve biobanks from the early stages of study design and planning, as biobankers can provide unique insight and solutions to preserve the integrity, fitness, and traceability of the biospecimens and associated data. Just as pilot phases are an excellent method of identifying challenges and
opportunities for improvements in a workflow, frequent QA/QC steps followed by dynamic implementation of improvements throughout the duration of the project allow for problems and challenges to be identified and addressed in a timely manner. Additionally, large-scale longitudinal studies can greatly benefit from the expertise of personnel such as quality assurance and marketing and communication specialists to ensure the fitness-for-purpose and optimized use of their specimens. This collaborative approach leads to more accurate and reproducible results for researchers and enhanced sustainability for biobanks, which over time will benefit the general public.

Declarations

Ethics approval and consent to participate
All procedures for dogs enrolled into the Precision and TRIAD cohorts, including Informed Owner Consent, were approved by the TAMU Institutional Animal Care and Use Committee (IACUC; Precision: Animal Use Protocol 2021-0316 CAM, TRIAD: Animal Use Protocol 2021-0317 CAM).

Consent for publication
Not applicable.

Author contributions
Lara Moutham: Conceptualization; Data Curation; Formal Analysis; Investigation; Methodology; Validation; Visualization; Writing—Original Draft. Marta Castellano: Conceptualization; Funding Acquisition; Project Administration; Supervision; Writing—Review and Editing. DAP Consortium: Conceptualization; Data Curation; Funding Acquisition; Investigation; Methodology; Project Administration; Resources; Software; Supervision; Validation; Writing—Review and Editing.

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Availability of data and materials
Dog Aging Project curated data is available at https://dogagingproject.org/open_data_access/

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Supplemental material
Supplemental material for this article is available online.

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