*home*RNA: A self-sampling kit for the collection of peripheral blood and stabilization of RNA

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ABSTRACT: Gene expression analysis (e.g., targeted small gene panels, transcriptomics) from whole blood can elucidate mechanisms of immune function and aid in the discovery of biomarkers. Conventional in-clinic venipuncture offers only a small snapshot of our broad immune landscape as immune responses may occur outside of the time and location parameters available for conventional venipuncture. A self-operated method that enables flexible sampling of liquid whole blood coupled with an immediate stabilization of cellular RNA is instrumental in facilitating capture and preservation of acute or transient immune fluxes. To this end, we developed *home*RNA: a kit that allows for self-collection of peripheral blood (~0.5 mL) and immediate stabilization of cellular RNA, using the Tasso-SST™ blood collection device paired with a specially designed stabilizer tube containing RNA*later*™. To assess the usability and feasibility of *home*RNA for self-collection and stabilization of whole blood RNA, we conducted a pilot study (n = 41 participants) where we sent *home*RNA to participants aged 21-69, located across 10 US states (94% successful blood collections, n = 51). Among participants who successfully collected blood, 91% reported no or minimal pain/discomfort using the kit (n = 35), and 77% reported easy or somewhat easy stabilization protocol. Total RNA yield from the stabilized samples ranged between 0.24 µg and 5.99 µg (mean = 1.65 µg), while RNA Integrity Number (RIN) values were above 7.0 (mean = 7.9), indicating limited RNA degradation. Results from this study demonstrate the self-collection and RNA stabilization of whole blood with *home*RNA by participants themselves, in their own home.

INTRODUCTION:

Remote and contact-free laboratory testing is rapidly emerging as the new standard in patient care and clinical research, especially in light of the COVID-19 pandemic. However, blood sample collection remains a challenging procedure to perform remotely as venipuncture is resource-intensive, physically uncomfortable, and inflexible in regard to collection time and location.[1, 2] Remote self-administered blood collection, on the other hand, offers many practical advantages, including 1) expanded lab testing for rural and remote medicine applications (i.e., telemedicine), 2) convenience for clinical research studies as well as the ability to recruit participants that are not able to come to the clinic (due to work schedules, caregiver responsibilities, mobility challenges, etc.), 3) the ability to capture acute and transient biomarker fluxes (e.g., immediately following an acute exposure, an asthma attack, or a flare in an autoimmune disease), and 4) opportunities to conduct longitudinal research studies that require frequent sample collections from the same individual over a short time course (e.g., daily blood collections); to-date these applications have been limited due to the logistical challenges associated with in-person venipuncture. Here, we will describe *home*RNA: a new technology to enable at-home collection and stabilization of whole blood cellular RNA for transcriptomics.

An important example of an existing technology aimed at remote blood sampling is the use of dried blood spot (DBS) sampling. In DBS sampling, a lancet-based finger prick is used to draw blood, which is applied to a sampling paper and left to dry. The sampling paper containing the DBS is then mailed back to the lab for analysis.[3] This technology has been applied to a variety of applications, including diagnostics and screening,[4-9] therapeutic drug monitoring,[1, 10, 11]and other mechanistic biomolecule analysis.[12, 13] Due to the increased use of DBS and convenience for remote sampling, tremendous research and development have been undertaken to improve the consistency and analysis of DBS samples.[5, 14-16] However, a liquid blood sample can provide a greater volume of blood (>100 µL). An increased sample volume may be desirable for applications such as genomics, transcriptomics, or the detection of rare analytes. Further, if adequately stabilized, liquid samples may provide a greater quantity and better quality of the desired analyte than DBS samples, such as a higher yield of minimally degraded total whole blood RNA.[17] Another burgeoning class of blood sample collection devices is lancet-based devices that collect from capillary beds in the arm. The user activates the lancet by pushing a button, which then causes blood to flow into a collection receptacle.[18-21] The Tasso-SST™ blood collection device used in this study falls under this category of sampling method. These devices collect larger volumes (>100 µL) than traditional finger-prick DBS sampling, and the sample is kept in liquid form rather than dried on paper. Moreover, these devices are simple to use, and users report less pain while using these compared to a finger prick or traditional venipuncture.[18, 19]

There is an obvious advantage to eliminating the need for a phlebotomist; consequently, there have been many technological advances in self-blood collection. However, blood collection is often only the first step in blood-based laboratory tests. In a traditional outpatient or research setting, after a phlebotomist draws blood, it is processed soon after in a lab by a technician. Blood is not a static tissue sample; it contains living cells that can continue reacting to changes in their *ex vivo* environment. Post-collection sample handling is critical, and there have been numerous studies determining the best way to store, stabilize and handle blood samples for various target analytes.[22-24] For transcriptomics profiling, RNA stabilization in liquid whole blood is particularly critical. Degradation of RNA by ribonucleases and rapid fluxes (induction and decay) of mRNA transcripts in response to the post-collection *ex vivo* environment can be highly unfavorable for research intended to understand *in vivo* cellular expression landscapes. Further, these *ex vivo* changes can lead to an inaccurate representation of the *in vivo* transcriptome in question[25, 26]

In a traditional outpatient venipuncture setting, stabilization of whole blood RNA is accomplished by collecting venous blood directly into vacutainers containing RNA stabilizers (e.g., Tempus™ or Paxgene™) or immediately pipetting anti-coagulated blood into RNA*later*™ containing vials. This procedure is incompatible with a self-sampling regime, as users cannot be expected to pipette their own blood or do venipuncture into a vacutainer tube on themselves. To fully enable remote sampling and transcriptomics profiling of liquid blood samples, one must eliminate the need for a phlebotomist *and* enable the patient or research participants to act as their own laboratory technician, allowing them to perform necessary steps to stabilize their blood sample without the use of pipettes, gloves, or syringes. In the present manuscript, we accomplish this goal of both collection *and* RNA stabilization. We combine a commercially available lancet-based blood sampling device (Tasso-SST™), a liquid RNA stabilizer (RNA*later*™), and a custom-engineered fluid transfer and stabilizer tube into a single sampling unit that can be mailed to study participants. Participants collect a liquid sample (~ 0.1 - 0.5 mL) of whole blood, stabilize it, and ship it back to the laboratory for analysis. To demonstrate our liquid stabilization technology, we chose to target total whole blood cellular RNA with RNA*later*™. However, our technology is broadly generalizable in that a researcher interested in using a different stabilizer or targeting a different class of biomarkers can replace the stabilizer in the stabilizer tube with another liquid stabilizer.

To assess the usability and feasibility of this sampling methodology, we conducted a pilot study (*n* = 41 participants) to answer two fundamental research questions: 1) is the design and instructions for the kit comprehensive and user-friendly enough to allow users to collect and stabilize a sample of their own blood without in-person training?, and 2) is the stabilization process sufficient to enable isolation of high-quality RNA suitable for standard gene expression analyses from a variety of post-collection storage temperatures and duration? We sent this kit to

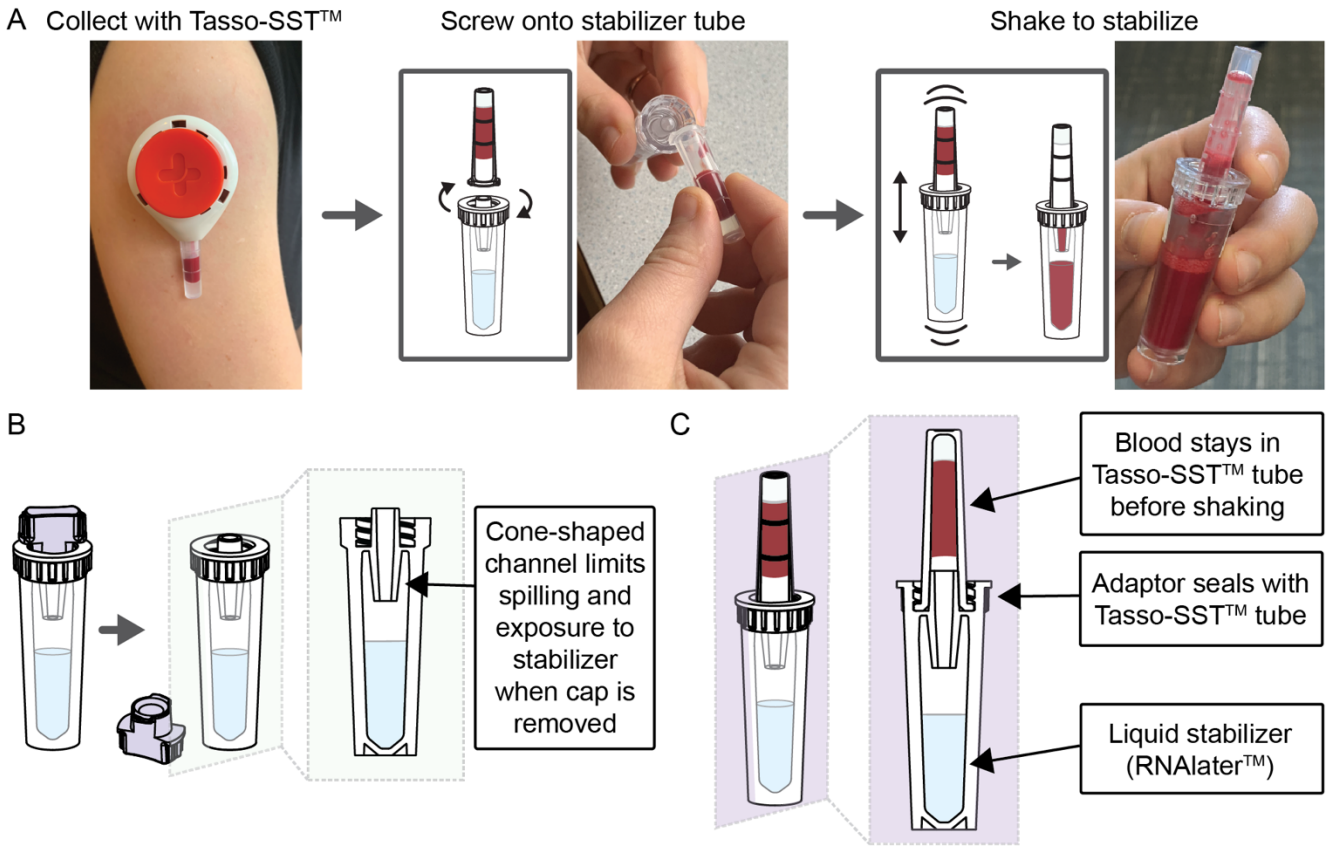

Figure 1: Workflow and design of *home*RNA blood collection and RNA stabilization kits. A) Workflow for *home*RNA, where blood is first collected using the Tasso-SST™, screwed on tightly to the stabilizer tube, and then shaken to mix with the stabilizer, RNA*later*™. B) Cross-sectional schematic of the stabilizer tube after the cap has been removed. C) Cross-sectional schematic depicting the Tasso-SST™ blood collection tube containing blood attached.

41 participants, aged between 21 and 69 and living across 10 different US states. We demonstrated successful blood collection and RNA stabilization measured by total RNA yield and RNA integrity number (RIN) value. Our kit and methodology open the potential for a new class of transcriptomics studies, enabling increased sampling frequency for longitudinal studies and access to populations that have been historically hard to reach.

MATERIALS AND METHODS:

Development and assembly of the *home*RNA blood collection and stabilization kit

Fabrication of the RNA stabilizer tube

The RNA stabilizer tube is assembled from three individual components: 1) a reagent vial to house the liquid stabilizer 2) an adaptor that interfaces both the reagent vial and the Tasso-SST™ blood tube and 3) a vial closure cap. Preliminary design iterations were generated on SolidWorks and 3D printed on a Form 3 3D printer (Formlabs) using clear resin (Formlabs). Specific features of the final stabilizer tube design include a cone channel feature in the adaptor piece (Fig. 1B) to prevent contact between stabilizer reagent and the user during blood stabilization. The final tube design was injection molded out of polycarbonate (PC: Makrolon 2407) by Protolabs, Inc (Maple Plain, MN). For assembly, all components of the stabilizer tube are first cleaned via sonication in 70% ethanol (v/v) for 30 min and air dried. The adaptor was bonded onto the reagent vial using a UV curing glue (Damn Good®). Bonded parts were cured for 60 min at 395-405 nm UV Lamp (Quans). The stabilizer tube was filled with 1.4 mL of RNA*later*™ (Thermo Fisher) as the stabilizing reagent, capped, and checked for leakage due to bonding defects before distribution to study participants. RNA stabilizer tubes used in the feasibility pilot study were prepared within a week of being mailed to study participants.

Kit components and assembly

All components included in the *home*RNA blood kit are listed in Table S1 and shown in Fig. S1. The RNA stabilizer tube was labeled with a unique sample code and packaged into a transport bag with an absorbent material. A heat pack was included to increase blood flow to the upper arm. The stabilizer tube insert was designed to immobilize the stabilizer vial containing blood in a 50 mL conical tube during transport. The insert was designed on SolidWorks and 3D printed on a Form 3 3D printer (Formlabs) using clear resin. All kit components were placed in a rigid custom design mailer box fabricated via die-cutting (The BoxMaker, Inc.). A temperature strip (Propagate Pro) was affixed onto the outer side of the mailer box for temperature recording by study participants. A mailer bag with pre-printed return shipping label was also included with the outgoing package for specimen return to the lab. In brief, iterations were made to improve overall usability of the kit and clarity of the instructions for use. We included the final version of the instructions for use (IFU) in the SI.

RNA stabilization, isolation, and gene expression analysis

RNA stabilization and isolation

For in-lab RNA stabilization experiments, fresh (< 8 hours post blood draw) whole venous blood drawn into EDTA-coated vacutainers (BD) was purchased from Bloodworks Northwest (Seattle, WA). Upon receipt, anti-coagulated whole blood was promptly transferred into either Tempus™, PAXgene®, or RNA*later*™ stabilizing reagents at their manufacturer's recommended stabilizer : blood ratios (v/v) of 2:1, 2:1, and 2.6:1 respectively. Stabilized blood was then incubated at various temperature ranges (4ºC, ambient, 30ºC, and 37ºC) and for a range of time periods (0-8 days) specific to each experiment as described in the results. At the end of the incubation period, total RNA was isolated from Tempus™-, PAXgene®-, and RNA*later*™- stabilized blood using Tempus Spin RNA Isolation Kit (Thermo Fisher), PAXgene® Blood RNA Kit (PreAnalytiX), and Ribopure™ - Blood RNA Isolation Kit (Thermo Fisher), respectively, according to manufacturer's protocol, and eluted in 50-100 µL volume.

For the at-home blood collection and stabilization feasibility study study, Tasso-SST™ collected blood was stabilized in RNA*later*™ by the human subjects using the RNA stabilizer tube that interfaces with the Tasso-SST™ blood tube. Total RNA was isolated using the Ribopure™ - Blood RNA Isolation Kit (Thermo Fisher) according to manufacturer's protocol and eluted in 50-100 µL volume. RNA concentrations were obtained on a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific). RNA integrity number (RIN) values were obtained on a Bioanalyzer 2100 (Agilent) using the RNA 6000 Nano Kit (Agilent). Isolated RNA was stored at -80ºC until ready for further analyses.

Pilot study: feasibility and usability assessment of the *home*RNA kit

Participant characteristics

This study was approved by the University of Washington Institutional Review Board (IRB) under protocol STUDY00007868. All study procedures were performed after informed consent was obtained. A total of 41 healthy volunteers between ages 18-75 years old and weighing between 105-230 pounds (assigned female at birth) and 135-250 pounds (assigned male at birth) were recruited via word of mouth or email to participate in the pilot study. Pregnant individuals or those currently breastfeeding, individuals with skin disorders (e.g., scabbing, psoriasis) on the upper arm, individuals with a blood platelet or coagulation disorder or currently on blood platelet or anticoagulant medications, individuals who are immunosuppressed or on immunosuppressive medication, and individuals who reside in a correctional facility were excluded from the study.

Human subjects study design

General study design:

Study participants were enrolled in groups in order to iterate on the general usability of the *home*RNA kit, specifically on the kit components and the clarity of the IFU (Table S2). The study enrolled a total of five groups. In each group, participants were asked to self-collect and stabilize blood from their upper arm using the *home*RNA blood collection and stabilization kit. Each participant was also asked to complete a user experience survey that was designed to guide further improvements to kit components and sampling parameters (e.g., ease of use, clarity of the IFU, mailing logistics, ambient temperature at collection site, etc.) Based on feedback, improvements were implemented in subsequent groups. Participants were asked to package their

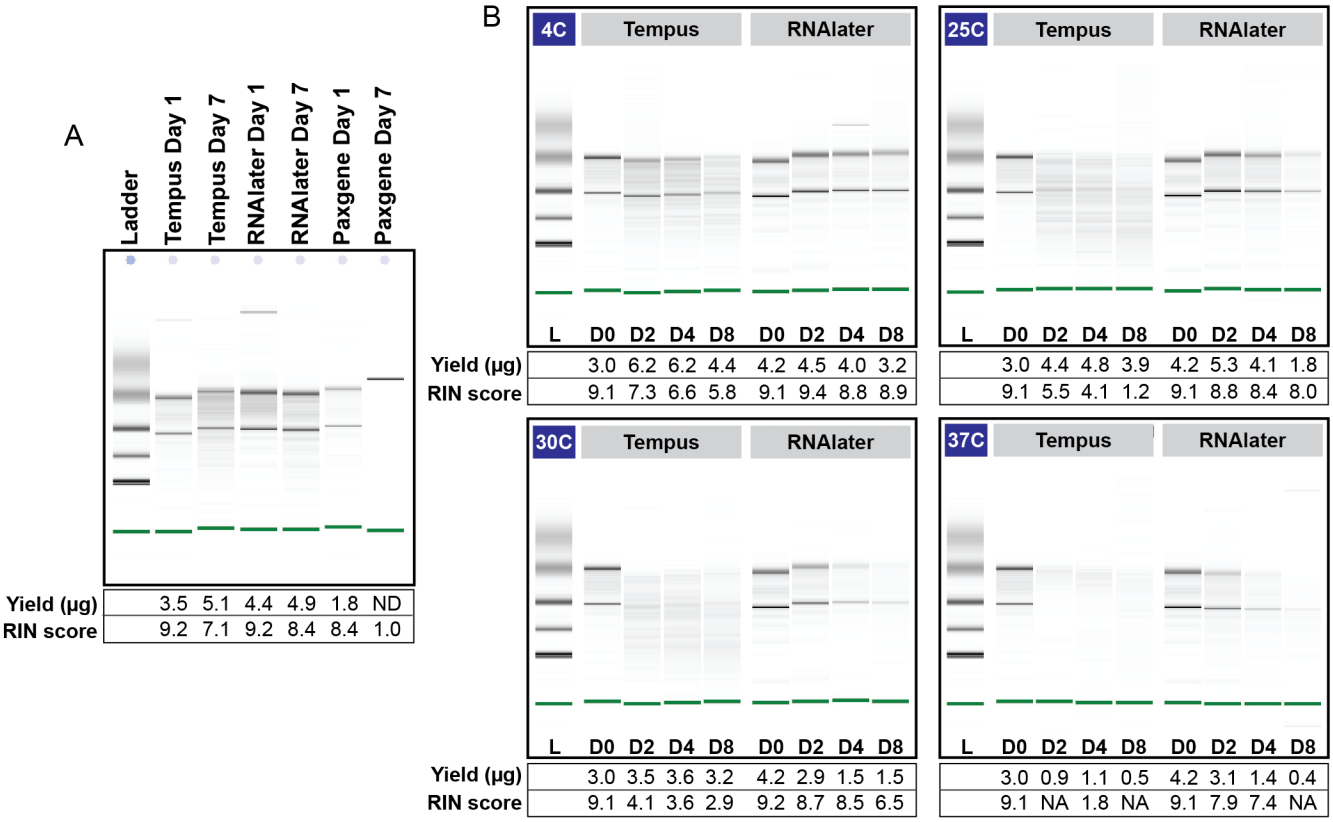

Figure 2: Performance of select RNA stabilizers on total RNA yield and RNA quality. A) Digital gel image, total yield, and RIN scores of RNA isolated from blood stabilized in Tempus, Paxgene® and RNA*later*™ for seven days at ambient temperature. B) Effect of storage temperature and duration on total RNA yield and quality (RIN value) of samples stabilized in Tempus™ vs. RNA*later*™. Digital gel image of Tempus and RNA*later*™ stabilized blood stored at 4C, 25C, 30C and 37C over the 8 days.

stabilized blood samples to be returned to the lab for analysis using the provided return mailer bag. Except for samples from group 1, where samples were picked up by the study team, all stabilized blood samples were mailed using next day delivery courier services (UPS). Unless specified, returned samples are stored at -20ºC until ready for RNA extraction. Study data were collected and managed using REDCap electronic data capture tools[27] hosted at the Institute of Translational Health Sciences. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources

Group-specific study design:

In order to assess effect(s) of storage length on total RNA yield and quality, participants in groups 1 (n = 4) and 2 (n = 5) were given two *home*RNA kits to be used, one on each arm. Participants were instructed to use the second kit promptly upon completion of the first kit and to complete a user experience survey. One vial of stabilized blood from each participant was frozen at -20ºC immediately upon return to the lab, while the other was stored at ambient temperature for an additional three days prior to RNA extraction. Participants in groups 3-5 were given one *home*RNA kit and asked to perform the collection and stabilization procedure and complete a user experience survey. The first three groups were recruited locally (greater Seattle area), however, to assess feasibility of the sampling pipeline across a wider geographical distribution, individuals from across the contiguous United States were recruited as participants for groups 4 (n = 13) and 5 (n = 16).

RESULTS AND DISCUSSION:

Self-sampling kit for peripheral blood collection and RNA stabilization: overarching considerations of the *home*RNA blood kit

The ability to self-collect blood and immediately stabilize RNA opens up new opportunities to probe immune responses to time- and location-specific stimuli outside of traditional venipuncture collection limitations. We present a home blood collection and RNA stabilization kit (*home*RNA) to remotely self-collect and stabilize blood. The two main components of *home*RNA include the Tasso-SST™, which collects approximately 100-500 µL of blood from the upper arm and a stabilizer tube, designed to screw onto the detachable blood collection tube from the Tasso-SST™ device. Figure 1A summarizes the general workflow for collection and stabilization using *home*RNA. To operate the Tasso-SST™, the device is first applied to the upper arm (Fig. 1A), where it is held in place by an adhesive. The user then presses a red activator button deploying a lancet that quickly punctures the skin. Blood is then drawn into a detachable collection tube, which holds up to approximately 500 µL of blood. To stabilize the freshly drawn blood, the collection tube containing the blood is detached from the Tasso-SST™ device and screwed tightly onto the stabilizer tube, and the connected tubes are shaken to thoroughly mix the blood with the stabilizer. The stabilized blood sample is then packaged and mailed back to the lab for analysis.

The Tasso-SST™ was chosen as a method for blood collection due to its ability to be self-administered, its general ease of use, and the larger blood volume (>100µL) it draws when compared to other devices in its category, making it suitable for applications requiring a greater amount of starting material (e.g., RNA sequencing (RNAseq)). Compared to other blood collection methods, users report much lower pain thresholds when using the Tasso-SST™ or similar devices that collect from the upper arm.[18-21] The authors note that the serum separator tube (SST) gel (included in the Tasso-SST™ collection tube) is not necessary for RNA stabilization and analysis. In fact, a device or tube containing EDTA or another anticoagulant would be preferable to prevent clotting, and we note varying degrees of clotting observed in our returned samples. At the time of the study, the Tasso-SST™ was available for purchase. Therefore, it was chosen as the device to use as an initial proof of concept for demonstrating remote blood collection and RNA stabilization.

Design of the stabilizer tube

The two primary design considerations for the stabilizer tube were 1) preventing exposure of the stabilizer solution (Fig. 1B) and 2) sealing tightly to the Tasso-SST™ collection tube so when vigorously mixed, the blood and stabilizer mixture does not leak or splash (Fig. 1C). These design parameters are critical for home use, particularly for ensuring that both the user's blood and stabilizer remained contained within the tubes throughout the stabilization process. Because of the small volume and shape (i.e., long and skinny) of the Tasso-SST™ blood collection tube, the blood sample remains in the tube even when tipped upside down due to surface tension. This observation combined with a no-spill cone feature in the stabilizer tube allowed the user to easily tip the tubes sideways to connect them (Fig. 1A) without causing either liquid (the stabilizer or the blood sample) to spill. However, the blood's tendency to remain in the Tasso-SST™ blood collection tube presented a non-trivial engineering challenge for mixing. To be stabilized, the blood needed to interface with the stabilizer liquid in the opposite tube (Fig. 1C). However, when attached and shaken up and down, the surface tension is broken as the two liquid interfaces (the blood and the stabilizer) come into contact, allowing for mixing. Because this mechanism for mixing required vigorous mixing across the two tubes' attachment point, a tight seal was critical. If the seal is not tight, blood and stabilizer will splash, leak, or otherwise get outside the connected tubes as soon as the shaking began. Leaking could also happen during the sample's return if the seal were inadequate. To achieve a tight seal, the stabilizer tube piece that attaches to the Tasso-SST™ collection tube was based on the design of the cap included with the Tasso-SST™.

Surface tension was also utilized to design the opening of the stabilizer tube to achieve the first objective of preventing exposure to the stabilizer. The stabilizer tube was engineered with a fluidic cone-shaped channel at the connection point between the stabilizer tube and the Tasso-SST™ tube (Fig. 1B). This fluidic cone takes advantage of surface tension to create a valve such that the stabilizer solution remains in the stabilizer tube when the tube is inverted. This feature protects the user from direct contact with the stabilizer solution and allows them to screw the

stabilizer tube into the Tasso-SST™ blood tube without spilling. Figure 1 illustrates schematic cross-sections of the stabilizer tube open (Fig. 1B) and attached to the Tasso-SST™ collection tube (Fig. 1C). Additional details on the design and fabrication of the stabilizer tube can be found in the supplementary information.

All pieces that comprised the stabilizer tube (tube, adapter, and cap) were injection molded out of polycarbonate, which was chosen as it is commonly used in biological laboratory consumables and is known to be inert to most biological samples and reagents. Due to the nature of the injection molding process required for the adaptor piece (which included an internal thread feature), polystyrene and polypropylene, other commonly used materials in laboratory consumables, could not be used. The other two parts, the cap and vial, were also made from polycarbonate so all pieces would be made of the same material to account for consistent material shrinkage amongst all the pieces during the injection molding process.

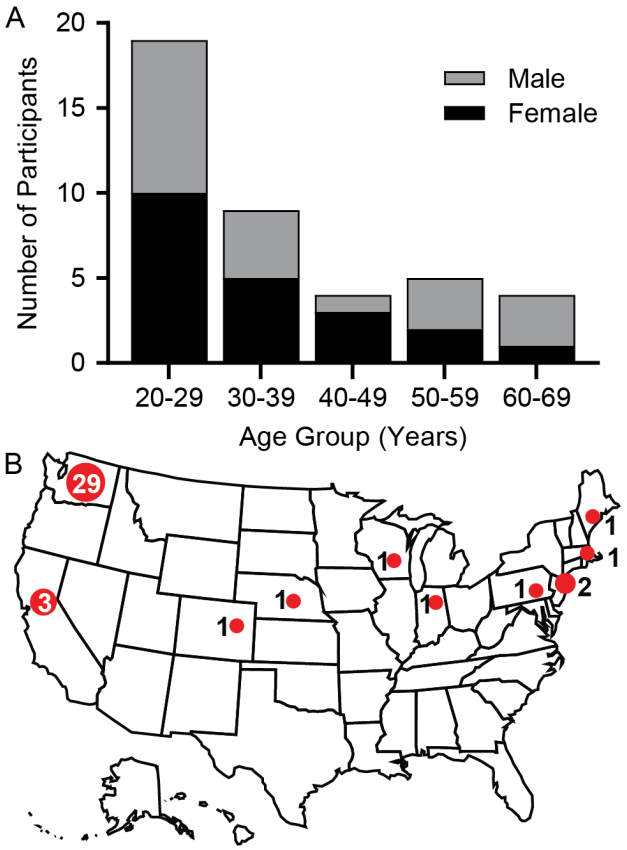

Figure 3: Demographics and Location of Participants. A) Age range and genders of study participants. B) Locations by state of study participants.

Stabilization of blood using RNA*later*™ resulted in higher RNA yield and quality compared to other stabilizers over broad storage conditions

We assessed three RNA stabilizers commonly used in gene expression studies (Tempus™, PAXgene®, or RNA*later*™) for both yield and quality of the total RNA isolated from stabilized blood samples over broad storage conditions. Both Tempus™ and PAXgene® are often used in blood gene expression studies due to the commercial availability of these stabilizers in vacutainer tubes allowing for a direct draw of venous blood into the stabilizers. On the contrary, RNA*later*™ is widely used to stabilize transcripts in laboratory specimens and extracted tissues but not commonly used in blood gene expression studies due to the lack of commercially available RNA*later*™ vacutainer tubes. However, the in-house assembly of stabilizer tubes used in this study prompted us to assess all three common stabilizers for preserving transcripts from blood. These three stabilizers were assessed for both storage temperature and length, the two variables that could significantly affect post-collection RNA yield and quality. In this study, RNA integrity number (RIN) values obtained on a Bioanalyzer 2100 coupled with its corresponding electropherogram profile are used to measure RNA quality. The RIN value is determined based on an algorithm that analyzes the electrophoretogram obtained from the capillary electrophoresis. RIN values range from 1-10, where 10 represents entirely intact and non-degraded RNA (Schroeder 2006). This part of the study was performed in-lab using blood collected from venous draws so that we could expose blood samples collected from one donor to controlled temperatures for fixed periods.

Preliminary experiments showed that blood stabilized in RNA*later*™ offered the highest RIN value (RIN = 8.4) compared to both Tempus™ (RIN = 7.1) and PAXgene® (RIN = 1.0) after 7 days of storage at ambient temperature (Fig. 2A; see Fig. S4A for electropherogram profiles). Given these preliminary results, both RNA*later*™ and Tempus™ were assessed further for performance at a broader range of storage temperatures. As depicted in Fig. 2B (see Fig. S4B for electropherogram profiles), stabilization of blood using RNA*later*™ yielded better RIN values at higher temperatures. These parameters could be experienced with remote user-administered sampling methodologies. Furthermore, the lack of corrosive (tartaric acid) and toxic (guanidine hydrochloride)

stabilizing chemicals in RNA*later*™ makes it an attractive choice for home-use or user-administered procedures. Due to high observed efficacy in stabilization for variable time and temperature profiles in our initial in-lab testing, coupled with user safety considerations, we chose to incorporate RNA*later*™ into the *home*RNA stabilizer tubes to assess stabilization of peripheral blood drawn with the Tasso-SST™ in the pilot study.

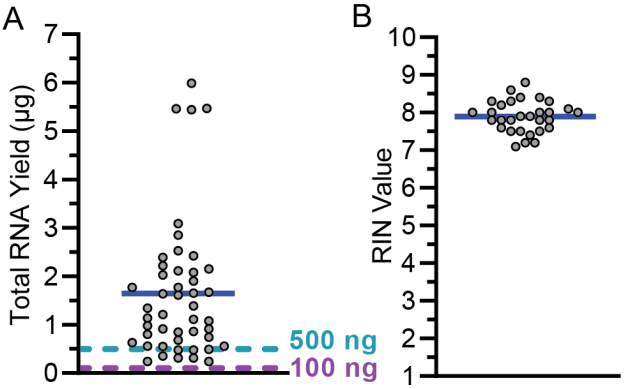

Figure 4: RNA yield and RIN value of peripheral blood samples self-collected and stabilized with *home*RNA. A) Total RNA yield of samples and B) RIN value of peripheral blood samples collected and stabilized by participants in their home using *home*RNA

Analysis of whole-blood RNA returned from the *home*RNA kit reveals feasibility for disseminated whole blood sampling and RNA stabilization

We enrolled 41 participants in a pilot feasibility and usability study to demonstrate self-blood collection and RNA stabilization outside of a clinical or research setting. Participants were mailed kits to their home where they collected, stabilized, and returned their own blood sample based solely on provided instructions in the kits and instructional video (see SI). Samples were returned to the laboratory for analysis via mail. Therefore, they had to remain stable throughout the shipping and the variability of temperature, pressure changes, and other mechanical stress inflicted during shipping.

Upon returning to the lab, total RNA was extracted from all stabilized blood samples and assessed for yield and quality (RIN values). Roughly 83% and 100% (n = 47) of blood samples returned from the pilot study offered a total RNA yield greater than 500 ng (a comfortable minimal cut-off value for large-scale transcriptomics analyses) and 100 ng (a comfortable minimal cut-off value for expression analyses of a small panel of targeted genes), respectively (Fig. 4A). These cut-off values for total yield obtained immediately after extraction may vary across studies depending on the choice of analysis methods (e.g., RT-PCR, digital droplet PCR, RNAseq, xMAP® and nCounter® technologies) and pre-analysis sample processing steps (e.g., globin depletion, RNA species enrichments) that will incur further yield losses. Based on our pilot study data, all self-drawn and self-stabilized peripheral blood samples using the *home*RNA blood kit offered sufficient yield for targeted small gene panel profiling. A significant portion (83%, n = 47) also have sufficient yield for genome-wide profiling analysis methods.

We obtained RIN values for 64% of all isolated RNA (n = 47) samples (Fig. 4B). We note that the 36% (n = 47) of samples that were not scorable did not afford a RIN value primarily due to the low total yield, resulting in low RNA concentrations in these samples. For samples that did not afford RIN values, an inspection of the digital gel images of these samples showed 65% (n = 17) contains intact ribosomal RNA bands depicting good RNA integrity (data not shown). RIN values of all scorable RNA samples range between 7.0 - 9.0, with 47% (n = 30) of samples affording RIN values greater than 8.0. Similar to the minimum cut-off value for yield, RIN values and their suitability for downstream gene expression analyses vary widely based on the source of the tissue or sample from where the RNA is isolated. For example, formalin-fixed paraformaldehyde embedded (FFPE) tissues and tissues containing high levels of ribonucleases (e.g., blood, liver, spleen, and kidney tissues) often afford lower RIN values due to the high degradation potential in these tissue types. A RIN value of 7.0 is a typical minimal cut-off value for RNAseq applications,[17, 28] which has more stringent requirements for quality than other gene expression analysis. For highly degraded samples such as FFPE tissues that often have RIN values as low as 2.0, the fragment size distribution index (DV_{200}) is frequently used to assess RNA quality and determine sample suitability for downstream gene expression analyses. Despite whole blood being rich in ribonucleases, the remote self-collection and stabilization process of the *home*RNA blood kit still afforded high RIN values (RIN > 7.0) that, by themselves, render these samples suitable for a variety of gene expression analysis. Therefore, a DV_{200} index assessment was not necessary for these samples. As there is interest in

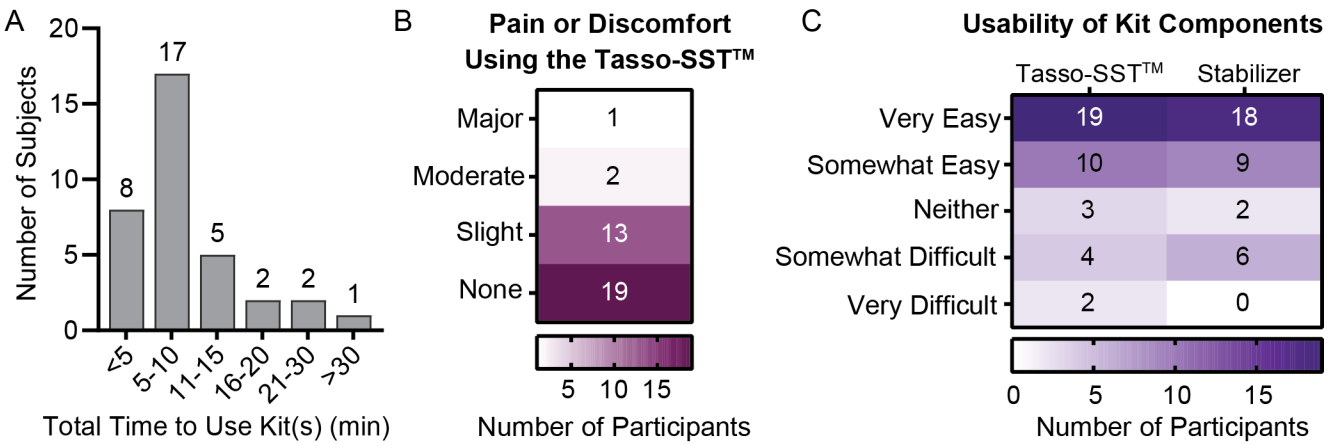

Figure 5: Survey responses to assess kit usability. A) Total self-estimated time it took each participant to use the kit from start to finish. B) Participant ratings for level of pain or discomfort using the Tasso-SST™. C) Participant ratings for the usability of the different kit components.

using RNAseq for lower yield or degraded samples (such as FFPE), there are many published techniques on methods to do so.[28-30] The yield and quality of the RNA extracted from blood samples from our pilot study are well within the parameters for targeted small gene panel profiling, and many of the samples even reach the higher thresholds set by sequencing facilities, conferring the convenience of outsourcing RNAseq for researchers interested in using our *home*RNA kit for their own out-of-clinic transcriptomics studies. Importantly, when compared to emerging remote self-sampling methodologies such as dried blood spotting, the *home*RNA collection process affords a better yield and quality that allows for a broader range of flexibility in analysis methodologies.[17, 31]

Kit performance is robust across participant demography (locations, age) and mailing groups.

To assess the geographic distribution feasibility of this remote-sampling methodology, *home*RNA blood kits were mailed from our lab (Seattle, WA) to various residential destinations in the West Coast, Midwest, and East Coast of the United States (Fig. 3B). Additionally, we mailed *home*RNA to various residential housing types in urban, suburban, and rural areas, including single-family unit houses and large multi-family apartment complexes (where packages are typically held in the lobby or mailroom). Demonstrating sampling from rural areas is important to expand research in places where participants traditionally needed to travel to phlebotomy labs located in other towns or cities. Such expansion would enable research into immune events that may be more commonly triggered in rural populations, such as exposure to agricultural chemicals or wildfires.

*home*RNA kits were sent out in five independent mailing groups from May to September 2020 (Table S3). For each group, most of the samples were returned and obtained a yield that could be used for downstream expression analysis (Fig. S5). From a usability perspective, differences between groups in terms of total RNA yield and RIN values were minimal (Fig. S5), suggesting robustness to the remote sampling methodology afforded by the *home*RNA kit; slight variations in the instructions (e.g., changes in wording, updated graphics, the inclusion of an instructional video) did not dramatically change results, suggesting the kit itself was relatively simple and intuitive to use. Finally, the RNA analysis parameters (total RNA yield and RIN values) were not significantly different across a range of age groups (Fig. S6). While we demonstrated robustness across these parameters, we note that the pilot study did not collect information on participants' race or ethnicity, or socioeconomic status. For future studies, we intend to evaluate the usability of the *home*RNA kit across a more diverse population and in geographical regions or during seasons that can incur more considerable variations in the high/low ambient temperatures.

Participant survey responses indicate good usability of *home*RNA

Ease of use and participant perception is critical for compliance, particularly for using this method in future longitudinal (multi-sample) studies. Usability was assessed through a user experience survey that the participants were asked to complete

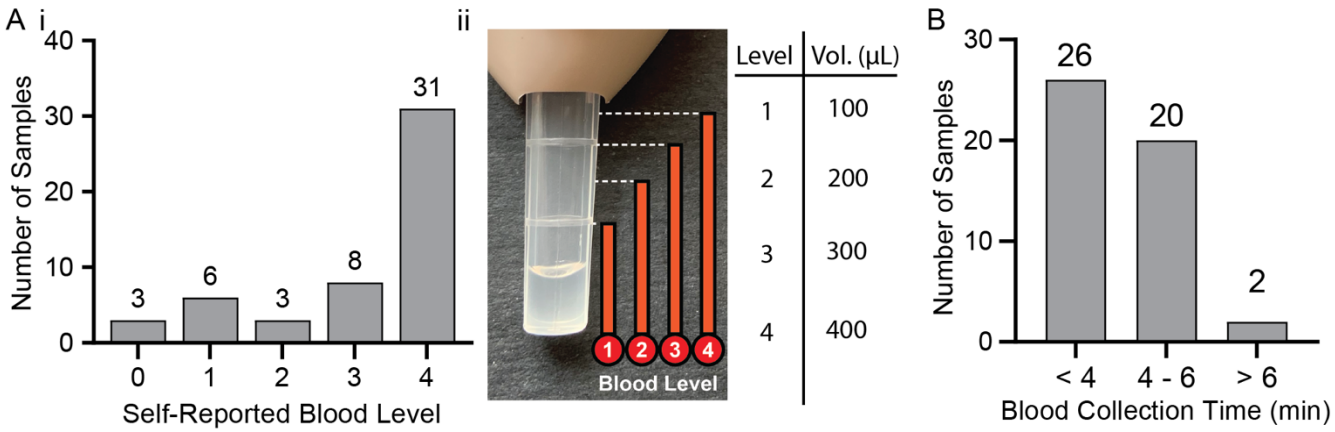

Figure 6: Survey responses to assess kit performance. A) i) Participant responses when asked to estimate the approximate blood level they filled the Tasso-SST™ blood collection tube based on the ii) picture provided in the online survey. The volumes corresponding to each level are noted in Aii. B) Participant responses when asked how long they left the Tasso-SST™ blood collection tube on their arm - this includes time before collection and after collection has stopped before removal of the device.

after using the kits. This survey was also used as a mechanism for feedback from the participants in order to iterate upon the instructions and kit components. Perception of the kits in terms of the time it takes to complete it, ease of use, and pain or discomfort were assessed (Fig. 5). Metrics for measuring the performance of the kit were also assessed, including asking the participants to estimate how much blood was collected and the time the Tasso-SST™ was left on the arm, e.g., the blood collection time (Fig. 6).

The majority of the participants who successfully collected blood using the Tasso-SST™ finished using their kits in less than 10 minutes (71%, *n* = 35, Fig. 5A). Most participants (54%, *n* = 35) reported no pain or discomfort while using the Tasso-SST™, whereas an additional 13 participants (37%, *n* = 35) reported minimal pain or discomfort. Only 3 participants found the pain or discomfort to be rated as moderate (*n* = 2) or major (*n* = 1). Regarding the participant's perception of how easy the kits were to use, most of the participants reported the Tasso-SST™ and stabilizer tube to be easy to use or somewhat easy to use (76%, *n* = 38 and 77%, *n* = 35 for the Tasso-SST™ and stabilizer tube respectively). Participants in group 1 were excluded from this analysis due to their proximity to the project; participants who failed to collect blood were excluded from the pain and stabilizer tube usability but were included in the usability for the Tasso-SST™ (3 out of 42 total enrollment). In summary, feedback with regards to the pain experienced and usability was positive.

Participant survey responses on device performance show sufficient yield even at a low sample volume

To assess the possible correlation between estimated blood collection volume and total RNA yield, participants were asked to estimate the levels of blood drawn into the Tasso-SST™ blood collection tube based on a provided blood tube image (depicted in Fig. 6Aii). Estimated volumes for the four levels are as follow: Level 1 = 100 µL, Level 2 = 200 µL, Level 3 = 300 µL and Level 4 > 400 µL. The majority of the participants reported blood collection at Level 4 (65% *n* = 48 samples). The reported blood level versus the RNA yield is plotted in Fig. S7A. While there are very few samples at lower reported blood levels (Level 1 and 2), there does not appear to be a strong correlation between blood level reported and yield. This could be due to inaccuracies in reporting by participants, individual variability in RNA yield, or loss during the sample processing or blood clotting in the stabilizer tube. Notably, participants who reported collecting a low volume (Level 1 ~100 µL) of blood still had RNA yields >100 ng, with one sample as high as 2.85 µg. We have also included data on blood collection time, reported blood volume, and RNA yield in the SI (Fig. S7B and S8). In short, all collections, irrespective of reported collection levels, afforded RNA yield sufficient for downstream gene expression analyses.

CONCLUSION:

Our *home*RNA kit will enable translational researchers to ask fundamentally different biological and clinical questions than have been accessible with clinic-based transcriptomics. Given the flexibility of this sampling system and low sample volume, future studies involving frequent sampling and sampling around a specific event (e.g., disease flare, environmental or pathogen exposure) can be employed to better elucidate hard-to-capture expression signatures of the immune response. For example, we hope that our sampling platform enables us to observe and study early, transient, and dynamic changes in both the innate and adaptive arm of the immune system throughout the various stages of an infection in order to guide treatment or transmission control measures.

Critically, with *home*RNA, multiple samples can be taken from the same individual more readily than with current methods, enabling easier comparison to a individual's own baseline. Coupling this with the ability to sample virtually anywhere, studies into a person's individualized response (that is, compared to their own baseline) to an exposure or event in their daily environment are possible. Finally, we are excited to expand this technology to disseminated diagnostics, therapeutics, and clinical research into lower resource or rural settings, which are often far from a phlebotomy clinic.

CONFLICTS OF INTEREST:

The authors acknowledge the following potential conflict of interests: ABT: ownership in Stacks to the Future, LLC. EB: ownership in Stacks to the Future, LLC, Salus Discovery, LLC, and Tasso, Inc., and employment by Tasso, Inc. Technologies from Stacks to the Future, LLC and Salus Discovery, LLC are not included in this publication. The blood collection device used in this publication is from Tasso, Inc; the terms of this arrangement have been reviewed and approved by the University of Washington in accordance with its policies governing outside work and financial conflicts of interest in research.

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