Implementation of Novel Remote Sampling Tools in Longitudinal Studies for Increased Participation and Accessibility

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Abstract

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Traditional clinical studies are often conducted in-person at large medical centers or universities, which can create logistical barriers for prospective participants. One way of circumventing these barriers is by adopting a remote study model where sample collection can be done in participants' homes. The work presented herein illustrates the process of developing, piloting, and implementing tools for remote clinical research with the goal of making participation in clinical studies more accessible. In chapter 1, I introduce the current state of health research and remote studies. In chapter 2, we present the effects of the shipping process (from participant back to the lab) on the integrity of the RNA extracted from homeRNA-stabilized whole blood samples, with an emphasis on the considerations of RNA degradation from exposure to high temperatures. This work includes controlled in-lab experiments where samples are exposed to known temperatures (25℃, 37℃, 40℃, 45℃, and

50°C) for known time intervals (hours to days), as well as a real-life shipping experiment where blood from a single donor is sent to volunteers across the United States during the summer months. Here, we use the RNA Integrity Number (RIN) and sequencing data from 3' mRNA-seq as metrics for successful preservation of sample RNA in the shipping process with the homeRNA platform. In chapter 3, we outline the process of updating the existing homeRNA platform to interface with a commercially available BD microtainer tube and to accommodate larger blood volumes (up to 1.5 mL). We pilot this new device, homeRNAmax, for usability in naïve users and demonstrate feasibility of implementation in a real-world clinical study. Finally, in chapter 4, I investigate the utility of remote research and homeRNA for reaching Underrepresented, Underserved, and Underreported (U3) populations in parallel to tracking early infection biomarkers in a cohort of 40 COVID-19+ women. The biospecimen collection is accompanied by comprehensive user experience surveys which are then examined to better understand factors contributing to the high participant retention (n = 39/40, 98%). The work presented herein illustrates the entire process of implementing new remote clinical tools, from their inception to their use in human subject studies. Taken together, I intend this compilation to serve as a roadmap for researchers and clinicians developing new remote sampling tools and as an instructive guide on how to make participation in clinical research more accessible.

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Dedication

To my grandma.

Chapter 1 | Introduction

Portions of this introduction have been excerpted or paraphrased from Chapters 2-4 of this dissertation.

1.1 Current State of Human Health Research

Human health is a complex and intricate research topic. Contemporary explorations of the human body usually employ one of the following three methods: animal models, tissue engineering, and clinical studies.[1–7] In recent years, animal models have been scrutinized because of the ethical implications and unsatisfactory relevance to human health.[8,9] Tissue engineering, on the other hand, has developed rapidly, creating more physiologically representative in vitro models that have potential to answer mechanistic questions about basic biology. However, a major limitation of tissue engineering is the inability to accurately capture the interplay between different tissues and organs in the body. While some microfluidic lab-on-a-chip platforms have attempted to address this issue[10,11], more work remains to be done before these models can be used to reliably predict human response to clinical interventions. As such, clinical studies remain the predominant investigative method in the field.

Clinical studies are commonly conducted in-person at research sites such as universities and large medical centers. The visits often consist of specimen collection, surveys, or interviews.[5–7,12–14] While the in-person model remains the most reliable method for sampling and data generation, there exist considerable barriers to participation for many people.[15,16] These barriers include reliable access to transport, commute times, scheduling difficulties, discomfort in hospital settings, and discomfort with specimen collection.[15,17–21] It can therefore be inferred that these barriers have prevented a portion of eligible populations from participating in research, limiting the relevance of the findings to the broader population.

The underreporting of certain demographic groups in clinical research is a well-documented phenomenon.[21–24] Owing to these gaps in the data, much of the existing literature is not representative of the larger population of the United States. Lack of clinical data of specific groups results in poorer standards of care and poorer (or even ineffective) treatment options. In efforts to make healthcare as effective as possible, there has been a push in recent years to supplement the existing data and bridge the gaps in knowledge through recruitment of more diverse

cohorts and studies that focus on underrepresented populations.[23,25–27]

While mistrust of medical providers is documented in populations underrepresented in clinical research, it appears that it does not impact their willingness to participate in clinical studies; instead, it is likely that logistical barriers play a stronger role in limiting access to participation.[18,28] This is in part because clinical research is traditionally conducted in person during regular working hours. Moving the study site from clinics and universities to participants' homes offers increased flexibility and offers a way of circumventing common logistical barriers to participation.[29,30]

1.2 Longitudinal Studies

When studying the human immune system, it is imperative to collect samples at multiple time points to generate datasets that capture the biological response of interest. If, for example, sampling is done only once and in person, this decreases the likelihood of collecting a sample when the immune response is most active. Furthermore, immune responses to unpredictable events–either exogenous (e.g., exposure to wildfire smoke) or endogenous (e.g., migraine flares)–are particularly challenging to characterize when only collecting a single time point. Consequently, many clinical research studies employ a longitudinal design, where samples from a participant are collected repeatedly over an extended period of time, often lasting months to years.[31–34] Combined with a transcriptomic endpoint (RNA sequencing data), longitudinal studies enable the study of the immune system, particularly the immune response to pathogens, environmental exposures, or endogenous signals.[34–37]

1.3 Biospecimen Selection, Collection, and Stabilization

An integral component of clinical study design is the selection of appropriate biospecimens. These can include collection of saliva and urine (non-invasive)[38,39], blood draws (moderately invasive)[40–42], or tissue biopsies (highly invasive).[43] The selection of sampling methods in a study will depend on the analyte of interest as well as available resources for collection, storage, and processing.

In this work, the primary biofluid is blood. Blood is selected because it comes into contact with nearly all parts of the body and offers a snapshot of the immune system at that point in time.[44,45] Further, because of the routine nature of blood collection, there are well-established and relatively simple methods for sample collection. The two most common methods for blood collection include fingersticks and venous draws. Fingerstick collection can be self-performed and involves creating a small incision with a sharp blade on one of the fingers, resulting in a few drops of blood (~100 μL).[40,46,47] Venous draws must be performed on-site by trained phlebotomists and can collect larger volumes of blood (up to 40 mL).[41,48] Recently, novel devices for remote self-collection of blood have become commercially available, offering a new possibility for remote research. These include platforms such as the Tasso+ (Tasso, Inc.) and Tap Micro (YourBio Health) which can collect blood volumes up to 1 mL.

As described in the previous section, RNA is a key analyte of interest in longitudinal studies. In cells, RNA is the intermediate between DNA and protein synthesis and the number of RNA transcripts of a particular gene indicate the level of activation of said gene.[37,44,49] When tracked over time, changes in RNA transcripts correspond to an increase in activation (upregulation) or decrease of activation (downregulation) of a particular gene. Since the RNA in blood comes primarily from white blood cells, it is a useful proxy for studying immune system responses to particular stimuli.[37,44,45,49]

However, in the absence of stabilization, RNA is prone to enzymatic degradation, resulting in skewed expression profiles and, thereby, inaccurate conclusions.[50] RNA degradation has been an active area of investigation and there exist several methods for sample preservation. For example, fingerstick blood collection is often paired with dried blood spots (DBS), where the blood is smeared in designated locations on a piece of cardboard and left to dry. Enzymatic activity is significantly decreased in solid state, thus limiting digestion of RNA. Cost-effective and easy to use, DBS have long been employed in research and are an invaluable tool for remote sample collection.[51–54] One limitation of DBS is the low volumes and resulting analyte of interest.[55] Another common method for RNA preservation is the addition of liquid stabilizing agents such as PAXgene and RNA*later*. Commercial RNA stabilizer composition is often proprietary and their mechanisms of action are not well characterized, however, they have been shown to successfully inhibit RNA degradation and are able to preserve

larger volumes of blood than DBS.[56,57]

1.4 Remote Blood Collection and Stabilization

The question of RNA preservation is particularly important for remote studies, since the sample may spend a long time in transit from the time of collection to time of retrieval. To this end, our group has developed the homeRNA kit.[20] The homeRNA kit consists of two main components: a Tasso device for self-collection of < 1 mL of blood from the upper arm and a custom-engineered stabilizer tube with RNA*later*. With the ability to both collect blood and stabilize RNA remotely, homeRNA enables the implementation of fully remote longitudinal studies with transcriptomic readouts and the investigation of time-sensitive immune responses such as: after exposure to a pathogen but prior to development of symptoms[32], during autoimmune disease flares, or following exposure to environmental stressors like wildfire smoke.[31]

1.5 Outline of Remote Study Structure

Most remote studies with biospecimen collection will have a similar outline. The following description is representative of the homeRNA-based studies contained in the subsequent chapters of this thesis and is summarized in Fig. 1. Following Institutional Review Board (IRB) approval, studies are advertised in one or more of the following ways: (1) physical media such as flyers, (2) digital media such as social media posts, or (3) word of mouth recruitment and recommendations by clinicians. Participants will then follow instructions to register using online surveys and are screened for specific study requirements. Those who meet the inclusion criteria are then invited into the study and informed consent is obtained. Once enrolled, a study kit with all necessary materials needed for sample collection is sent to the participants, who collect samples either on a set schedule or in response to specific events. As part of each sampling, participants are asked to record sampling conditions and information about the blood draws including length of collection time, collected sample volume, and any issues in the sampling process. Participant surveys will also often ask about ongoing symptoms and their severity, which

can inform when participants are instructed to collect samples. After collection, samples are shipped back to a central lab for further processing and analysis.

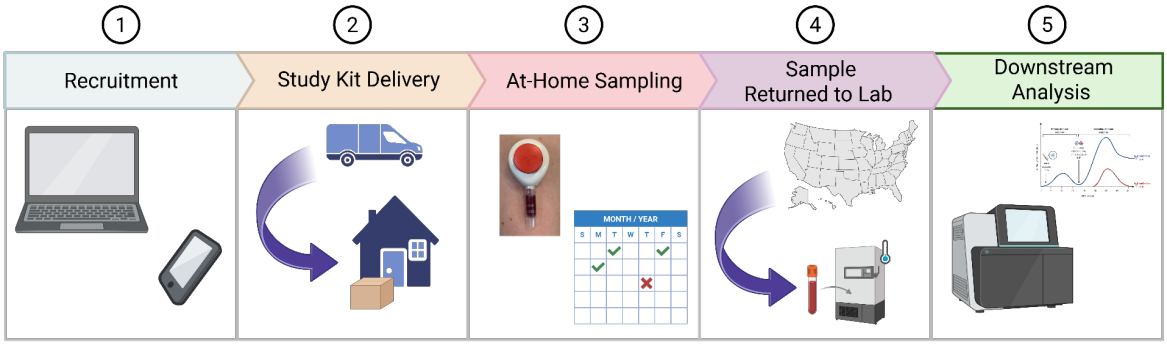

Figure 1.1. Steps of a general remote longitudinal study with homeRNA. Prospective participants complete online screening surveys to verify eligibility (1). If inclusion criteria are met and once informed consent is obtained, participants are sent study kits (2) with all materials necessary for sample collection. At specified times, participants self-sample and stabilize blood using the homeRNA kit (see *Biospecimen Selection, Collection, and Stabilization* below for details) (3). After collection, samples are shipped back to the central lab where they are stored in freezers until further processing (4). Finally, RNA is extracted from the blood samples and undergoes quality control prior to sequencing. This figure was made with Biorender.

1.6 Summary of Research

I propose that remote study design can be leveraged to increase both accessibility and retention in clinical research. I explore the validity of this claim in three chapters:

In chapter 2, I demonstrate robustness of homeRNA to temperature degradation and successful preservation of RNA. This section consists of in-lab temperature controlled experiments, as well as a real-world experiment to elucidate the conditions samples experience in transit back to the central lab. The shipped samples are sequenced to verify that there is no selective degradation of RNA transcript in the shipping process.

In chapter 3, I illustrate the process of development and piloting of an updated homeRNA device. Here, the original homeRNA design is updated to interface with commercially available blood collection tubes (BD microtainers) and accommodate the increased blood volumes. This work also attempts to characterize the fluid physics of the stabilizer tube spill-proof feature. Finally, this chapter describes the process of piloting a novel tool for remote sampling in naïve users and subsequent implementation in a real-world clinical study.

In Chapter 4, I show the utility of a remote sampling model for increased participation and retention of underrepresented populations. Leveraging the homeRNA platform, a remote and longitudinal study is conducted. Participation was limited to women of underrepresented, underserved, and underreported backgrounds, who tested positive for the SARS-CoV-2 virus. Despite the intensive study requirements consisting of self-sampling of blood and comprehensive surveys over 6 months, excellent (98%) retention of U3 participants is reported.

Taken together, this work describes the three main phases of developing novel clinical tools for remote sample collection: development and piloting, stress-testing, and implementation in real-world studies. Furthermore, these chapters demonstrate that homeRNA-based longitudinal studies have the potential to bolster participation and retention of underrepresented populations, and, as such, offer one potential solution for generating more representative datasets in clinical research.

1.7 References

(1) Fluri, F.; Schuhmann, M. K.; Kleinschnitz, C. Animal Models of Ischemic Stroke and Their Application in Clinical Research. *Drug Des. Devel. Ther.* **2015**, *9*, 3445–3454. https://doi.org/10.2147/DDDT.S56071.

(2) Ruggeri, B. A.; Camp, F.; Miknyoczki, S. Animal Models of Disease: Pre-Clinical Animal Models of Cancer and Their Applications and Utility in Drug Discovery. *Biochem. Pharmacol.* **2014**, *87* (1), 150–161. https://doi.org/10.1016/j.bcp.2013.06.020.

(3) Shafiee, A.; Atala, A. Tissue Engineering: Toward a New Era of Medicine. *Annu. Rev. Med.* **2017**, *68* (Volume 68, 2017), 29–40. https://doi.org/10.1146/annurev-med-102715-092331.

(4) *Trends in Tissue Engineering for Blood Vessels - Nemeno-Guanzon - 2012 - BioMed Research International - Wiley Online Library*. https://onlinelibrary.wiley.com/doi/full/10.1155/2012/956345 (accessed 2026-04-07).

(5) Singh, A. K.; Singh, A.; Singh, R.; Misra, A. Remdesivir in COVID-19: A Critical Review of Pharmacology, Pre-Clinical and Clinical Studies. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2020**, *14* (4), 641–648. https://doi.org/10.1016/j.dsx.2020.05.018.

(6) Moschos, S. J.; Mantzoros, C. S. The Role of the IGF System in Cancer: From Basic to Clinical Studies and Clinical Applications. *Oncology* **2002**, *63* (4), 317–332. https://doi.org/10.1159/000066230.

(7) Hidalgo, M.; Bruckheimer, E.; Rajeshkumar, N. V.; Garrido-Laguna, I.; De Oliveira, E.; Rubio-Viqueira, B.; Strawn, S.; Wick, M. J.; Martell, J.; Sidransky, D. A Pilot Clinical Study of Treatment Guided by Personalized Tumorgrafts in Patients with Advanced Cancer. *Mol. Cancer Ther.* **2011**, *10* (8), 1311–1316. https://doi.org/10.1158/1535-7163.MCT-11-0233.

(8) Robinson, N. B.; Krieger, K.; Khan, F. M.; Huffman, W.; Chang, M.; Naik, A.; Yongle, R.; Hameed, I.; Krieger, K.; Girardi, L. N.; Gaudino, M. The Current State of Animal Models in Research: A Review. *Int. J. Surg.* **2019**, *72*, 9–13. https://doi.org/10.1016/j.ijsu.2019.10.015.

(9) Ingber, D. E. Is It Time for Reviewer 3 to Request Human Organ Chip Experiments Instead of Animal Validation Studies? *Adv. Sci.* **2020**, *7* (22), 2002030. https://doi.org/10.1002/advs.202002030.

(10) Schimek, K.; Busek, M.; Brincker, S.; Groth, B.; Hoffmann, S.; Lauster, R.; Lindner, G.; Lorenz, A.; Menzel, U.; Sonntag, F.; Walles, H.; Marx, U.; Horland, R. Integrating Biological Vasculature into a Multi-Organ-Chip Microsystem. *Lab. Chip* **2013**, *13* (18), 3588–3598. https://doi.org/10.1039/C3LC50217A.

(11) Picollet-D'hahan, N.; Zuchowska, A.; Lemeunier, I.; Gac, S. L. Multiorgan-on-a-Chip: A Systemic Approach To Model and Decipher Inter-Organ Communication. *Trends Biotechnol.* **2021**, *39* (8), 788–810. https://doi.org/10.1016/j.tibtech.2020.11.014.

(12) LaVange, L. M.; Koch, G. G.; Schwartz, T. A. Applying Sample Survey Methods to Clinical Trials Data. *Stat. Med.* **2001**, *20* (17–18), 2609–2623. https://doi.org/10.1002/sim.732.

(13) Guidi, J.; Fava, G. A.; Bech, P.; Paykel, E. The Clinical Interview for Depression: A Comprehensive Review of Studies and Clinimetric Properties. *Psychother. Psychosom.* **2010**, *80* (1), 10–27. https://doi.org/10.1159/000317532.

(14) Clement, J. Analysis of Clinical Interviews: Foundations and Model Viability. In *Handbook of Research Design in Mathematics and Science Education*; Routledge, 2000.

(15) Robiner, W. N.; Yozwiak, J. A.; Bearman, D. L.; Strand, T. D.; Strasburg, K. R. Barriers to Clinical Research Participation in a Diabetes Randomized Clinical Trial. *Soc. Sci. Med.* **2009**, *68* (6), 1069–1074. https://doi.org/10.1016/j.socscimed.2008.12.025.

(16) Marcantonio, E. R.; Aneja, J.; Jones, R. N.; Alsop, D. C.; Fong, T. G.; Crosby, G. J.; Culley, D. J.; Cupples, L. A.; Inouye, S. K. Maximizing Clinical Research Participation in Vulnerable Older Persons: Identification of Barriers and Motivators. *J. Am. Geriatr. Soc.* **2008**, *56* (8), 1522–1527. https://doi.org/10.1111/j.1532-5415.2008.01829.x.

(17) Meropol, N. J.; Buzaglo, J. S.; Millard, J.; Damjanov, N.; Miller, S. M.; Ridgway, C.; Ross, E. A.;

Sprandio, J. D.; Watts, P. Barriers to Clinical Trial Participation as Perceived by Oncologists and Patients. *J. Natl. Compr. Canc. Netw.* **2007**, *5* (8), 753–762. https://doi.org/10.6004/jnccn.2007.0067.

(18) Luebbert, R.; Perez, A. Barriers to Clinical Research Participation Among African Americans. *J. Transcult. Nurs.* **2016**, *27* (5), 456–463. https://doi.org/10.1177/1043659615575578.

(19) Rigatti, M.; DeGurian, A. A.; Albert, S. M. "Getting There": Transportation as a Barrier to Research Participation Among Older Adults. *J. Appl. Gerontol.* **2022**, *41* (5), 1321–1328. https://doi.org/10.1177/07334648211072537.

(20) Haack, A. J.; Lim, F. Y.; Kennedy, D. S.; Day, J. H.; Adams, K. N.; Lee, J. J.; Berthier, E.; Theberge, A. B. homeRNA: A Self-Sampling Kit for the Collection of Peripheral Blood and Stabilization of RNA. *Anal. Chem.* **2021**, *93* (39), 13196–13203. https://doi.org/10.1021/acs.analchem.1c02008.

(21) Ford, J. G.; Howerton, M. W.; Lai, G. Y.; Gary, T. L.; Bolen, S.; Gibbons, M. C.; Tilburt, J.; Baffi, C.; Tanpitukpongse, T. P.; Wilson, R. F.; Powe, N. R.; Bass, E. B. Barriers to Recruiting Underrepresented Populations to Cancer Clinical Trials: A Systematic Review. *Cancer* **2008**, *112* (2), 228–242. https://doi.org/10.1002/cncr.23157.

(22) Sedano, R.; Hogan, M.; Mcdonald, C.; Aswani-Omprakash, T.; Ma, C.; Jairath, V. Underrepresentation of Minorities and Underreporting of Race and Ethnicity in Crohn's Disease Clinical Trials. *Gastroenterology* **2022**, *162* (1), 338-340.e2. https://doi.org/10.1053/j.gastro.2021.09.054.

(23) Bains, A.; Osathanugrah, P.; Sanjiv, N.; Chiu, C.; Fiorello, M. G.; Siegel, N. H.; Peeler, C. E.; Distefano, A. G.; Lee, H. J.; Ness, S.; Desai, M. A.; Titelbaum, J. R.; Pira, T.; LaMattina, K. C.; Christiansen, S. P.; Cabral, H. J.; Subramanian, M. L. Diverse Research Teams and Underrepresented Groups in Clinical Studies. *JAMA Ophthalmol.* **2023**, *141* (11), 1037–1044. https://doi.org/10.1001/jamaophthalmol.2023.4638.

(24) Noah, B. A. The Participation of Underrepresented Minorities in Clinical Research. *Am. J. Law Med.*

2003, *29* (2–3), 221–245. https://doi.org/10.1017/S0098858800002823.

(25) Hartwell, C.; Lovell, S.; Hess, J. J.; Dolan, K.; Vickery, J.; Errett, N. A. Barriers and Facilitators to State Public Health Agency Climate and Health Action: A Qualitative Assessment. *BMC Public Health* **2023**, *23* (1), 145. https://doi.org/10.1186/s12889-023-14996-2.

(26) Magesh, S.; John, D.; Li, W. T.; Li, Y.; Mattingly-App, A.; Jain, S.; Chang, E. Y.; Ongkeko, W. M. Disparities in COVID-19 Outcomes by Race, Ethnicity, and Socioeconomic Status: A Systematic-Review and Meta-Analysis. *JAMA Netw. Open* **2021**, *4* (11), e2134147. https://doi.org/10.1001/jamanetworkopen.2021.34147.

(27) Weiner, M. W.; Veitch, D. P.; Miller, M. J.; Aisen, P. S.; Albala, B.; Beckett, L. A.; Green, R. C.; Harvey, D.; Jack Jr, C. R.; Jagust, W.; Landau, S. M.; Morris, J. C.; Nosheny, R.; Okonkwo, O. C.; Perrin, R. J.; Petersen, R. C.; Rivera-Mindt, M.; Saykin, A. J.; Shaw, L. M.; Toga, A. W.; Tosun, D.; Trojanowski, J. Q.; Initiative, A. D. N. Increasing Participant Diversity in AD Research: Plans for Digital Screening, Blood Testing, and a Community-Engaged Approach in the Alzheimer's Disease Neuroimaging Initiative 4. *Alzheimers Dement.* **2023**, *19* (1), 307–317. https://doi.org/10.1002/alz.12797.

(28) Wendler, D.; Kington, R.; Madans, J.; Wye, G. V.; Christ-Schmidt, H.; Pratt, L. A.; Brawley, O. W.; Gross, C. P.; Emanuel, E. Are Racial and Ethnic Minorities Less Willing to Participate in Health Research? *PLOS Med.* **2005**, *3* (2), e19. https://doi.org/10.1371/journal.pmed.0030019.

(29) Stewart, J.; Krows, M. L.; Schaafsma, T. T.; Heller, K. B.; Brown, E. R.; Boonyaratanakornkit, J.; Brown, C. E.; Leingang, H.; Liou, C.; Bershteyn, A.; Schwartz, M. D.; Agrawal, V.; Friedman-Klabanoff, D.; Eustace, S.; Stankiewicz Karita, H. C.; Paasche-Orlow, M. K.; Kissinger, P.; Hosek, S. G.; Chu, H. Y.; Celum, C.; Baeten, J. M.; Wald, A.; Johnston, C.; Barnabas, R. V. Comparison of Racial, Ethnic, and Geographic Location Diversity of Participants Enrolled in Clinic-Based vs 2 Remote COVID-19 Clinical Trials. *JAMA Netw. Open* **2022**, *5* (2), e2148325. https://doi.org/10.1001/jamanetworkopen.2021.48325.

(30) Cummings, S. R. Clinical Trials Without Clinical Sites. *JAMA Intern. Med.* **2021**, *181* (5), 680–684. https://doi.org/10.1001/jamainternmed.2020.9223.

(31) Haack, A. J.; Zheng, Y.; Brown, L. G.; Stefanovic, F.; Jeacopello, I.; Adams, K.; Kennedy, D. S.; Stolarczuk, J. E.; Takezawa, M. G.; Hassan, G. W.; MacDonald, J.; Bammler, T.; Edelson, J.; Lin, A.; Lim, F. Y.; Chaussabel, D.; Walker, E. G.; Errett, N. A.; Berthier, E.; Theberge, A. B. Longitudinal Transcriptomic Studies Using homeRNA: A Framework For Studying the Effects of the Exposome and Wildfire Smoke Using Self-Sampled and Stabilized Blood, 2024.

(32) Lim, F. Y.; Lea, H. G.; Dostie, A. M.; Kim, S.-Y.; Neel, T. L. van; Hassan, G. W.; Takezawa, M. G.; Starita, L. M.; Adams, K. N.; Boeckh, M.; Schiffer, J. T.; Hyrien, O.; Waghmare, A.; Berthier, E.; Theberge, A. B. homeRNA Self-Blood Collection Enables High-Frequency Temporal Profiling of Presymptomatic Host Immune Kinetics to Respiratory Viral Infection: A Prospective Cohort Study. *eBioMedicine* **2025**, *112*. https://doi.org/10.1016/j.ebiom.2024.105531.

(33) Santillan, M. K.; Leslie, K. K.; Hamilton, W. S.; Boese, B. J.; Ahuja, M.; Hunter, S. K.; Santillan, D. A. "Collection of a Lifetime: A Practical Approach to Developing a Longitudinal Collection of Women's Healthcare Biological Samples." *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2014**, *179*, 94–99. https://doi.org/10.1016/j.ejogrb.2014.05.023.

(34) Speake, C.; Whalen, E.; Gersuk, V. H.; Chaussabel, D.; Odegard, J. M.; Greenbaum, C. J. Longitudinal Monitoring of Gene Expression in Ultra-Low-Volume Blood Samples Self-Collected at Home. *Clin. Exp. Immunol.* **2017**, *188* (2), 226–233. https://doi.org/10.1111/cei.12916.

(35) Chaussabel, D.; Pulendran, B. A Vision and a Prescription for Big Data–Enabled Medicine. *Nat. Immunol.* **2015**, *16* (5), 435–439. https://doi.org/10.1038/ni.3151.

(36) Rinchai, D.; Deola, S.; Zoppoli, G.; Kabeer, B. S. A.; Taleb, S.; Pavlovski, I.; Maacha, S.; Gentilcore, G.; Toufiq, M.; Mathew, L.; Liu, L.; Vempalli, F. R.; Mubarak, G.; Lorenz, S.; Sivieri, I.; Cirmena, G.;

Dentone, C.; Cuccarolo, P.; Giacobbe, D. R.; Baldi, F.; Garbarino, A.; Cigolini, B.; Cremonesi, P.; Bedognetti, M.; Ballestrero, A.; Bassetti, M.; Hejblum, B. P.; Augustine, T.; Van Panhuys, N.; Thiebaut, R.; Branco, R.; Chew, T.; Shojaei, M.; Short, K.; Feng, C. G.; PREDICT-19 Consortium; Zughaier, S. M.; De Maria, A.; Tang, B.; Ait Hssain, A.; Bedognetti, D.; Grivel, J.-C.; Chaussabel, D. High–Temporal Resolution Profiling Reveals Distinct Immune Trajectories Following the First and Second Doses of COVID-19 mRNA Vaccines. *Sci. Adv.* **2022**, *8* (45), eabp9961. https://doi.org/10.1126/sciadv.abp9961.

(37) Chaussabel, D. Assessment of Immune Status Using Blood Transcriptomics and Potential Implications for Global Health. *Semin. Immunol.* **2015**, *27* (1), 58–66. https://doi.org/10.1016/j.smim.2015.03.002.

(38) Holm, A.; Aabenhus, R. Urine Sampling Techniques in Symptomatic Primary-Care Patients: A Diagnostic Accuracy Review. *BMC Fam. Pract.* **2016**, *17* (1), 72. https://doi.org/10.1186/s12875-016-0465-4.

(39) Bellagambi, F. G.; Lomonaco, T.; Salvo, P.; Vivaldi, F.; Hangouët, M.; Ghimenti, S.; Biagini, D.; Di Francesco, F.; Fuoco, R.; Errachid, A. Saliva Sampling: Methods and Devices. An Overview. *TrAC Trends Anal. Chem.* **2020**, *124*, 115781. https://doi.org/10.1016/j.trac.2019.115781.

(40) Rao, L. V.; Moiles, D.; Snyder, M. Finger-Stick Complete Blood Counts: Comparison Between Venous and Capillary Blood. *Point Care* **2011**, *10* (3), 120. https://doi.org/10.1097/POC.0b013e31822814e0.

(41) Giavarina, D.; Lippi, G. Blood Venous Sample Collection: Recommendations Overview and a Checklist to Improve Quality. *Clin. Biochem.* **2017**, *50* (10), 568–573. https://doi.org/10.1016/j.clinbiochem.2017.02.021.

(42) Brown, L. G.; Wei, X.; Milton, L. A.; Alizai, M. Y.; MacDonald, J. W.; Bammler, T. K.; Zeng, Y.; Robertson, I.; Adams, K. N.; Toh, Y.-C.; Chaussabel, D.; Berthier, E.; Haack, A. J.; Theberge, A. B. From Home to Transcriptome: Comparing the Transcriptomic Profile of Induced Immune Response via Lipopolysaccharide Stimulation in homeRNA and Venous Blood. *Anal. Chem.* **2026**. https://doi.org/10.1021/acs.analchem.5c06040.

(43) *Systematic Review of the Quality of Liver Biopsy Specimens | American Journal of Clinical Pathology | Oxford Academic*. https://academic.oup.com/ajcp/article-abstract/125/5/710/1759859 (accessed 2026-04-08).

(44) Chaussabel, D.; Pascual, V.; Banchereau, J. Assessing the Human Immune System through Blood Transcriptomics. *BMC Biol.* **2010**, *8*, 84. https://doi.org/10.1186/1741-7007-8-84.

(45) Müller, S.; Kröger, C.; Schultze, J. L.; Aschenbrenner, A. C. Whole Blood Stimulation as a Tool for Studying the Human Immune System. *Eur. J. Immunol.* **2024**, *54* (2), e2350519. https://doi.org/10.1002/eji.202350519.

(46) Doeleman, M. J. H.; Koster, A.-F.; Esseveld, A.; Kemperman, H.; Swart, J. F.; Roock, S. de; Groenestege, W. M. T. Comparison of Capillary Finger Stick and Venous Blood Sampling for 34 Routine Chemistry Analytes: Potential for in Hospital and Remote Blood Sampling. *Clin. Chem. Lab. Med. CCLM* **2025**, *63* (4), 747–752. https://doi.org/10.1515/cclm-2024-0812.

(47) Robison, E. H.; Mondala, T. S.; Williams, A. R.; Head, S. R.; Salomon, D. R.; Kurian, S. M. Whole Genome Transcript Profiling from Fingerstick Blood Samples: A Comparison and Feasibility Study. *BMC Genomics* **2009**, *10* (1), 617. https://doi.org/10.1186/1471-2164-10-617.

(48) Gonsalves, W. I.; Cornish, N.; Moore, M.; Chen, A.; Varman, M. Effects of Volume and Site of Blood Draw on Blood Culture Results. *J. Clin. Microbiol.* **2009**, *47* (11), 3482–3485. https://doi.org/10.1128/jcm.02107-08.

(49) Forabosco, P.; Pala, M.; Crobu, F.; Diana, M. A.; Marongiu, M.; Cusano, R.; Angius, A.; Steri, M.; Orrù, V.; Schlessinger, D.; Fiorillo, E.; Devoto, M.; Cucca, F. Transcriptome Organization of White Blood Cells through Gene Co-Expression Network Analysis in a Large RNA-Seq Dataset. *Front. Immunol.* **2024**, *15*. https://doi.org/10.3389/fimmu.2024.1350111.

(50) Houseley, J.; Tollervey, D. The Many Pathways of RNA Degradation. *Cell* **2009**, *136* (4), 763–776.

https://doi.org/10.1016/j.cell.2009.01.019.

(51) Baillargeon, K. R.; Brooks, J. C.; Miljanic, P. R.; Mace, C. R. Patterned Dried Blood Spot Cards for the Improved Sampling of Whole Blood. *ACS Meas. Sci. Au* **2022**, *2* (1), 31–38. https://doi.org/10.1021/acsmeasuresciau.1c00031.

(52) Fredolini, C.; Dodig-Crnković, T.; Bendes, A.; Dahl, L.; Dale, M.; Albrecht, V.; Mattsson, C.; Thomas, C. E.; Torinsson Naluai, Å.; Gisslen, M.; Beck, O.; Roxhed, N.; Schwenk, J. M. Proteome Profiling of Home-Sampled Dried Blood Spots Reveals Proteins of SARS-CoV-2 Infections. *Commun. Med.* **2024**, *4* (1), 55. https://doi.org/10.1038/s43856-024-00480-4.

(53) Lim, M. D. Dried Blood Spots for Global Health Diagnostics and Surveillance: Opportunities and Challenges. *Am. J. Trop. Med. Hyg.* **2018**, *99* (2), 256–265. https://doi.org/10.4269/ajtmh.17-0889.

(54) Wikström, F.; Olsson, C.; Palm, B.; Roxhed, N.; Backlund, L.; Schalling, M.; Beck, O. Determination of Lithium Concentration in Capillary Blood Using Volumetric Dried Blood Spots. *J. Pharm. Biomed. Anal.* **2023**, *227*, 115269. https://doi.org/10.1016/j.jpba.2023.115269.

(55) Zakaria, R.; Allen, K. J.; Koplin, J. J.; Roche, P.; Greaves, R. F. Advantages and Challenges of Dried Blood Spot Analysis by Mass Spectrometry Across the Total Testing Process. *EJIFCC* **2016**, *27* (4), 288–317.

(56) Weber, D. G.; Casjens, S.; Rozynek, P.; Lehnert, M.; Zilch-Schöneweis, S.; Bryk, O.; Taeger, D.; Gomolka, M.; Kreuzer, M.; Otten, H.; Pesch, B.; Johnen, G.; Brüning, T. Assessment of mRNA and microRNA Stabilization in Peripheral Human Blood for Multicenter Studies and Biobanks. *Biomark. Insights* **2010**, *5*, BMI.S5522. https://doi.org/10.4137/BMI.S5522.

(57) Chai, V.; Vassilakos, A.; Lee, Y.; Wright, J. A.; Young, A. H. Optimization of the PAXgene™ Blood RNA Extraction System for Gene Expression Analysis of Clinical Samples. *J. Clin. Lab. Anal.* **2005**, *19* (5), 182–188. https://doi.org/10.1002/jcla.20075.

Chapter 2 | Your Blood is Out for Delivery: Considerations of Shipping Time and Temperature on Degradation of RNA from Stabilized Whole Blood[1]

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Abstract: Remote research studies are an invaluable tool for reaching populations with limited access to large medical centers or universities. To expand the remote study toolkit, we previously developed homeRNA, which allows for at-home self-collection and stabilization of blood and demonstrated the feasibility of using homeRNA in high temperature climates. Here, we expand upon this work through a systematic study exploring the effects of high temperature on RNA integrity (represented as RNA Integrity Number, RIN) through in-lab and field experiments. Compared to the frozen controls (overall mean RIN of 8.2, n = 8), samples kept at 37°C for 2, 4, and 8 days had mean RINs of 7.6, 5.9, and 5.2 (n = 3), respectively, indicating that typical shipping conditions (~2 days) yield samples suitable for downstream RNA sequencing. Shorter time intervals (6 hours) resulted in minimal RNA degradation (median RIN of 6.4, n = 3) even at higher temperatures (50°C) compared to the frozen control (mean RIN of 7.8, n = 3). Additionally, we shipped homeRNA-stabilized blood from a single donor to 14 states and back during the summer with continuous temperature probes (7.1 median RIN, n = 42). Samples from all locations were analyzed with 3' mRNA-seq to assess differences in gene counts, with the data suggesting that

1 This chapter contains collaborative work. FS, LGB, EB, AJH, and ABT contributed to the conception and design of the study. FS, LGB, SN, VS, and AJH conducted the experiments. FS, LGB, SN, YZ, and VS performed the RNA extractions on samples and collected the RIN and yield data. FS, LGB, JM, TB, DR, DC, EB, AJH, and ABT analyzed/interpreted the data. FS, LGB, DR, AJH, and ABT prepared the figures and wrote the manuscript.

there was no preferential degradation of transcripts as a result of different shipping times, temperatures, and regions. Overall, our data support that homeRNA can be used in elevated temperature conditions, enabling decentralized sample collection for telemedicine, global health, and clinical research.

2.1 Introduction

Remote self-sampling studies have become more prevalent in the wake of the COVID-19 pandemic; however, their utility goes far beyond the convenience of at-home sampling in a pandemic setting [1–5]. Fully remote studies circumvent many logistical barriers that preclude underserved and rural populations from participating in clinical research. These barriers include access to clinics or study sites that can perform blood draws, need for trained phlebotomists, suitable transportation, and sufficient time outside of typical work schedules. Additionally, remote sampling is compatible with collection of time-sensitive and longitudinal samples, making it an invaluable tool for human health research [6–11].

Devices for remote blood sampling (such as lancet-based devices from Tasso (Seattle, WA), YourBio Health (Medford, MA), etc.) offer a user-friendly way to self-collect blood samples. To enable analysis of many blood analytes, remote sample stabilization is necessary, particularly for whole blood RNA. Without stabilization, RNAses in whole blood can trigger intracellular RNA transcript degradation pathways ex vivo that can alter gene expression levels [12–15]. To address this aspect of RNA degradation, our lab has developed the homeRNA kit which consists of a commercially available Tasso-SST upper-arm blood collection device (which collects up to 0.5 mL of blood) and a custom-engineered stabilizer tube containing RNAlater [9]. RNAlater is a commercially available RNA stabilization agent commonly used with biological tissue samples that inhibits RNase activity and stabilizes cellular RNA [16–19]. In previous homeRNA studies, we have demonstrated that RNAlater effectively stabilizes self-collected blood and yields RNA of sufficient quality (represented by RNA Integrity Number, RIN) and quantity for downstream transcriptome analyses [9–11, 20]. In these studies, samples experience variable shipping times and often are not stored in the freezer until >48 h after collection. In ongoing work, we use homeRNA to study the inflammatory response to wildfire smoke exposure, which includes sampling during hot

summer months across Western and Central U.S., and acute immune response to COVID-19 infection with nationwide sampling during all seasons [10, 11]. Since the US has vastly different climates depending on the time of year and location, it is important to consider how the shipping process may affect the integrity of RNA in remotely collected and stabilized blood samples. For example, our wildfire smoke exposure study took place over a 10-month period (before, during, and after wildfire smoke exposure) and many samples were collected during the summer months. Further, we have previously conducted a preliminary examination of homeRNA in the Western and Central U.S. and Qatar with the goal of validating its utility in high temperature settings [20].

While the majority of samples collected in our previous and ongoing studies have sufficient RNA quality and yield for transcriptomic analysis, it is important to further investigate if transcripts are preferentially degraded and could bias the interpretation of these results. A potential concern based on past remote study experiences is that rural communities experience longer shipping times which could adversely affect RNA quality. Similarly, there is a concern that remote studies investigating immune response in tropical climates or during summer months could suffer from heat-induced RNA degradation.

The existing body of research has primarily investigated the effects of cold and ambient temperatures on RNA integrity prior to isolation from whole blood samples, as well as stability of RNA following extraction [21–24]. However, few studies to date have systematically investigated exposure of blood to high temperatures (>37°C) and its effect on the resulting isolated RNA quality; one study from Sarathkumara et al. investigated the effect of exposing Tempus- and PAXgene-stabilized blood samples up to 40°C and found a decrease in RIN with prolonged exposure (up to 10 days) [25]. Additionally, Heneghan et al. observed a 5-10 fold increase in the rate of RNA transcript degradation from dried blood spots stored at 37°C, suggesting that higher temperatures may compromise the integrity of the transcriptome in dried blood spots [26]. Drawing on our past experiences with homeRNA and remote studies, we designed a set of temperature-controlled experiments on RNAlater-stabilized whole blood samples exposed to temperatures up to 50°C for up to 8 days. Additionally, we conducted a real-world shipping experiment and sequenced a subset of these samples using 3’ mRNA sequencing (3’ mRNA-seq) to better understand how variable exposure to different temperatures and shipping times can affect the quality of

the transcriptomic data. This work serves as a roadmap for developing future remote transcriptomic studies that take place in elevated temperatures (e.g., tropical climates, summer months) and lays the groundwork for understanding the role temperature degradation plays in the interpretation of these data.

2.2 Investigating RNA Integrity of RNAlater Stabilized Blood Samples in Temperature Controlled Experiments

To validate the utility of the homeRNA kit for preservation of RNA, we conducted a set of experiments to systematically probe the effects of storage conditions (time, temperature, repeat exposure). We chose times and temperatures to replicate scenarios similar to what samples would experience in a remote study conducted with the homeRNA platform [9-11, 20]. In a typical homeRNA study, participants are given instructions for self-collection and blood stabilization. Participants then package their stabilized samples for next- or same-day courier pickup. Many study participants opt to leave the samples on their front porch for a pickup window of 2 – 4 hours. Based on the location and time of year, samples may experience multiple hours outdoors in hot temperatures (>37°C). After pickup, packages typically take 1 – 2 days to be returned to the lab, depending on the participant's location; but in rare cases it has taken up to 2 weeks for a package to be returned [9]. Additionally, while in transit, samples may experience fluctuations in temperatures (e.g., prior to pickup, during transit, or in storage facilities) (Figure C2) that could affect the stability of the RNA and cause differential degradation of RNA transcripts. In this work, we aim to elucidate (1) if exposure to longer shipping times and higher temperatures results in increased degradation of RNA and (2) if the variable conditions in the shipping process result in preferential degradation of specific transcripts.

To model shipping times and temperatures that homeRNA samples may experience, we exposed RNAlater-stabilized venous blood samples to various temperatures (25°C, 37°C, 40°C, 45°C, or 50°C) and varying lengths of time (6 h, 1 day, 2 days, 4 days, or 8 days) in the lab. We place emphasis on the effects of higher temperature conditions (>37°C) since some of our ongoing homeRNA studies take place in hot climates or during the summer months. Remote studies can also take place in winter months or colder climates and may experience freezing with subsequent thawing prior to processing. Although some literature reports adverse effects of freeze-thaw cycles

on RNA integrity from whole blood samples, we have found that RNAlater-stabilized whole blood samples are minimally affected by a freeze-thaw cycle (Figure C3) [21, 27, 28].

2.3 Longer Term (2, 4, and 8 day) Exposure to High Temperatures (>37°C)

Our first experiment was set up to understand the effect of "longer-term" exposure to various temperatures and storage times (2, 4, or 8 days) of the stabilized blood samples and the resulting effects on RNA integrity. These time points were chosen to recapitulate potential scenarios we have seen with homeRNA. While we have opted for overnight shipping with private companies (UPS, FedEx) to minimize time samples spend in transit, we recognize that these are not available to all researchers who may want to use the homeRNA platform. As such, the longer time points (4 and 8 days) are valuable for an array of scenarios where samples spend extended periods in transit such as overseas shipping, or shipping from rural areas. Additionally, we chose to hold the samples at steady temperatures to understand temperature-dependent degradation of RNA. For this experiment, we chose three conditions to represent three different scenarios: (condition 1) the sample is left out on the porch for pickup immediately after collection, (condition 2) the sample is kept in a fridge prior to being left on the porch for pickup, and (condition 3) the sample is kept indoors (not refrigerated) overnight prior to being left outside for pickup. All samples were stored at -20°C until being extracted, which is known to be stable for prolonged periods of time per the manufacturer's information.

RNA Integrity Number (RIN) is a common metric used to measure the quality of RNA samples; RIN values are found using Agilent's 2100 Bioanalyzer which calculates the RIN through a proprietary algorithm, taking into account the relative peak height and shape of the 18S and 28S ribosomal RNA subunits in a given sample, as well as other features of the resulting electropherograms [29, 30]. There are some discrepancies in the literature about a cutoff RIN value that is suitable for sequencing, with values of 7 or 8 often cited [14, 31–33]. However, newer sequencing technologies such as 3' mRNA-seq and post-sequencing computational processing methods make possible the sequencing of samples with RINs as low as 3 [14, 32, 34–36]. Our data show that increasing temperatures results in greater RNA degradation (lower RIN values) in RNAlater-stabilized blood samples as

compared to samples immediately frozen after stabilization (Figure 2.1). Longer exposures resulted in additional degradation; for example, the mean RIN for the 50°C condition changed from 5.5 to 4.0 to 1.8 for the samples at 2, 4, and 8 days, respectively. When incubated for two days at any temperature, all samples had RINs that were suitable for downstream transcriptomic analysis (from 7.9 at 25°C to 5.5 at 50°C). Although notable degradation was observed for samples that were kept at 45°C and 50°C for 8 days, it is also important to note that samples in a remote study will be subject to variable temperatures and not a constant exposure to the high temperatures over several days. For example, it is highly unlikely that a sample would be exposed to 8 days at 50°C, the highest and longest condition that we tested. Moreover, samples that were kept at 25°C had a mean RIN of at least 7 across all time points, suggesting that RNAlater-stabilized samples at lower temperatures undergo limited degradation even at longer time scales (up to 8 days). Further, samples in condition 2 (refrigerated prior to shipment) gave similar RIN values as samples in condition 3 (kept at room temperature overnight). Taken together, these results suggest that the additional refrigeration step prior to sample shipping is not strictly necessary.

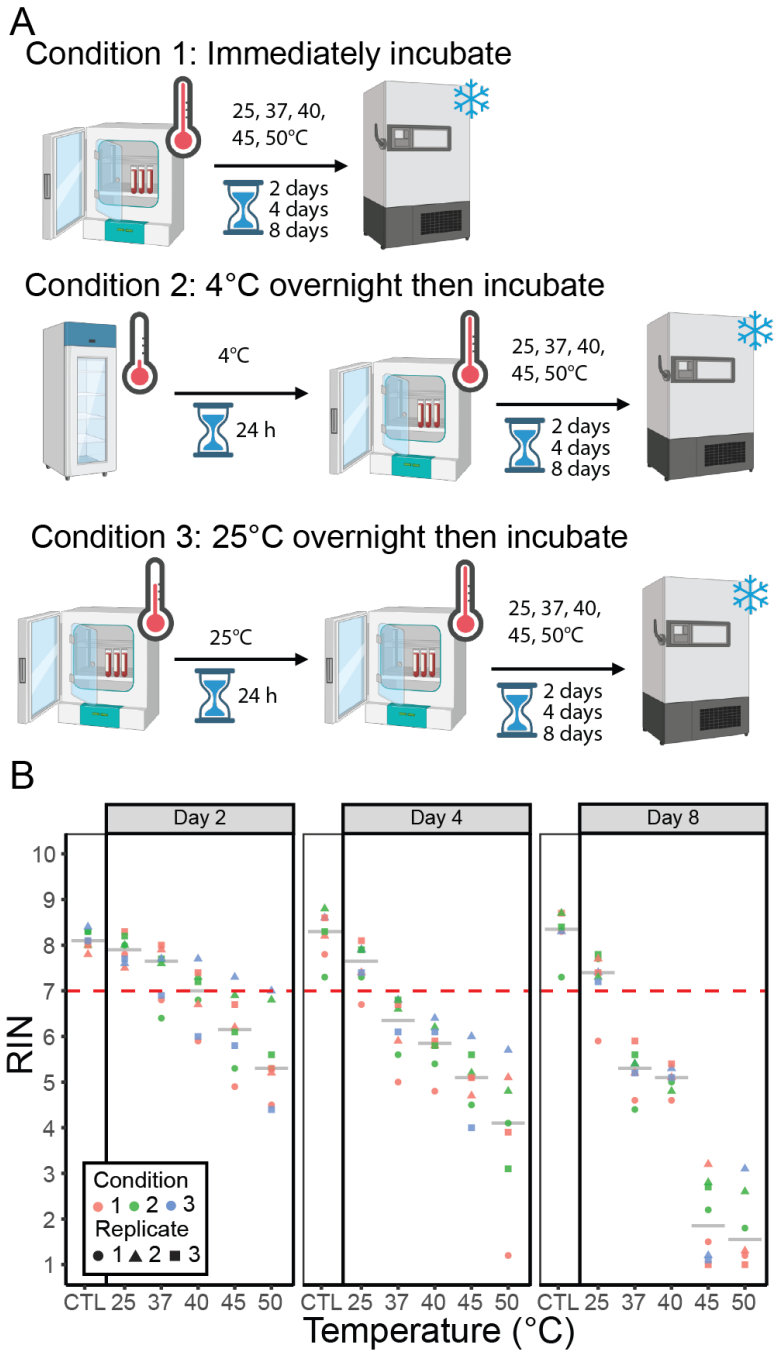

Figure 2.1. Exposure of RNAlater-stabilized whole blood samples to longer term (2, 4, and 8 day) high temperatures (>37°C). (A) Outline of experimental conditions. Condition 1, samples were immediately incubated at 25°C, 37°C, 40°C, 45°C, and 50°C for 2, 4, and 8 days. One sample was frozen at -20°C immediately after stabilizing with RNAlater; Condition 2, samples were incubated at 4°C for 24 hours then exposed to the same set of temperatures and times as condition 1. One sample was frozen at -20°C after incubation for 24 hours at 4°C; and condition 3, samples were incubated at 25°C for 24 hours prior to exposure to the same set of temperatures and times as condition 1. One sample was frozen at -20°C after incubation for 24 hours at 25°C. After each timepoint, one sample from every temperature was immediately frozen and kept at -20°C until ready for RNA extraction. Created with BioRender.com. (B) RNA quality of each extracted sample from each condition. For each condition, samples were extracted based on timepoints, resulting in three total extraction batches (day 2, 4, and 8) with 6 samples in each batch (condition control (CTL), 25°C, 37°C, 40°C, 45°C, and 50°C). Each condition was performed in triplicate. The gray crossbars signify the mean RIN at each time and temperature across all conditions and replicates.

2.4 Shorter Term (<2 days) Exposure to High Temperatures (>37℃)

Beyond investigating longer times samples may experience at high temperatures (>37℃) during shipping, we were also interested in shorter durations (within hours) of high temperature exposure. In homeRNA studies, participants will often opt to have the sample picked up by a courier service by leaving the sample on their front porch. Depending on location and time of year, the sample may be exposed to very high temperatures for several hours.

To better mimic the conditions homeRNA samples may experience, we replicated conditions from different scenarios prior to shipment. All experimental samples were kept at room temperature (25°C) overnight (16 hours) to emulate a study participant collecting their sample and leaving it at home until pickup. After this initial incubation, the experimental samples were either frozen or exposed to the same set of temperatures established in our previous longer-term experiment (25℃, 37℃, 40℃, 45℃, and 50℃) for 6 hours. The samples that were frozen after incubation at room temperature overnight (Condition 1, Figure 2.2) represent degradation prior to pickup. We tested this condition because in many of our remote studies participants collect their samples the day before the pickup is scheduled, with the stabilized blood kept at room temperature overnight. The data show that there was effectively no difference between these samples and the control frozen at -20° immediately after stabilization, suggesting that there is little-to-no degradation of RNA during overnight ambient temperature storage in remote studies. The next set of samples were exposed to varying temperatures following the initial incubation for 6 hours (Condition 2, Figure 2.2). Samples from this condition simulate stabilized blood that sits at a participant's front door prior to pickup. The mean RIN scores ranged from 6.4 to 7.2 across the different temperatures and only one sample at 25°C yielded a RIN of 4.9 and one at 50°C had a RIN of 5.2. We include 25°C as one of the temperature conditions for incubation to capture study participants who either leave their sample to be picked up indoors in an apartment lounge or mail room or choose to drop their sample off at a UPS store. Finally, condition 3 had an additional overnight room temperature incubation as a representation of specimens that do not get picked up the day after sampling. These samples matched closely to condition 2, further

indicating that additional storage at room temperature (up to 38 hours) results in negligible degradation of RNA in homeRNA samples.

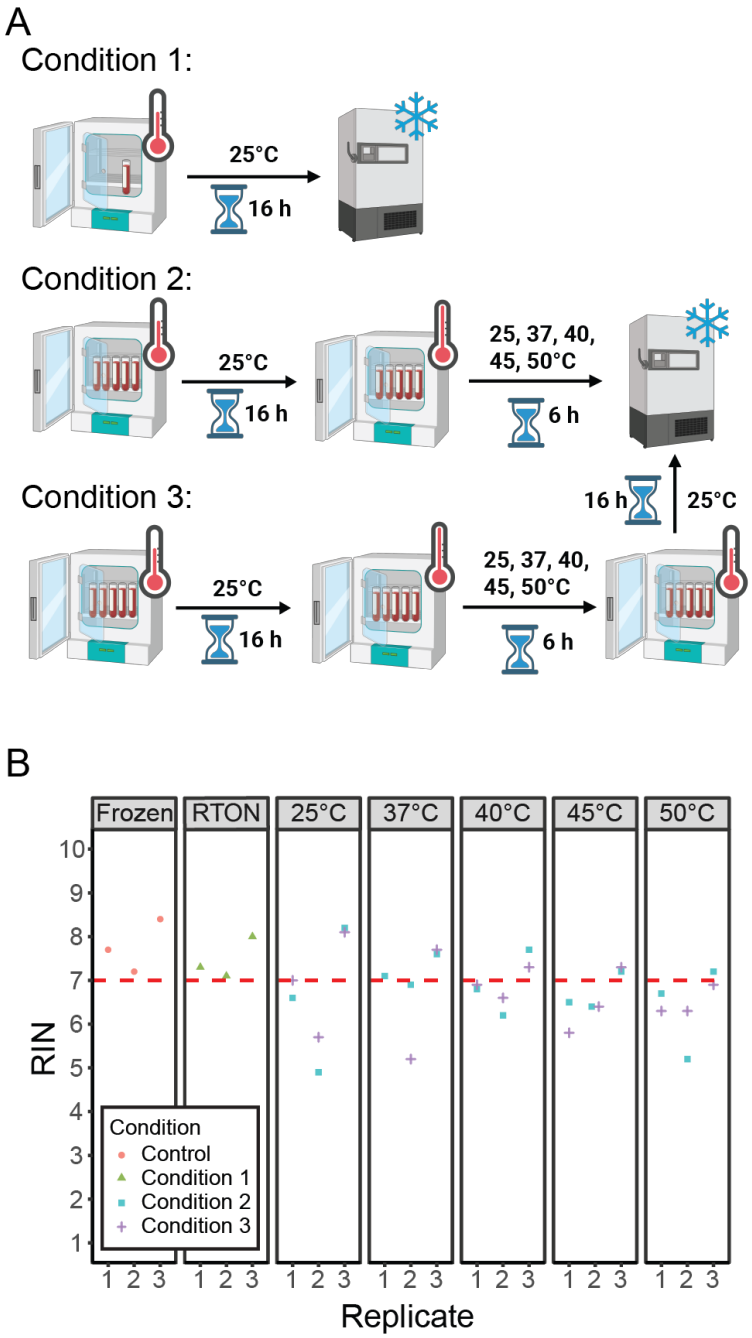

Figure 2.2. Exposure of RNAlater-stabilized whole blood samples to shorter term (<2 days) high temperatures (>37°C). (A) Outline of experimental conditions. One sample was frozen at -20°C immediately after stabilizing with RNAlater to be used as a baseline control. Condition 1 kept one sample at 25°C for 16 hours and then frozen at -20°C; this is referred to as room temperature overnight (RTON). Condition 2 incubated samples at 25°C for 16 hours and then exposed one sample each to 25°C, 37°C, 40°C, 45°C, and 50°C for 6 hours. Condition 3 incubated samples at 25°C for 16 hours and then exposed one sample each to 25°C, 37°C, 40°C, 45°C, and 50°C for 6 hours. After incubation at the different temperatures, all samples were incubated at 25°C for 16 hours then frozen at -20°C. Created with BioRender.com. (B) Resulting RNA quality of each extracted sample from each condition. Each condition was performed in triplicate. Amongst each replicate, all samples across all conditions were

extracted in the same batch, resulting in an extraction batch with 12 samples (immediately frozen, one sample from condition 1, 5 samples from condition 2, and 5 samples from condition 3).

2.5 Shipping of RNAlater-Stabilized Blood Across United States with Continuous Temperature Monitoring

While the in-lab temperature-controlled experiments capture a large range of time scales, we were interested in monitoring continuous temperature fluctuations that would be experienced in real shipping conditions (e.g., packages moving from doorstep to shipping vehicle, samples moving between air-conditioned areas to areas without air conditioning, etc.). To this end, we designed an experiment where blood aliquots from a single venous draw were stabilized using the homeRNA kit and shipped out to volunteers across 14 different states. These states are colored green in Figure 2.3A and include Washington (WA), New Mexico (NM), North Carolina (NC), Minnesota (MN), Maine (ME), Massachusetts (MA), Kansas (KS), Illinois (IL), Georgia (GA), Colorado (CO), California (CA), Arizona (AZ), Hawaii (HI), and Nebraska (NE). These samples were shipped at the same time during the summer in August of 2022. While temperature spikes or dips are possible year round, we chose the summer to ship these samples to ensure the samples would experience elevated temperatures. We aimed to include a diverse set of states reaching most regions of the U.S. including those located in regions with warm summers (HI, CA, AZ, GA, and NC). The locations include both urban and rural locations, reflected by the Rural-Urban Commuting Area (RUCA) codes (Table C1). Additionally, our samples were also shipped to an island in Hawaii, which requires longer shipping times to and from Seattle, Washington (location of study lab) than locations within the contiguous U.S.

Each package also included an Elitech RC-5+ continuous temperature probe (Figure C1) that recorded the temperature from the moment the samples were prepared for shipment in the lab, through the shipping process to the volunteer, to the samples being returned to our lab. These temperature data are plotted in Figure C2 and summarized in Table C1. The maximum temperature spike we observed was 45.1°C in the sample shipped to NE, which was the most rural of our shipping locations (highest RUCA score) and took 7 days to be returned to the

lab. Although HI did not have as high of a RUCA score, the samples shipped to this location took the longest to return to the lab (8 days). Most shipping locations (10 out of the 14 states) took 3 days to return to the lab.

Further, the RIN values from different locations followed a similar trend as the stabilized blood in the temperature-controlled experiments, where longer times and exposure to higher temperatures resulted in lower RIN values. Notably, AZ, NE, and HI had the lowest mean RINs (6.1, 5.5, and 6.2, respectively) and were the locations that took the longest to be returned to the lab. In addition, all three of these states reached temperatures greater than 40°C and spent the longest time above 30°C, with the samples sent to NE spending 55 h above 30°C. Conversely, locations such as CA had similar exposure to a maximum temperature of 44.4°C but had a RIN of 7.0. Even though the maximum temperatures were the same, the longer times in transit and higher median temperatures are the likely causes for the lower RIN from the NE samples.

It is also worth noting that long-term storage at room temperature results in some RNA degradation. The In-Lab sample, which was kept in a 25ºC incubator for the duration of the experiment (8 days), exhibited RINs similar to those of AZ, HI, and NE (that took 7 – 8 days to return). The In-Lab sample also had lower RINs than the other samples from shipped locations (e.g., WA, NM, NC, MN) that were returned within 4 days. This result indicates that RNAlater-stabilized blood samples will undergo some RNA degradation even at room temperature after prolonged periods of time and also that short-term temperature spikes do not markedly affect RIN.

In the context of remote studies, the data obtained from the real-world shipping experiment is both encouraging and informative. Even the most degraded sample (NE, RIN = 5.5) had RNA of sufficient quality for 3'RNA sequencing (see 3' mRNA-seq section below). Additionally, all samples were shipped in two directions (to the participant and back to the lab), whereas samples collected in remote studies using homeRNA are only shipped from the participants back to the lab. As such, we expect less degradation to occur in other homeRNA studies since the samples will only be shipped in one direction.

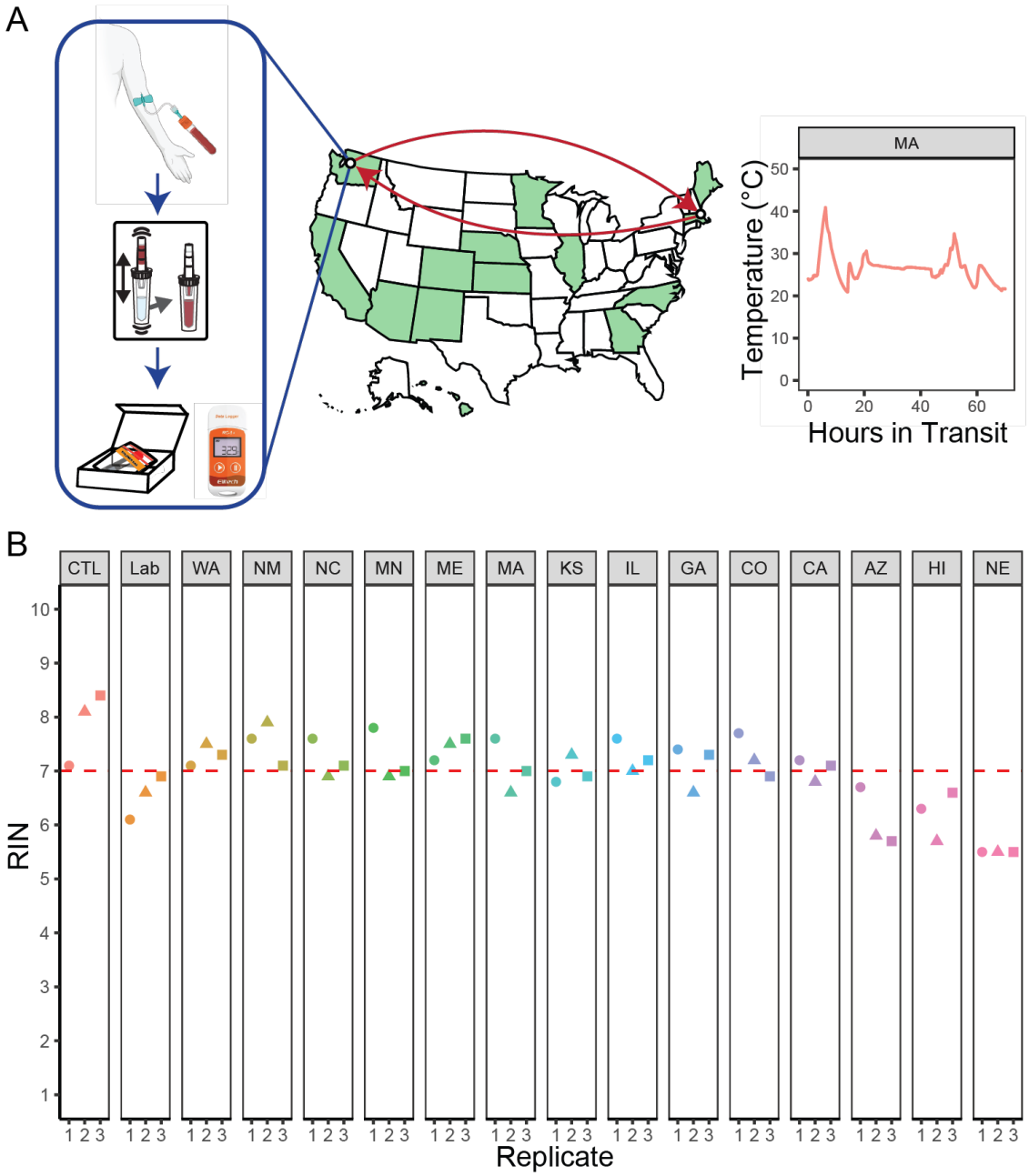

Figure 2.3. All samples across different shipping locations had RINs suitable for RNA sequencing. (A) Blood from a venous draw from a single participant was aliquoted into the Tasso blood collection tube and stabilized using the homeRNA kit in triplicates and shipped to 14 different U.S. states. The homeRNA kits were packaged as in our other homeRNA studies within a cardboard box and mailer bag, and a continuous temperature probe was directly attached to the samples [9–11, 20]. Once returned to the lab, the samples were kept frozen until ready for further processing. The RNA was extracted from the blood and tested for integrity. Created with BioRender.com. (B) The resulting RINs are plotted categorized by shipping location. The twoletter abbreviations

in the figure correspond to U.S. state abbreviations (see text above). The shape of the data point (circle, triangle, square) corresponds to the extraction batch. Control condition (CTL) was immediately frozen after stabilization, while the in-lab condition (Lab) was kept in a 25℃ incubator until frozen at -20℃ at the time the last sample (Hawaii, HI) was received (8 days).

2.6 3' mRNA Sequencing of Real-World Shipping Experiment

Finally, we look for selective degradation of RNA fragments from 3'mRNA-seq data. Since all samples in the shipping experiment came from a single blood draw, we can assume that any differences in the measured transcriptome can be attributed to shipping-related degradation. To probe this question further, we selected 20 samples to perform 3' mRNA-seq analysis, including all samples from extraction batch 3. Additionally, we chose to sequence all three control samples to establish a baseline as well as all three samples from the location with the most degraded samples (NE).

Our first approach is to reference the sequencing data of the samples against a well-characterized immune response signature from the BloodGen3 module repertoire [37, 38]. Each BloodGen3 module consists of a set of genes ranging from 12 to 169 with an average of 37.1 genes per module; these modules have been previously associated with responses to immune-modifying therapies or pathogenic processes [37]. By calculating enrichment scores (which summarizes the prevalence of a given gene in a module) using the gene set variation analysis (GSVA) method for each of the 382 modules across all samples, we aimed to break down the results and provide a more granular understanding of the immune response landscape [39]. Principal component analysis (PCA) of these scores revealed minimal clustering patterns based on shipping location (Figure 2.4A), indicating a lack of significant variability within the shipped samples. While the In-Lab samples (kept at 25ºC incubator for the duration of the experiment, 8 days) did exhibit some clustering, the Control sample (immediately frozen) clustered with the rest of the shipping locations. It is possible that the clustering of the In-Lab samples can be attributed to the fact that they experienced no temperature fluctuations nor agitation (Figure C2B). However, it is important to note that the overall variability captured by the PC1 is only 16.25% and PC2 is 8.97% and that the

distribution of the points is relatively tight. The proximity of the Control to the rest of the samples also indicates that there is very little variability arising from the shipping process. The heatmap visualization of enrichment scores across various modules and samples (Figure 2.4B) and the focused analysis of three key module groups (module aggregates A28, A35, and A37, corresponding to interferon, inflammation and circulating erythroid cells signatures, respectively) (Figure 2.4C) further supported this observation, with only minor variations in enrichment scores across individual samples. These findings highlight the relative homogeneity of our samples and indicate little variation depending on the sample location.

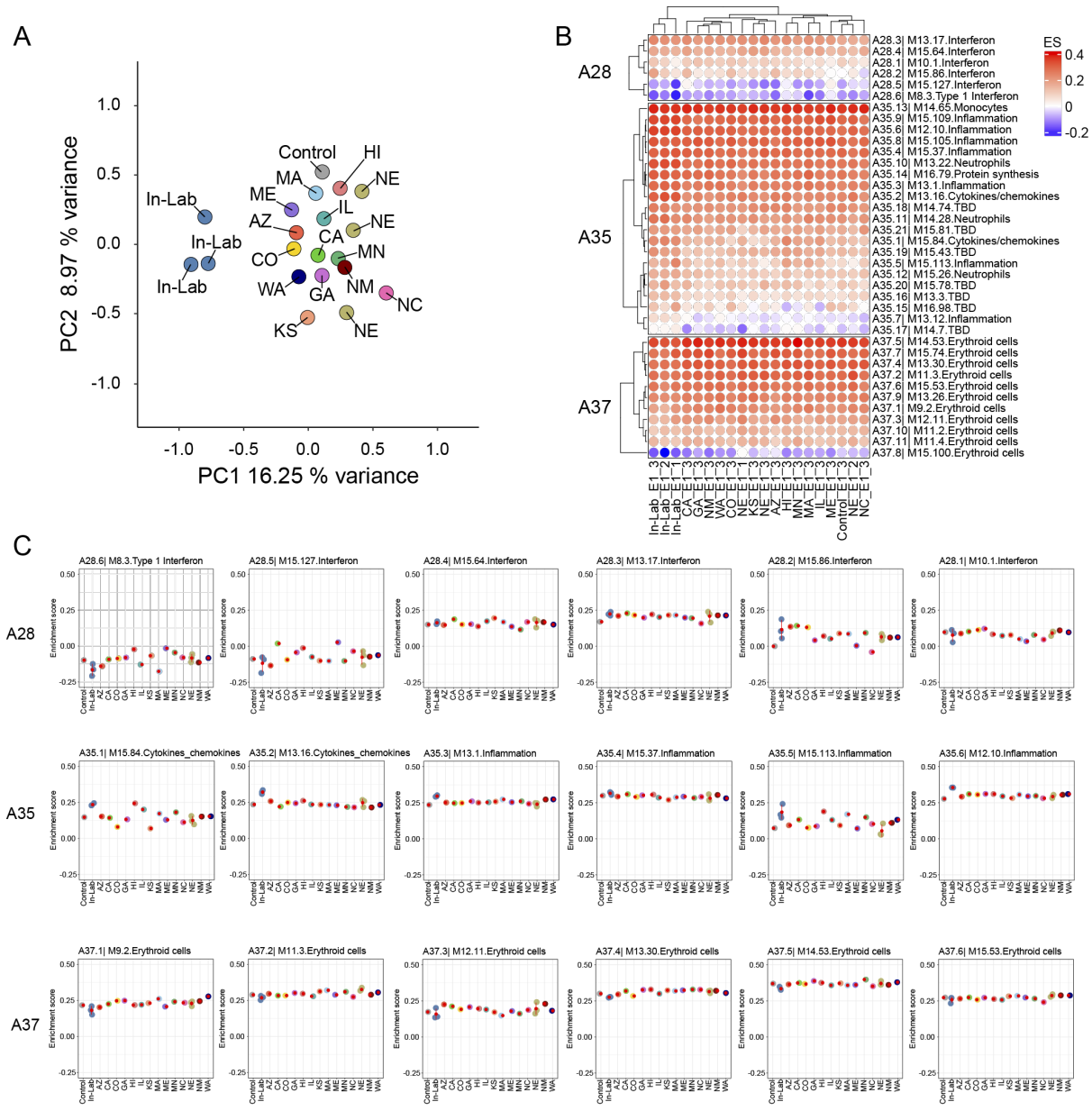

Figure 2.4. Analysis of Module Enrichment Scores in Samples. (A) PCA of sample data by location. This PCA plot provides a visualization of the data variance, with each point representing a sample's projection on the two principal components labeled based on sample condition (shipping location, control (frozen immediately), or in-lab sample (stored at 25°C for 8 days)). (B) Heatmap of enrichment scores across conditions. Enrichment scores for various biological modules are depicted in a heatmap format, with rows representing different modules (e.g., interferon response, inflammation) and columns representing individual samples. The color gradient indicates the level of enrichment from decreased (blue) to increased (red). (C) Detailed module enrichment scores for groups A28, A35, and A37. The dot plots show the enrichment scores for selected modules within the three groups. Individual sample scores are plotted on the x-axis against their corresponding enrichment score on the y-axis.

Further, to contextualize the observed variation in gene expression in our samples, we include results from a previously published COVAX study, which investigated the immune response to COVID-19 mRNA vaccine [42]. By comparing the enrichment scores of six interferon response modules (M8.3, M10.1, M13.17, M15.64, M15.86, and M15.127, comprised in the module aggregate A28) at different timepoints before and after vaccination, we can observe the differences in signal variability occurring due to a well-controlled biological signal. The dot plot in the supporting information (Figure C4) demonstrates a clear increase in interferon module enrichment scores following vaccination, with peak responses observed at days 2 and 3. This distinct pattern of vaccine-induced immune activation serves as a reference point for interpreting the variability observed in our samples (labeled as UW in Figure C4). Assuming that the stabilized blood exhibits minimal DNA transcription ex vivo, any large difference in transcript counts can be attributed to degradation during the shipping process. The tight clustering of our samples compared to the large differences in the COVAX data suggests that the variability introduced by temperature exposure during shipping is much smaller in magnitude than the changes induced by a biological signal, such as an immune response elicited by vaccination. Taken together, this indicates that there is minimal RNA degradation of homeRNA-stabilized samples owing to variable exposure to temperature and shipping times.

We performed additional analyses that confirmed that there was minimal differential degradation barring two genes (MMP9 and ADGRG3, Figure B5). We also considered how time in transit and time spent above 30ºC impact RIN (Figures C6 and C7) and found minimal effects on RNA integrity (see Supplementary Text in Appendix C for more details).

In summary, based on our 3' mRNA-seq dataset, there is little evidence that differences in shipping conditions result in consistent changes in the measured mRNA expression levels in blood RNA from a single donor. We expect that samples in other remote studies using homeRNA that have similar RINs will also exhibit negligible transcript-specific degradation from variable shipping conditions as measured by 3' mRNA-seq. It is important to note that traditional RNA-seq methods may yield different results owing to differences in read depth and genes detected. Further work is needed to conclusively determine if the homeRNA system is completely resilient to preferential degradation of transcripts when exposed to variable shipping conditions, however, the current work shows promising results.

2.7 Conclusion

We expand upon existing literature on RNA stability in cold storage conditions and dried blood spots by investigating the effects of variable time and temperature exposure on RNAlater-stabilized samples, with a systematic study on the effects of high temperature exposure and shipping time on liquid blood samples. In the temperature-controlled experiments, we found that longer storage times (4 and 8 days) at high temperatures (>37°C) resulted in lower RIN values, while shorter exposures (<6 hours) to high temperatures resulted in minimal RNA degradation. We also used homeRNA-stabilized blood samples in a real-world shipping experiment. All shipped samples were sequenced using Lexogen's QuantSeq 3' mRNA technology, with minimal differences in the transcriptome measured.

This study illustrates the utility of the homeRNA platform in high temperature settings. It also serves as a guide for other researchers who aim to conduct remote sampling studies that use RNA as a readout and rely on shipping via courier service for sample retrieval. Our findings strongly suggest that there is limited preferential

degradation of transcripts in the shipping process and, as such, give us confidence in interpreting transcriptomic data from homeRNA-stabilized whole blood samples. These results will inform ongoing homeRNA studies and will be critical for future study design. Future work will use larger sample sizes, longer read depth sequencing such as total RNA-seq to further substantiate these findings, and work in low- and middle-income countries (LMICs) with different courier methods and climates.

2.8 Materials and methods

2.8.1 Participant Recruitment and Demographics

Healthy adult volunteers (18 years or older) were recruited in Seattle, Washington via word of mouth under a protocol approved by the University of Washington Institutional Review Board study number STUDY00014133. Written informed consent was obtained from all participants.

2.8.2 Exposure to Longer Term (2, 4, and 8 days) High Temperatures (>37°C)

A venous blood draw was performed and promptly stabilized with RNAlater at the manufacturer's recommended ratio (2.6 mL RNAlater per 1 mL blood). For the first biological replicate, blood was acquired via venipuncture from Bloodworks and stabilized within 1-2 hours. For the second and third replicate, blood was collected via venipuncture in lab under study number STUDY00014133. After stabilization with RNAlater, the stabilized blood was aliquoted in 1.8 mL samples to replicate the maximum volume of homeRNA (0.5 mL blood and 1.3 mL RNAlater). After stabilization, each sample was stored according to the following conditions: (1) immediately incubated at set temperatures (25°C, 37°C, 40°C, 45°C, and 50°C) for 2, 4, and 8 days, (2) kept at 4°C for 24 hours then incubated at set temperatures (25°C, 37°C, 40°C, 45°C, 50°C) for 2, 4, and 8 days, and (3) kept at 25°C for 24 hours then incubated at set temperatures (25°C, 37°C, 40°C, 45°C, 50°C) for 2, 4, and 8 days. Each condition had a corresponding control: (1) frozen immediately at -20°C, (2) incubated at 4°C for 24 hours then frozen at -20°C, (3) incubated at 25°C for 24 h then frozen at -20°C. One sample at each temperature and condition was removed after 2, 4, and 8 days such that there was a single replicate for each temperature and condition on each day of removal. At these timepoints, the blood was moved from the incubators to the -20°C freezer until ready

for RNA isolation. For each condition, RNA from the stabilized blood samples was extracted based on timepoints, resulting in three total extraction batches based on day removed after high temperature exposure (day 2, 4, and 8) with six samples in each batch (condition control, 25℃, 37℃, 40℃, 45℃, and 50℃). The experiment was repeated two more times for a total of three replicates from two donors (replicate 2 and 3 from the same donor, with the blood drawn on separate days).

2.8.3 Exposure to Shorter Term (<2 days) High Temperatures (>37°C)

A venous blood draw was performed in lab under study number STUDY00014133 and promptly stabilized with RNAlater at the manufacturer's recommended ratio (2.6 mL RNAlater per 1 mL blood). The stabilized blood was then aliquoted to 1.8 mL samples. One sample was immediately frozen at -20℃ as the control. The rest of the samples were then treated according to the following conditions: (1) one sample kept at room temperature (25℃) overnight (16 h), then frozen (-20℃), (2) five samples kept at room temperature (25℃) overnight (16 h), moved to incubators of corresponding temperatures (25℃, 37℃, 40℃, 45℃, 50℃) for 6 h, then frozen (-20℃), (3) five samples kept at room temperature (25℃) overnight (16 h), moved to incubators of corresponding temperatures (25℃, 37℃, 40℃, 45℃, 50℃) for 6 h, moved back to room temp (25℃) for 24 h, then frozen (-20℃). This resulted in a total of one replicate for each temperature and condition combination. The stabilized blood samples were stored at -20℃ until ready for RNA isolation and assessment. All 12 samples from each replicate were extracted in one batch. The experiment was repeated two more times for a total of three replicates from three separate blood draws from one donor.

2.8.4 Effects of a Freeze-Thaw Cycle on RNA Stability

For this experiment, blood from a venous blood draw is stabilized using RNAlater according to manufacturer instructions. The blood is then aliquoted into 9 samples which are separated into three conditions: (1) control which is immediately frozen, (2) room temperature (25°C) for 24 h (room temperature overnight, RTON) then frozen, and (3) freeze-thaw (FT) where the samples were frozen immediately after stabilization for 24 h, then kept at room temperature for 24 h before being frozen again. The samples were frozen at -20°C for the duration of the experiment then moved to -80°C until further processing. The experiment was performed two more times for a

total of three replicates. Once the RNA was extracted the samples were tested for RNA integrity using the Agilent 2100 Bioanalyzer.

2.8.5 Shipping of RNAlater-Stabilized Blood Across United States with Continuous Temperature Monitoring

A venous blood draw was performed in the lab under study number STUDY00014133. The blood sample was aliquoted into 48 Tasso-SST tubes at 0.5 mL each. The blood was then stabilized by attaching the Tasso-SST tubes to a custom-designed stabilizer tube with 1.3 mL RNAlater and shaken to mimic blood stabilization in homeRNA kits. The remaining blood was stabilized in bulk (2.6 mL RNA later per 1 mL blood), aliquoted into 1.8 mL samples, and immediately frozen at -20℃ as controls. The 48 homeRNA-stabilized samples were then placed in 50 mL conical tubes fitted with custom adapters as described in Haack et al. [9]. The 50 mL tubes were then adhered together using tape in sets of three such that three technical replicates would be sent to each shipped location. Each set had an RC-5+ continuous temperature monitor (Elitech) directly attached with lab tape (see Figure B1A), with temperatures being recorded every 2 minutes.

These constructs were then placed in a biohazard bag and into the box used for homeRNA kits (see Haack et al., 2021) prior to being packaged into a UPS LabPak. The full setup is shown in Figure C1. The complete packages were shipped to volunteers in 14 different states across the United States via UPS. The states included: Washington (WA), New Mexico (NM), North Carolina (NC), Minnesota (MN), Maine (ME), Massachusetts (MA), Kansas (KS), Illinois (IL), Georgia (GA), Colorado (CO), California (CA), Arizona (AZ), Hawaii (HI), and Nebraska (NE). One additional package was kept in a 25℃ incubator (In-Lab/Lab). Upon delivery, the volunteers were instructed to keep the packages inside overnight at room temperature and adhere the return labels to the package. For pickup, most volunteers left the package outside on the front porch, two volunteers left the package indoors in a mailroom, and one volunteer directly handed the package to the UPS courier. Most samples were picked up within 1 – 2 days from the volunteer and shipped back to the lab. Shipping return times ranged from overnight to 4 days after pickup. Upon return to the lab, the packages were immediately placed into a -20℃ freezer. Once all the packages were returned, the samples that were kept in the lab in a 25℃ incubator were also frozen at -20℃. All samples were stored at -20℃ until ready for RNA isolation. RNA was extracted from all

shipped homeRNA-stabilized blood samples in three extraction batches, with one technical replicate from each shipping location and in-lab baseline samples (immediately frozen and constant 25℃ sample), resulting in 16 samples extracted in each batch.

2.8.6 Visualization of Temperature Over Time Across Shipping Locations

The data from the temperature probe directly attached to the samples is assumed to have readings closest to the "true" temperature the samples would experience in transit and only the data from this probe are visualized and used for downstream analysis. Since the temperature probes were not stopped until all packages arrived, the data needed to be filtered prior to graphing. The data were filtered using the R dplyr package such that the time did not exceed 200 hours (close to the maximum time for the last sample returned to lab) and was above 10°C given that the lowest temperature reading prior to being frozen was closer to 20°C for all samples. The temperature was then plotted against time (hours) to illustrate the variability in shipping process and temperature fluctuations.

RNA Isolation and Assessment of total cellular RNA integrity and yield

For all samples, total cellular RNA was isolated using the Ribopure Blood RNA Isolation Kit (Thermo Fisher) according to manufacturer's protocol and eluted in two 50 µl aliquots. Isolated RNA was stored at -80°C until ready for further analysis. RNA Integrity number (RIN) values were obtained on a Bioanalyzer 2100 (Agilent) from the first 50 µl elution. The Agilent 2100 Bioanalyzer uses proprietary, built-in software to calculate RIN values, which is based on the relative peak height and shape of the 18S and 28S rRNA fragments in the resulting electropherogram after separation. All samples were assessed using the RNA 6000 Nano Kit (Agilent). For the in lab temperature-controlled experiments, RNA concentration was measured from the first 50 µl elution aliquot using a Cytation 5 Multi-Mode Reader (Agilent Biotek Instruments) with a Take3 Micro-Volume Plate at the wavelengths of 260, 280, and 320 nm. For the field experiments, RNA concentration was measured from the first 50 µl elution using a Qubit 4 Fluorometer using the RNA High Sensitivity (HS) Assay kit (Invitrogen).

RNA Sequencing and Analysis

2.8.7 3' mRNA-seq of Real-World Shipping Experiment

After isolation the samples were tested for RIN scores on the Agilent 2100 Bioanalyzer (see Assessment of total cellular RNA integrity and yield section above and Figure C8 for resulting electropherograms). A 100 ng of total RNA from 20 selected samples were sequenced by the Lexogen facility (Vienna, Austria) using 3' mRNA-sequencing technology (Lexogen QuantSeq 3' mRNA-seq V2 Library Prep Kit FWD with UDI) on an Illumina NextSeq 2000 instrument according to the manufacturers' standard instructions. The 16 samples from the third batch were selected for sequencing as the third batch had the greatest proportion of the median RIN values amongst the three replicates from each location. Additionally, all three replicates from the baseline sample (frozen immediately after stabilization) and all three replicates from the location with the lowest RIN (NE, mean RIN of 5.5) were sent for sequencing. In total, 20 samples (16 from batch 3, 2 additional from NE, 2 additional from In-Lab) were sent for 3' mRNA sequencing. Prior to library preparation, all samples were treated with Ambion DNase I (Invitrogen) for 10 minutes at 37℃ followed by heat inactivation with EDTA at 75C for 10 minutes. Libraries were prepared using 17 ng of RNA as input. Libraries were amplified for 18 cycles and were quality controlled on a Fragment Analyzer device using the DNF-474 HS NGS Fragment kit (1-6000 bp) (Agilent). The samples were sequenced to a read depth of 5 million reads.

2.8.8 Transcriptomic Analysis of Individual Gene Expression Counts

Summarized counts/genes were imported into R and analyzed using the limma-voom pipeline, which uses conventional weighted linear regression to model the data [40]. The matrix of gene counts was filtered to remove those genes with consistently low expression, and then converted to log counts/million counts, using estimated library sizes calculated using the trimmed mean of M-values (TMM) method [41]. The logCPM values have a dependence between the mean and variance which is estimated as part of the limma-voom pipeline and used to estimate observation-level weights. These weights are used as part of the weighted line regression to adjust for heteroskedasticity. We fit a model that includes time spent at or above 30°C for each sample, the RIN (to adjust for RNA quality), and five surrogate variables computed using the Bioconductor sva package. The surrogate variables are intended to adjust for unobserved variability and have been shown to increase power to detect

differences [43]. We then tested for any apparent changes in gene expression that correlate with exposure to high temperatures.

2.8.9 Module Enrichment Score Calculation

Enrichment scores were calculated using the gene set variation analysis (GSVA) method for each of the 382 transcriptional modules from the BloodGen3 repertoire across all samples [37–39].

2.8.10Principal Component Analysis

Principal component analysis (PCA) was performed on the module enrichment scores to visualize the variance among samples, labeled by their condition (shipping location, in-lab sample, or control).

2.8.11 Heatmap Visualization

Module enrichment scores were represented in a heatmap format, with rows corresponding to different modules associated with biological processes such as interferon response and inflammation, and columns representing individual samples. The color gradient of the heatmap indicates the level of enrichment, ranging from decreased (blue) to increased (red).

2.8.12 Dot Plot Visualization

For a more detailed analysis, we focused on specific module groups (A28, A35, and A37) and plotted their enrichment scores using dot plots. The x-axis represents individual samples, while the y-axis denotes the corresponding enrichment score for each sample within the selected modules.

2.8.13 Interferon Response Kinetics Analysis

We used data from the COVAX study (Rinchai et al [42]), to illustrate the kinetics of six interferon response modules (M8.3, M10.1, M13.17, M15.64, M15.86, and M15.127) at different timepoints before and after administration of COVID-19 mRNA vaccine [42]. Enrichment scores for these modules were plotted for each sample across the various timepoints.

2.9 References

1. Maass, K. F. *et al.* Leveraging patient-centric sampling for clinical drug development and decentralized clinical trials: Promise to reality. *Clinical and Translational Science* **15**, 2785–2795 (2022).
2. Harahap, Y., Diptasaadya, R. & Purwanto, D. J. Volumetric Absorptive Microsampling as a Sampling Alternative in Clinical Trials and Therapeutic Drug Monitoring During the COVID19 Pandemic: A Review. *Drug Design, Development and Therapy* **14**, 5757–5771 (2020).
3. Fuller, G. *et al.* Feasibility of Patient-Centric Remote Dried Blood Sampling: The Prediction, Risk, and Evaluation of Major Adverse Cardiac Events (PREMACE) Study. *Biodemography and Social Biology* **65**, 313–322 (2020).
4. Weiner, M. W. *et al.* Increasing participant diversity in AD research: Plans for digital screening, blood testing, and a community-engaged approach in the Alzheimer's Disease Neuroimaging Initiative 4. *Alzheimer's & Dementia* **19**, 307–317 (2023).
5. Koulman, A. *et al.* The development, validation and application of remote blood sample collection in telehealth programmes. *Journal of Telemedicine and Telecare* **30**, 731–738 (2024).
6. Buck Louis, G. M. *et al.* Designing prospective cohort studies for assessing reproductive and developmental toxicity during sensitive windows of human reproduction and development – the LIFE Study. *Paediatric and Perinatal Epidemiology* **25**, 413–424 (2011).
7. Garcia-Beltran, W. F. *et al.* Remote Fingerstick Blood Collection for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Antibody Testing. *Archives of Pathology & Laboratory Medicine* **145**, 415–418 (2020).
8. Laven-Law, G. *et al.* Hot Mail: Temperature Exposure during Mail Return of an Immunochemical Fecal Occult Blood Test. *Clinical Chemistry* **69**, 615–626 (2023).
9. Haack, A. J. *et al.* homeRNA: A Self-Sampling Kit for the Collection of Peripheral Blood and Stabilization of RNA. *Analytical Chemistry* **93**, 13196–13203 (2021).

10. Lim, F. Y. *et al.* Longitudinal home self-collection of capillary blood using homeRNA correlates interferon and innate viral defense pathways with SARS-CoV-2 viral clearance (2023).
11. Lim, F. Y. *et al.* homeRNA self-blood collection by exposed close contacts enables high-frequency temporal profiling of the pre-symptomatic host immune kinetics to respiratory viral infection (2023).
12. Donohue, D. E. *et al.* Gene expression profiling of whole blood: A comparative assessment of RNA-stabilizing collection methods. *PLOS ONE* **14**, e0223065 (2019).
13. Opitz, L. *et al.* Impact of RNA degradation on gene expression profiling. *BMC Medical Genomics* **3**, 36 (2010).
14. Gallego Romero, I., Pai, A. A., Tung, J. & Gilad, Y. RNA-seq: impact of RNA degradation on transcript quantification. *BMC Biology* **12**, 42 (2014).
15. Houseley, J. & Tollervey, D. The Many Pathways of RNA Degradation. *Cell* **136**, 763–776 (2009).
16. Micke, P. *et al.* Biobanking of fresh frozen tissue: RNA is stable in nonfixed surgical specimens. *Laboratory Investigation* **86**, 202–211 (2006).
17. Florell, S. R. *et al.* Preservation of RNA for Functional Genomic Studies: A Multidisciplinary Tumor Bank Protocol. *Modern Pathology* **14**, 116–128 (2001).
18. Chowdary, D. *et al.* Prognostic gene expression signatures can be measured in tissues collected in RNAlater preservative. *The Journal of molecular diagnostics: JMD* **8**, 31–39 (2006).
19. Kruse, C. P. S., Basu, P., Luesse, D. R. & Wyatt, S. E. Transcriptome and proteome responses in RNAlater preserved tissue of Arabidopsis thaliana. *PLOS ONE* **12**, e0175943 (2017).
20. Brown, L. G. *et al.* At-home blood collection and stabilization in high temperature climates using homeRNA. *Frontiers in Digital Health* **4**, 903153 (2022).
21. Huang, L.-H. *et al.* The effects of storage temperature and duration of blood samples on DNA and RNA qualities. *PLOS ONE* **12**, e0184692 (2017).
22. Das, K. *et al.* Stabilization of Cellular RNA in Blood During Storage at Room Temperature: A Comparison of Cell-Free RNA BCT® with K3EDTA Tubes. *Molecular Diagnosis & Therapy* **18**, 647–653 (2014).

23. Tsui, N. B., Ng, E. K. & Lo, Y. D. Stability of Endogenous and Added RNA in Blood Specimens, Serum, and Plasma. *Clinical Chemistry* **48**, 1647–1653 (2002).

24. Kim, S. J. *et al.* Effects of Storage, RNA Extraction, Genechip Type, and Donor Sex on Gene Expression Profiling of Human Whole Blood. *Clinical Chemistry* **53**, 1038–1045 (2007).

25. Sarathkumara, Y. D. *et al.* The Effect of Tropical Temperatures on the Quality of RNA Extracted from Stabilized Whole-Blood Samples. *International Journal of Molecular Sciences* **23**, 10609 (2022).

26. Heneghan, N., Fu, J., Pritchard, J., Payton, M. & Allen, R. W. The effect of environmental conditions on the rate of RNA degradation in dried blood stains. *Forensic Science International: Genetics* **51**, 102456 (2021).

27. Beekman, J. M. *et al.* Recovery of microarray-quality RNA from frozen EDTA blood samples. *Journal of Pharmacological and Toxicological Methods* **59**, 44–49 (2009).

28. Muth, D. C., Powell, B. H., Zhao, Z. & Witwer, K. W. miRNAs in platelet-poor blood plasma and purified RNA are highly stable: a confirmatory study. *BMC Research Notes* **11**, 273 (2018).

29. Mueller, O., Lightfoot, S. & Schroeder, A. RNA integrity number (RIN)–standardization of RNA quality control. (2016).

30. Schroeder, A. *et al.* The RIN: an RNA integrity number for assigning integrity values to RNA measurements. *BMC Molecular Biology* **7**, 3 (2006).

31. Jahn, C. E., Charkowski, A. O. & Willis, D. K. Evaluation of isolation methods and RNA integrity for bacterial RNA quantitation. *Journal of Microbiological Methods* **75**, 318–324 (2008).

32. Sigurgeirsson, B., Emanuelsson, O. & Lundeberg, J. Sequencing Degraded RNA Addressed by 3' Tag Counting. *PLoS ONE* **9**, e91851 (2014).

33. Hitzemann, R. *et al.* Genes, behavior and next-generation RNA sequencing. *Genes, Brain and Behavior* **12**, 1–12 (2013).

34. Puchta, M., Boczkowska, M. & Groszyk, J. Low RIN Value for RNA-Seq Library Construction from Long-Term Stored Seeds: A Case Study of Barley Seeds. *Genes* **11**, 1190 (2020).

35. Johnson, M. T. J. *et al.* Evaluating Methods for Isolating Total RNA and Predicting the Success of Sequencing Phylogenetically Diverse Plant Transcriptomes. *PLOS ONE* **7**, e50226 (2012).

36. Li, S. *et al.* Multi-platform assessment of transcriptome profiling using RNA-seq in the ABRF next-generation sequencing study. *Nature Biotechnology* **32**, 915–925 (2014).

37. Altman, M. C. *et al.* Development of a fixed module repertoire for the analysis and interpretation of blood transcriptome data. *Nature Communications* **12**, 4385 (2021).

38. Rinchai, D. *et al.* BloodGen3Module: blood transcriptional module repertoire analysis and visualization using R. *Bioinformatics* **37**, 2382–2389 (2021).

39. H¨anzelmann, S., Castelo, R. & Guinney, J. GSVA: gene set variation analysis for microarray and RNA-Seq data. *BMC Bioinformatics* **14**, 7 (2013).

40. Law, C. W., Chen, Y., Shi, W. & Smyth, G. K. voom: precision weights unlock linear model analysis tools for RNA-seq read counts. *Genome Biology* **15**, R29 (2014).

41. Robinson, M. D. & Oshlack, A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biology* **11**, R25 (2010).

42. Rinchai, D. *et al.* High–temporal resolution profiling reveals distinct immune trajectories following the first and second doses of COVID-19 mRNA vaccines. *Science Advances* **8**, eabp9961 (2022).

43. Leek, J. T. & Storey, J. D. Capturing Heterogeneity in Gene Expression Studies by Surrogate Variable Analysis. PLOS Genetics 3, e161 (2007).

Chapter 3 | To the homeRNAmax: Developing an Improved Blood Self-Collection and Stabilization Platform for Remote Transcriptomic Studies[2]

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Abstract: Shifting human subjects research from research sites to participants' homes removes barriers to participation. Previously, we developed homeRNA, a kit for stabilization of RNA in self-collected blood using a custom-engineered tube containing RNA stabilizer fluid. The stabilized RNA is extracted and used for downstream gene expression analysis. Here, we introduce homeRNAmax, which improves our original design by interfacing with a commercially available blood collection tube (BD Microtainer), allowing homeRNAmax to be used with any blood collection method that uses this tube and doubling the possible sample volume that can be collected and stabilized compared to the original homeRNA. Through a pilot study (n=19 participants), we show that homeRNAmax (with the Tasso+ blood collection device) produces RNA samples of sufficient quality (mean

[2] This chapter contains collaborative work. FS, ME, ST, EB, ABT, and AJH contributed to the design of the homeRNAmax platform. CAC, KML, AOO generated CAD versions of the original device and helped with 3D printing. FS, ME, and LAK prototyped and tested the homeRNAmax devices. ME generated designs for injection molding. FS and ME conducted experiments for spill-proof feature. FS conducted the implementation study with human subjects including recruitment, consenting, developing and implementing REDCap, and participant communications.. IHR and DWHC helped with shipping and receiving of samples. DWHC contributed to collection of evaporation data. FS, ME, and SHN extracted RNA from homeRNAmax-stabilized blood samples and measured RNA yield and RIN. JB developed theory of spill proof feature. CCC, KC, JA, SE, RHH, JUS conducted the clinical trial. KNA and IHR oversaw the IRB approval process, overall human subjects regulatory design, and compliance in study implementation. FS, ME, and LAK contributed to making of the figures. FS, ME, and ABT contributed to writing and editing of the manuscript.

RIN=7.8) and yield (mean yield=1.93 μg) for downstream analysis and can reach participants across the United States, who generally (n=17/19) found the homeRNAmax kit easy to use. We also present RNA integrity data from an ongoing longitudinal clinical study using homeRNAmax in rheumatology (mean RIN=7.1). A key aspect of the homeRNA and homeRNAmax platforms is a fluidic feature that prevents the RNA stabilizer from spilling, for which we developed a theoretical model. In brief, fluid in the tube is suspended due to a balance of pressures; an increase in air volume within the tube reduces the air pressure above the fluid, creating a small vacuum, and preventing fluid leakage. Overall, we show that homeRNAmax is a user-friendly, effective tool for remote blood RNA stabilization.

3.1 Introduction

Gene expression analyses using RNA are established methods of monitoring health and disease over time, allowing for immunological studies of infectious diseases and cancers[1–5]. Blood is commonly used for such studies, but blood collection has historically presented challenges for participation in transcriptomics studies because venous blood draws have to be performed by a trained phlebotomist in a clinic, leading to barriers caused by scheduling, aversion to needles, or proximity to a clinic. Shifting sample collection from a clinic to a participant's home therefore addresses barriers to participation in transcriptomics studies.

Dried blood spots (DBS) are an established method of blood sampling for biomolecule analysis and are an integral tool for remote studies[6–8]. DBS are a very important part of the remote study toolkit[9–13]; however, degradation of RNA after collection–especially in elevated temperature conditions–can limit accurate gene expression profiling[8,14,15]. Hence, we saw the need for a technology that is robust to elevated temperatures and varied shipping conditions.

To this end, we previously developed a custom-designed platform that facilitates the stabilization of RNA in whole blood samples collected at home. The homeRNA kit[16] makes frequent longitudinal sample collection logistically less challenging compared to clinic-based studies by using a lancet-based upper-arm blood collection device (Tasso, Inc.), removing the need for a trained phlebotomist and the need to milk the collection site (as would be required with DBS sampling using a finger stick). The collected blood is stabilized using a custom-

engineered stabilizer tube which holds RNA*later*, a commercially available RNA stabilizing agent. homeRNA has been successfully used to investigate gene expression related to SARS-CoV-2 infection[17,18] and wildfire exposure[19], has captured a well-established inflammatory response comparable to that observed in venous blood samples[20], and has been shown to be robust in some high temperature climates, shipping scenarios[21,22], and extended temperature exposure through simulated lab experiments[22]. While our lab uses RNA*later* (ThermoFisher Scientific) to stabilize RNA in blood, homeRNA can be used to stabilize other bioanalytes such as DNA by using a different stabilizer. homeRNA was designed to interface specifically with the Tasso-SST blood tube, as selection of upper-arm blood collection devices was limited at the time of its invention. The recent discontinuation of the Tasso-SST and replacement with the Tasso+ and Tasso Mini presents an opportunity and a need to update homeRNA for broader usability with blood collection tools and downstream analyses.

Here, we introduce the homeRNAmax platform and characterize the spill-resistant feature of the stabilizer tube with a theoretical model. homeRNAmax improves upon the original design by interfacing with the commercially available BD Microtainer blood collection tube instead of a device-specific tube, allowing homeRNAmax to be used with multiple blood collection devices such as the Tasso+ (Tasso, Inc.), the Tasso Mini (Tasso, Inc.), the TAP® Micro Select (YourBio Health), and the RedDrop One (RedDrop Dx). The BD Microtainer also holds up to 1 mL of blood and is available with many coatings, including dipotassium ethylenediaminetetraacetic acid (K_2EDTA) and lithium heparin, doubling the possible sample volume compared with the original homeRNA and opening up a new range of possibilities for downstream analyses and a range of new study designs to probe questions previously out of reach.

3.2 Experimental Section

3.2.1 Design process for the RNA stabilizer tube

Like the original homeRNA, the RNA stabilizer tube was assembled from three components: 1) a reagent vial to house the liquid stabilizer, 2) an adapter that interfaces both the reagent vial and the BD Microtainer (BD), and 3) a vial closure cap. Preliminary design iterations using the same dimensions of the spill-resistant feature as in the original homeRNA[16], but with an expanded volume to accommodate additional stabilizing fluid, were

generated on Solidworks and 3D printed using a Form 3B 3D printer (Formlabs) and a CADworks ProFluidics 285D 3D printer (CADworks). These preliminary designs were evaluated for leakage and spillage using a simulation of the mechanical agitation of the shipping process; three assembled tubes were filled with 3.6 mL RNA*later* (Thermofisher Scientific), then inverted three times for three seconds each. They were then dropped from approximately three feet three times, and were shaken in a shipping box for 30 seconds. Designs were considered to have "passed" this drop test if minimal spillage occurred in observation (maximum of a few droplets of fluid, approximately 20 μL). Specific features of the stabilizer tube design include a cone nozzle feature in the adapter (Figure 3.1B) to prevent contact between the stabilizer reagent and the user during blood stabilization (the "spill-resistant" fluidic feature), and an aperture at the top with dimensions to allow the BD Microtainer to fit snugly with the homeRNAmax setup without making the removal of the cap difficult for the user. Engineering drawings of the stabilizer tube components can be found in the Supplementary Information (Figure B1).

3.2.2 Design considerations for injection molding of the RNA stabilizer tube

The adapter and the vial components were injection molded out of polycarbonate (PC: Makrolon 2407), and the vial cap was injection molded out of polypropylene (PP: RTP Permastat 100 Anti-static) by Protolabs, Inc. (Maple Plain, MN). Several design considerations were optimized to include features important for the process of injection molding. Briefly, these include 1) Adding at least a 0.5 degree draft to all vertical surfaces in the adapter and the vial to aid in removal from the mold, (the part of the cap that interfaces with the adapter was left undrafted to ensure a tight seal with the adapter), 2) flipping the direction of draft partway down the adapter to allow easier removal of both sides of the mold, 3) thickening of the vial and adapter pieces to allow for ejector pin zones, 4) removing sections of plastic in the cap to prevent warping, and 5) designing the adapter piece to avoid any overhangs, allowing for a two-piece mold. The vial and the adapter pieces were both made of polycarbonate to ensure consistent material shrinkage, and because polycarbonate is a common material for biochemical samples containers. The cap was made out of polypropylene because the added flexibility of the polypropylene mimics the BD Microtainer and helped ensure a tight seal between the cap and the adapter.

3.2.3 Fabrication of the RNA stabilizer tube

For assembly, all components of the stabilizer tube were injection molded by Protolabs, Inc. as described above and cleaned via sonication in 70% ethanol (v/v) for 30 minutes and air dried. The adapter was bonded onto the reagent vial using a UV curing glue (Dymax MD® 1450-M-UR-SC) (Figure B2). Bonded parts were cured for 45 minutes at 405 nm in a Formcure (Formlabs). The stabilizer tube was filled with 3.6 mL of RNA*later* as the stabilizing reagent, capped, and checked for leakage due to bonding defects before distribution to study participants.

3.2.4 Measuring evaporative loss from stabilizer tubes

The stabilizer tube was assembled according to the section above (see *Fabrication of the RNA stabilizer tube*) before being filled with 3.6 mL of RNA*later*. Since the manufacturer recommended ratio is 1 mL of blood to 2.6 mL of RNA*later*, 3.6 mL of RNA*later* was chosen to accommodate any loss due to evaporation or shipping, as well as collected blood volumes above 1 mL, ensuring stabilization in a variety of scenarios. The tubes were sealed with the injection molded caps and their initial mass was recorded. The filled stabilizer tubes were then placed upright and kept on a lab benchtop. The mass of the stabilizer tube was then recorded once per week for a total of 10 weeks (Figure B3). The change in mass from initial mass is calculated and converted to volume, using a density of 1.2125 g/mL for RNA*later*. The resulting volume is then subtracted from 3.6 mL and converted to a percentage, representing the percent RNA*later* loss from evaporation for each tube at each timepoint.

3.2.5 Development of the stabilizer tube theoretical model

The theoretical model was prepared using MATLAB and Microsoft Excel.

For testing the stabilizer tubes for leakage, four stabilizer tubes were filled with 2.00 mL, 4.00 mL, 6.00 mL, and ~6.70 mL (completely filled) with DI water. The height of the fluid within the tube was measured using a caliper, and all the tube nozzles were dried using a Kimwipe. All tubes were then inverted, and the heights of the fluid above the nozzles were measured using a caliper. Tubes were observed for leakage for 10 minutes. Tubes were

considered to have “leaked” if any amount of fluid left the tubes during the observation period, which did not happen in any of the cases (reported in Table S1).

3.2.6 User experience pilot study

This study was IRB approved under University of Washington STUDY00007868. For the pilot study, we recruited participants using Facebook ads. We selected individuals who had not previously seen or used a Tasso device (by self-report) and who were not affiliated in any way with the Bioanalytical Chemistry for Medicine and the Environment (BCME) lab (Theberge lab). Individuals were eligible if they were healthy, adults aged 18 or older, and able to have lancet blood sampling (collected by self-report). Prospective participants were screened for age, gender, race and ethnicity, and state of residence. Balancing these factors to match the greater population of the United States as closely as possible, people who were selected were then sent an email with a link to the informed consent form (ICF). Upon completion of the ICF, participants would be considered as “enrolled” and were instructed to complete a survey with their shipping information. The study kit consisted of (1) instructions for sampling and sample return (Figures S4-S5), (2) a Tasso+, (3) Tasso warming pad, (4) an alcohol wipe, (5) a bandaid, and (6) a homeRNAmax stabilizer tube which was filled with 3.6 mL of RNA*later* on the day of shipment (Figures S6 and Table S2). The kits were packed inside a United Parcel Service (UPS) LabPak alongside another, pre-labeled UPS LabPak for sample return. The packages were sent using the UPS next day air service and the tracking information was directly shared with the participants. Once a package was delivered to a study participant, another email with study instructions (see SI) was sent that included a link to an instructional video prepared by our lab. The participants then completed their sampling one time according to the instructions and completed a short usability survey (See SI). The sample was then sealed back in the Tasso box, placed into the spare LabPak, and kept indoors overnight. The following morning, participants left their samples at the designated pickup location and the study team scheduled a UPS pickup. Once the samples arrived back to the lab, they were stored at -20°C until further processing.

In the pilot study outlined above, participants self-collected blood using the Tasso+ device, which is commercially available and collects up to 1 mL of blood. The Tasso+ device uses BD Microtainers for blood collection. In the

pilot study, we opted to use the K_2EDTA coated BD Microtainer tube to limit blood coagulation prior to stabilization. Participants were instructed to collect blood for five minutes or when the blood reached the collar of the BD Microtainer (1 mL), whichever happened first; see instructions for use (IFU) in the Supplemental Information (Figure B4) for more information. Once the blood was collected, participants connect the BD Microtainer to the stabilizer tube containing 3.6 mL of RNA*later* and shake them to mix the blood and stabilizer.

3.2.7 Longitudinal clinical study

This study was IRB approved under s22-00484 and is reviewed by the New York University (NYU) School of Medicine IRB. We screened and recruited participants through their NYU rheumatologist. Individuals were eligible if they were switching from their first biologic medication or starting a biologic medication for the first time, were aged 12 or older, and had a diagnosis of psoriatic arthritis (PsA) or psoriasis (PsO). Prospective participants also had to be clinically active, as determined by a rheumatologist. At the time of enrollment, done in person, the ICF was reviewed by the clinical research staff with the prospective participant, and a hard copy was signed. A copy of the signed ICF was provided to the participant for their records following the enrollment appointment. During this visit, participants collected two samples using the homeRNAmax kit, one as a practice guided by the study team and one performed on their own. Only one of these samples is processed while the other is banked. Following the two in-clinic samples, participants completed additional samples remotely which were collected on a weekly basis with the homeRNAmax kits. Participants switching medications could be invited to repeat the study for another set of samples. All kits, including those collected on the day of enrollment, were mailed overnight via FedEx to ensure consistency. The study is ongoing and the data presented in this manuscript include samples collected and analyzed through May of 2026.

3.2.8 RNA isolation and quantification

Sample RNA extraction was performed using the blood RiboPure RNA Purification Kit and following the manufacturer suggested protocol. Because homeRNAmax sample volumes exceeded the maximum capacity for this kit, the samples were split into two aliquots and extracted at the same time. Prior to the elution step, these two aliquots were combined into a single sample to have a uniform sample for analysis. The resulting RNA was

then analyzed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) to verify RNA integrity. The RNA yield was measured both on the BioTek Cytation 5 (Agilent Technologies, Inc.). Take3 application, as well as on the Qubit Flex Fluorometer (Thermo Fisher Scientific Inc.) for comparison.

For the longitudinal clinical study, samples were also extracted using the RiboPure RNA Purification Kit, but only half the sample was extracted while the other was banked. The RNA integrity was analyzed using either the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.) or the Agilent TapeStation (Agilent Technologies, Inc.) RNA yield was quantified using the Thermo Fisher NanoDrop spectrophotometer (ThermoFisher Scientific, Inc.). The RNA integrity presented in the main body of the manuscript reflects only the data from the Bioanalyzer (n = 34/44) for ready comparison with our pilot study; the remaining samples (n = 10/44) are shown in Figure B7. As noted in the figure caption, the yields presented in the main manuscript have been doubled as an estimate of total RNA from both aliquots, since only half the sample was extracted and analyzed.

3.3 Results and Discussion

3.3.1 Development of the homeRNAmax stabilizer tube

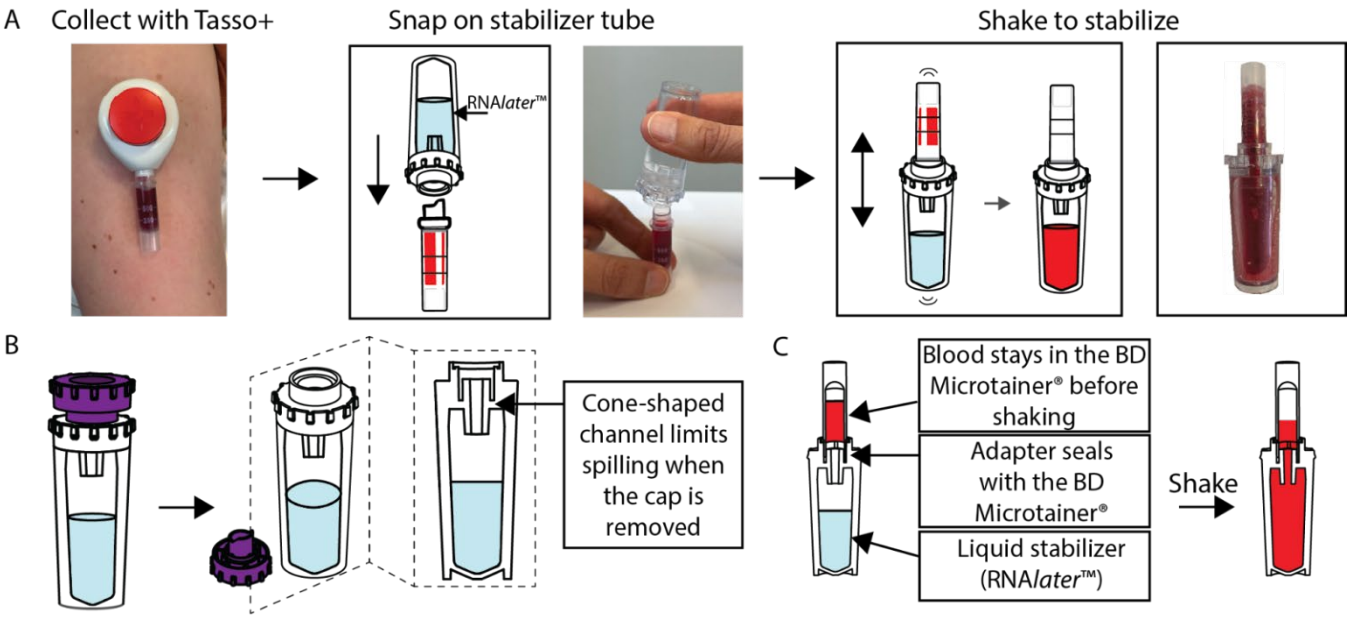

Figure 3.1. Workflow and features of homeRNAmax. A) describes the workflow for RNA stabilization of blood samples using homeRNAmax. To use our system, participants collect blood by following the directions for the Tasso+ device, connecting the BD Microtainer blood tube to the stabilizer tube filled with RNA*later*, then shake the blood into the RNA*later* to stabilize the RNA in the blood sample. B) Schematic of filled stabilizer tube. The features shown here are adapted from our original homeRNA system reported in Haack, Lim et al.[16], with adjustments made to interface with BD Microtainers. C) Cross section schematic of the stabilizer tube interfacing with a blood-filled BD Microtainer before and after shaking to stabilize.

The original homeRNA[16] was designed to accommodate the Tasso-SST blood tube, which could only hold up to 500 µL of blood. Our new design interfaces with the commercially available BD Microtainer tube, which holds

up to ~1 mL of blood. By interfacing with the BD Microtainer, homeRNAmax can be used with the Tasso+, Tasso Mini, and other blood collection devices that use the BD Microtainer as its blood tube, such as the YourBio TAP® Micro Select.

Several design considerations were made to ensure homeRNAmax performs comparably to the original homeRNA[16–18,21,22]. The dimensions of the cuvette had to be adjusted to hold at least 5 mL of fluid to accommodate the new blood volume of 1 mL, the RNA*later* required to stabilize 1 mL of blood, and extra RNA*later* to safeguard against issues such as evaporation or participants collecting additional drops of blood such that the total blood volume is >1 mL (see Experimental Section for more information). The homeRNAmax cuvette can reasonably hold up to ~6 mL of total volume (up to ~5 mL of stabilizer with ~1 mL of sample) as a consideration for applications other than RNA stabilization of blood that might require a larger volume. The homeRNAmax dimensions are also designed such that stabilized samples (i.e., the cuvette and adapter assembly connected to the blood tube) fit in a 50 mL conical vial, providing an easily accessible commercially available method to give additional protection for lab technicians and shipping personnel (although strictly speaking this is beyond the secondary containment required for shipping blood samples, which include only a watertight secondary container with absorbent material and a rigid box) The cuvette and adapter assembly is also short enough for current commercially available plastic pasteur pipettes to reach the bottom, allowing for the entire sample volume to be extracted without the risks associated with using glass pasteur pipettes. Considerations were also taken to allow for injection molding, including drafting vertical surfaces, avoiding overhang, and thickening the vial and adapter components to allow for ejector pin placement. These considerations are described in detail in the Experimental Section.

When considering the benefits of using the homeRNAmax platform in comparison to standard venous draws, it is important to evaluate aspects such as participant preferences, cost, and ability to sample at relevant and unpredictable times. We have previously achieved high retention rates across longitudinal studies with homeRNA platforms[18,19,23], with participants noting that at-home sampling and flexibility of the study were important factors in their continued participation[23]. Based on our experiences in injection molding and commercializing these types

of systems, we would expect a production price of homeRNAmax (inclusive of the Tasso blood collection device) to be $40-50 in the scales typically purchased by academic labs. Our typical UPS shipping costs with return overnight shipping are ~$35, and one can use less expensive options as our prior work with the original homeRNA platform shows there is sample stability over eight days at 25 °C. In contrast, the cost of phlebotomy supplies and PAXgene stabilizer tubes is ~$22, with mobile phlebotomists typically costing $75-150+. The cost of the homeRNAmax platform is therefore comparable to or less than at-home venous draws; higher volume manufacturing of homeRNAmax will further reduce costs. Moreover, the increased convenience (i.e., no need to schedule in advance) and privacy of self-collected blood make it more attractive than mobile phlebotomy for many participants. Finally, self-collection with homeRNA enables sampling at relevant time points (e.g., during wildfire smoke exposure[19], migraine events, rheumatoid arthritis flares) and throughout the course of infectious disease during quarantining[17,18].

3.3.2 Theoretical model of the stabilizer tube

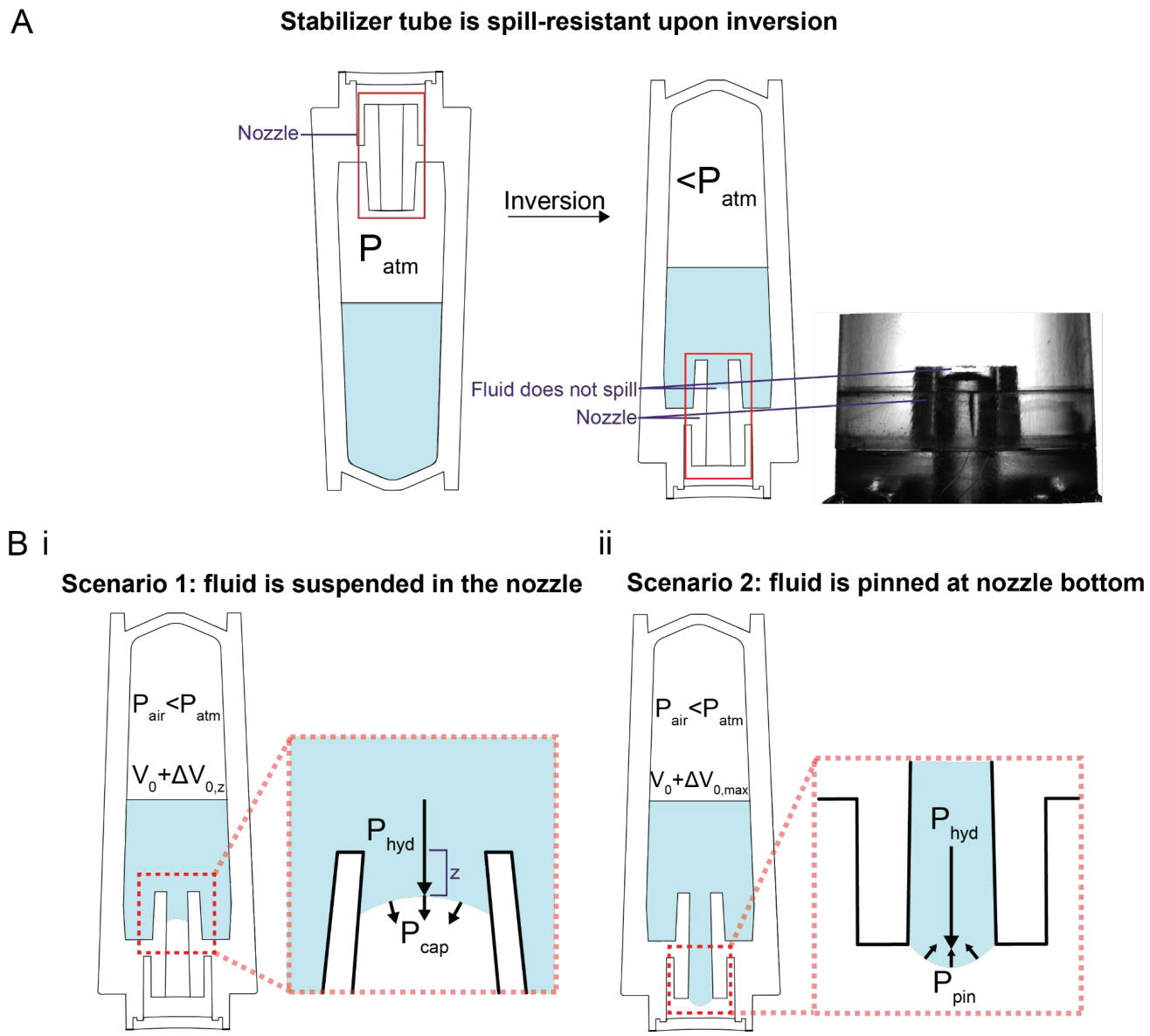

Figure 3.2. homeRNAmax stabilizer tube is spill-resistant due to a balance of pressures within the nozzle. (A) Schematic of stabilizer tube before (left) and after inverting (right). A close-up image of an inverted homeRNAmax tube showing a suspended meniscus within the nozzle is depicted to the right of the schematic. (B) Schematic of an inverted stabilizer tube at equilibrium when i) fluid stops in the nozzle and ii) fluid pins to the nozzle opening at equilibrium. The former is observed for the dimensions and volumes used in this work.

To understand the mechanism underlying the spill-resistant nozzle design on the homeRNAmax stabilizer tube (Table S1), we developed a model of pressures acting upon the liquid-air interface in the middle or bottom of the nozzle. Initially, fluid is added to the stabilizer tube when it is in an upright position (Figure 3.2A). Upon

inversion, the liquid is suspended in the middle (Figure 3.2Bi) or bottom (Figure 3.2Bii) of the nozzle when there is a balance of pressures at the liquid-air interface (i.e., $P_{balance}$=0). Inside the tube, we consider the hydrostatic pressure (P_{hyd}), the pressure of the trapped air (P_{air}), a capillary pressure at the interface between the liquid and the atmosphere within the nozzle (P_{cap}), and—if the liquid is at equilibrium at the bottom of the nozzle—a pressure of the fluid pinning to the opening of the nozzle (P_{pin}). The capillary pressure of the upper surface of the liquid is negligible since the tube diameter is larger than the capillary length. On the outside of the tube, the only pressure is the atmospheric pressure (P_{atm}). The balance of pressures can be expressed using the following equations in the case where the meniscus is suspended within the nozzle (1) and the case where the meniscus is pinned at the nozzle opening (2), respectively:

$$P_{balance} = P_{hyd} + P_{cap} + P_{air} - P_{atm} \quad (1)$$

$$P_{balance} = P_{hyd} - P_{pin} + P_{air} - P_{atm} \quad (2)$$

The notation in equations (1) and (2) is that the values for the individual pressures are always positive; the signs of each pressure indicate whether their contributions are upwards or downwards. The most important pressure for preventing spilling is the trapped air pressure, which decreases as the tube is inverted and is, as a result, lower than the atmospheric pressure. The expansion of the trapped air occurs when a small amount of fluid moves into the nozzle, lowering the pressure inside the tube.

This drop in pressure can be expressed using an equation derived from the Ideal Gas Law (3):

$$\Delta P_{air} \sim P_{atm}\left(1 - \frac{V_0}{V_{air}}\right) \quad (3)$$

where V_0 is the initial volume of air (i.e., the air volume before inversion), and V_{air} is the volume of the air after tilting. In the scenario described in Figure 3.2Bi, upon inverting the tube, the pressure decreases with the increasing air volume associated with the fluid entering the nozzle (equivalent to the volume of fluid in the nozzle, $\Delta V_{0,z}$), and creates a vacuum strong enough that the fluid is suspended in the middle of the nozzle. In this case, the drop in pressure opposes the hydrostatic and capillary pressures, arresting the meniscus within the nozzle and preventing spillage. This is the scenario that was observed experimentally for the dimensions of the stabilizer tube depicted in this work.

The most unfavorable situation for preventing leakage is when the meniscus is located at the bottom of the nozzle (Figure 3.2Bii) after tilting the tube. This scenario was never experimentally observed with the homeRNAmax device in the dimensions and volumes used in this work. However, it was observed using 3D printed stabilizer tubes where the height was increased to ~17 cm (as opposed to 4.8 cm for our actual stabilizer tube used in homeRNAmax) and the draft was removed. In these 3D printed tubes and at low liquid volumes, the effects of fluid movement into the nozzle resulted in less meaningful pressure changes. In this situation depicted in Figure 3.2Bii, pinning holds the fluid at the bottom of the nozzle, and the fluid leaks when pinning is exceeded (when the pendant drop exceeds a hemisphere). For further details on the development of the theoretical model, see Supplemental Information Appendix 1.

In some cases over timescales on the order of 10-20 minutes, additional meniscus movement has been observed after reaching equilibrium; we postulate that this movement is a result of effects related to temperature (e.g., evaporation, temperature changes over time). We have not observed any leakage as a result of these effects. Furthermore, in actual use cases, the tube is not left uncapped and inverted for longer than times on the order of seconds.

3.3.3 User experience pilot study

To validate the usability and robustness of the homeRNAmax platform, we conducted a human subjects pilot study of a total of 20 participants. For the pilot study, we were able to recruit a diverse cohort. A total of 319 individuals completed our screening survey, of whom 38 were invited based on the inclusion criteria. From

there, 23 signed the informed consent. The discussion below focuses on the 20 participants who completed the sampling and the survey. These participants were located across 20 states (including Hawaii) (Figure 3,3A), with a racial background similar to that of the larger United States population (Figure 3.3B), with a similar number of participants belonging to each sex and age bracket (Figure 3.3C).

Each of the 20 participants self-collected and stabilized one blood sample using the homeRNAmax kit. With the sampling, participants were asked to complete surveys about their experience. The majority of the participants (16) indicated that they felt no pain when using the Tasso+ device, and only 4 indicated that they experienced mild pain. (Figure 3.4A). No participant reported experiencing moderate or major pain. Additionally, the majority of participants found the Tasso+ device very easy to use (Figure 3.4B).

Of the 20 participants who were enrolled, only one was unable to collect blood. As a result, we did not collect data on usability of the stabilizer tube and blood level of this participant. The remaining 19 participants indicated very similar ease of use of the stabilizer tube (Figure 3.4B). Moreover, most of the participants were also able to use the kit within 5 to 10 minutes, making the sampling very time efficient.

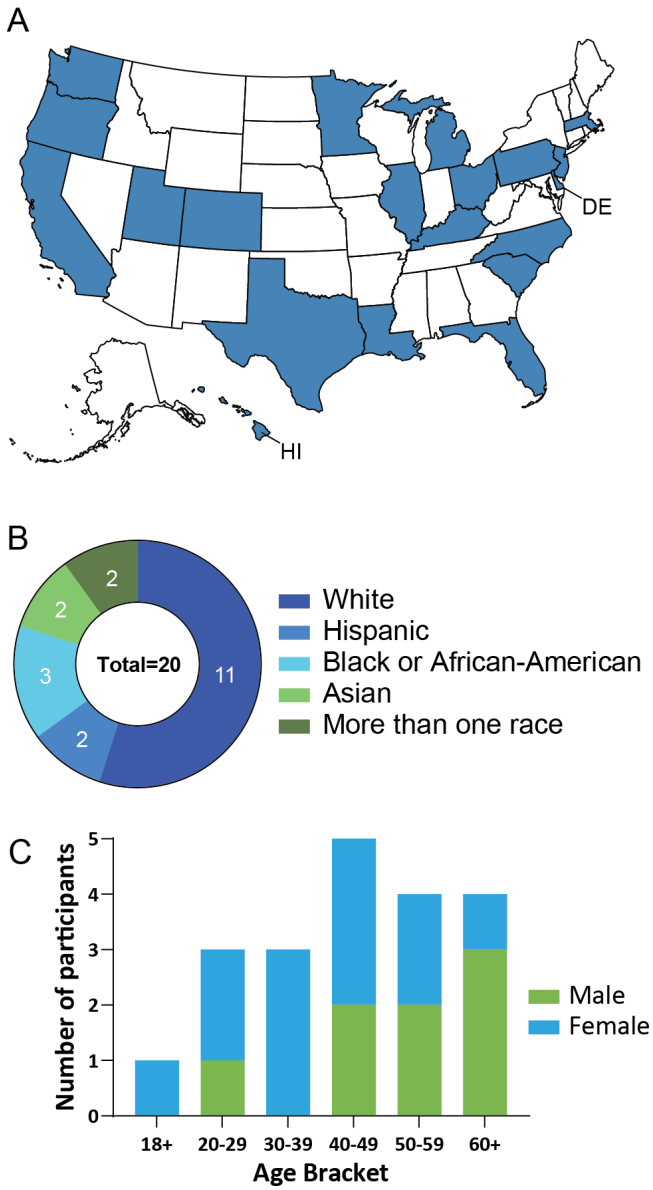

Figure 3.3 Summary of participant demographics. (A) US map showing distribution of participants across 20 states including Hawaii. Each shaded state represents one participant. (B) Distribution of races and ethnicities of participants. (C) Participant sex and age bracket. Participants were selected to represent an evenly distributed cohort, one that matches as closely as possible to the larger population of the United States.

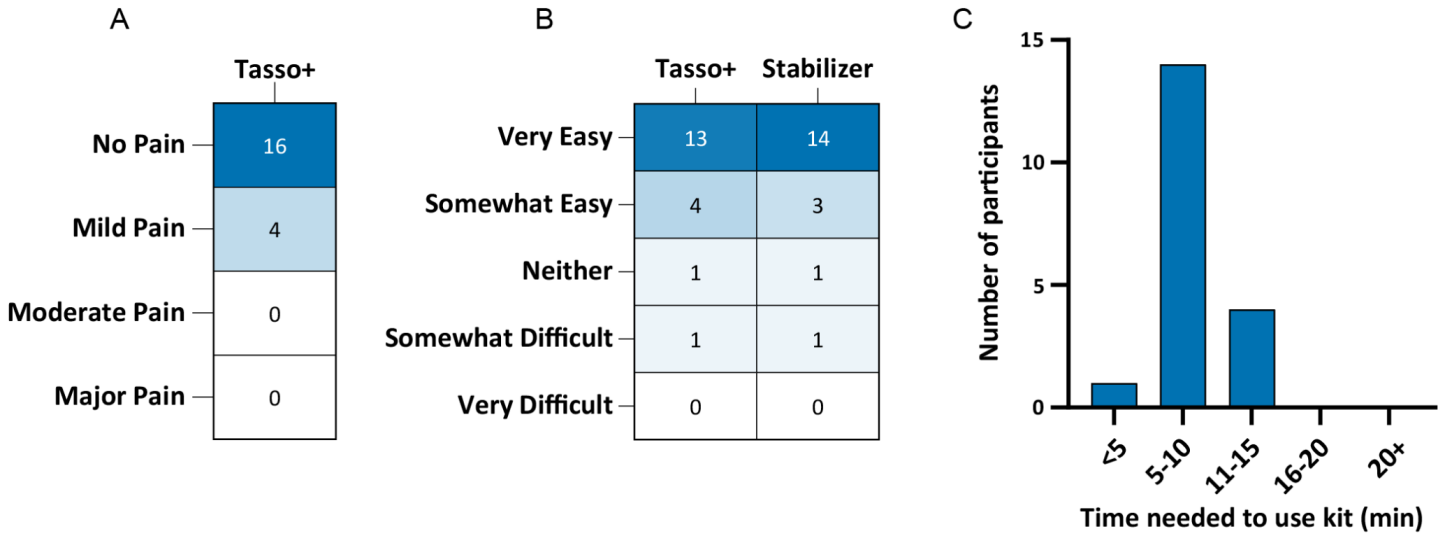

Figure 3.4 Participant-reported homeRNAmax usability data. (A) Pain level reported by each participant following the use of the Tasso+ device. (B) Participant-reported usability of the Tasso+ and homeRNAmax stabilizer tube following blood stabilization. (C) Time needed to use the kit, including reading instructions, collecting and stabilizing the sample, and packaging sample for pickup.

Most (n=15/20) participants collected at least 500 µL of blood, and 13 participants collected more than 500 µL. This means that 13 participants (of the 19 who successfully collected blood using the homeRNA platform) were able to collect more blood than the maximum volume of the original homeRNA platform[16] (Table S3). The homeRNAmax platform, with the Tasso+ device, reliably collected greater volumes of blood than the original homeRNA which used the Tasso-SST, although the majority of participants in our original homeRNA study collected the maximum possible blood volume (Figure B8).

As with the original device[16,21,22] validation, we used the Agilent Bioanalyzer 2100 to measure the quality of RNA using the RNA integrity number (RIN). RINs range from 1 to 10, where a RIN of 1 represents a completely degraded sample and a RIN of 10 a perfectly preserved RNA sample[24,25] (Figure B9). For this study, we used the Qubit Flex, which is specific to RNA, to measure the total yield of RNA in each of the samples.

Stabilization of homeRNAmax was similar to that of the original homeRNA device, with most (n=16/19) RIN values falling above 7 (Figure 5A) and most (17 out of 19) samples having a yield of more than 500 ng (Figure 5B), both of which are common requirements for Illumina RNA sequencing[19,26–28]. Although the RIN of some of the samples was below 7, which we have also seen in our prior work using the original homeRNA kit[16–19,21,22,29], advances in sample processing, sequencing technologies, and analysis methods can generate readouts for samples with lower RINs and yields[26,30–32]. Bioanalyzer electropherograms and a table of RNA yields can be found in the Supplementary Information (Figure B9, B10 and Table B3).

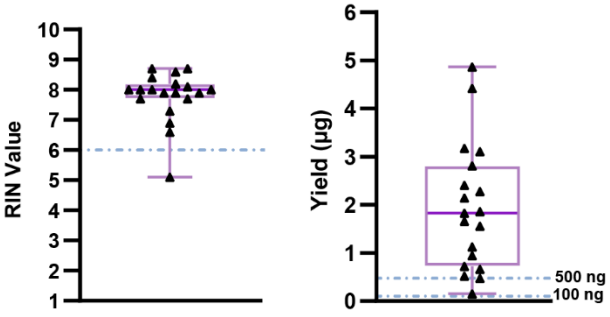

Figure 3.5. RNA integrity and yield of homeRNAmax-stabilized blood samples from a nationwide remote study. Both graphs are represented as standard box and whisker plots with the triangles representing each sample. (A) RNA integrity number (RIN) of homeRNAmax samples obtained using the Agilent 2100 Bioanalyzer. The dashed line represents a RIN of 6, a common cutoff for RNA sequencing technologies. (B) Yield measurements obtained using Qubit Flex. The dashed line represents 500 ng, a conservative cutoff for yield required for sequencing.

Although homeRNAmax collected more blood than the original homeRNA device[16,21], the distributions of RNA yields reported are relatively similar. This is expected from our past experiences using homeRNA, where we have seen that blood volume is not necessarily indicative of resulting RNA yield due to large inter-individual variation in RNA per unit volume of blood (for more information, see Figure B14 of Haack, Lim et al)[16]. However, within a person increased blood volume will still inherently increase RNA collected. When considering other homeRNA studies[16,22], the median RNA yield tends to fall around 2 and 3 µg (Figure B11), and the homeRNAmax-stabilized samples fall within that range. It is important to note that the yields reported in the original homeRNA paper were measured with NanoDrop, which uses absorbance of the sample at 260 nm to estimate concentration, and assumes

that the sample is pure RNA (has no contamination). From our experience, even with the RiboPure blood RNA extraction kits, there is oftenDNA contamination. The Qubit Flex, which was used to determine the yields in Figure 3.5B, relies on a dye that selectively binds to RNA and measures the fluorescence of the bounded dye. As a result, this measurement tool is considered to be more representative of the actual RNA yield in extracted samples. Although the resulting yields from the Take3, which also relies on absorbance, and the Qubit Flex in this study are similar, we note that the Take3 values are slightly higher, as would be expected from a less selective measurement. For comparison of the homeRNAmax platform with the original homeRNA platform, we consider the RNA yield readouts from the Take3 as well as the Qubit Flex. When considering the yields obtained from the Take3 (as a similar measurement method to that used in our original homeRNA manuscript[16], homeRNAmax performed similarly to or better than the original homeRNA (Figure B11), further supporting that the RNA yield is not directly tied to collected blood volume. Regardless of the instrument used to measure the yield, nearly all samples had yields higher than the minimum requirement of 500 ng needed for most sequencing technologies.

Additionally, while we have not seen a strong correlation between collected blood volume and resulting RNA yield in our other homeRNA studies (500+ samples[16–19,21,22,29]), the increased volume is useful for the RNA extraction process. Because homeRNAmax can have up to twice the sample volume of the original homeRNA, homeRNAmax samples can be aliquotted into two tubes. This allows one aliquot to be kept as whole blood in RNA*later* and banked in the freezer, while the other is extracted. The second banked aliquot can prove to be invaluable in the process of analyzing the data following a human subjects study, providing insurance should the other aliquot be lost due to laboratory error. Additionally, a banked second aliquot can be very useful in longer-term longitudinal studies, where samples are collected on the scale of months to years. In cases like these, it may be desirable to extract some of the earlier time points all together, and then have a second aliquot of the earlier time points that could be extracted later with additional time points. Here, having samples split into two aliquots allows them to be directly compared against another set of samples collected at later time points, while preserving extraction batches for direct comparison. Extracting samples at the same time allows for greater ease of comparison by limiting the differences due to variability

between extraction batches. There are many other instances where banking the aliquot may be of use, especially in longitudinal studies, that are dependent on the study design and question being asked.

3.3.4 Longitudinal clinical study

Finally, we demonstrate real-world application of the homeRNAmax device by implementing it into a clinical study. In this ongoing longitudinal study at NYU Langone Health, we have collected and analyzed 44 homeRNAmax samples in participants with psoriatic arthritis or psoriasis. The samples generally demonstrate good RINs and yields that match closely to our pilot study (Figure 3.6). The majority of the samples reported have a RIN greater than 6 and estimated yields greater than 500 ng. Even though some of the samples had RINs lower than the preferred cutoff (as low as 2.7), this kind of variation is expected given the number of samples collected and real-world conditions. In Figure 3.6A, we only represent a subset of the samples (n = 34/44) since these were analyzed using the Agilent Bioanalyzer and can be compared to the results of our user experience pilot study more readily. The remaining 10 samples were analyzed using the Agilent TapeStation and are summarized in Figure B7; all 10 of these samples had RIN values greater than 6. For details about the complete RIN and yield of the clinical study cohort, see Table S4.

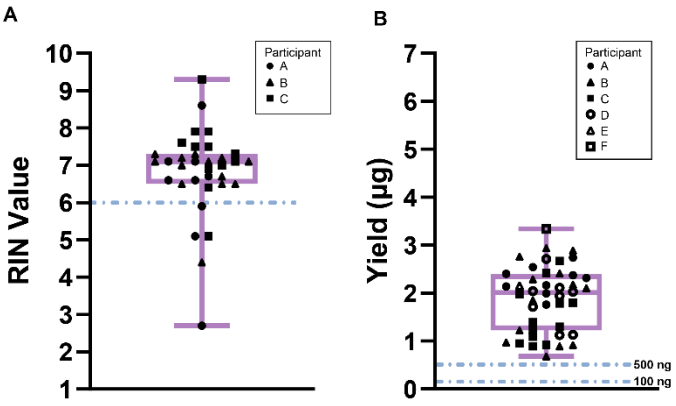

Figure 3.6. RNA integrity and yield of homeRNAmax-stabilized blood samples from a longitudinal clinical study conducted at NYU Langone Health. Both graphs are represented as standard box and whisker plots with data point shapes representing participant codes. Participants B and C are the same individual admitted into the study after switching medications. (A) RIN values of homeRNAmax sample subset (n = 34/44) obtained using the Agilent 2100 Bioanalyzer. The dashed line represents a RIN of 6, a common cutoff for RNA sequencing technologies. Remaining (n = 10/44) RNA integrity data measured with a Agilent TapeStation can be found in Table S4. (B)

Yield measurements obtained using the ThermoFisher NanoDrop. To estimate total yield in the full blood sample, the resulting values were doubled to account for only extracting half of the sample (see *Experimental Section* for details). The dashed line represents 500 ng, a conservative cutoff for yield required for sequencing.

3.4 Conclusion

homeRNAmax is the next generation of the homeRNA at-home blood self-collection and stabilization kit. It is able to collect up to two times the blood volume of the original kit, making for easier sample handling and enabling banking of RNA-stabilized samples. Like the original homeRNA, homeRNAmax is spill-resistant due to the cone-shaped nozzle geometry, which balances the total pressure of the liquid in the tube to the outside atmospheric pressure upon inversion. In the human subjects pilot feasibility study with a total of 20 participants, homeRNAmax was virtually painless (n = 16/20), easy to use (n = 13 for Tasso+ blood collection device and n = 14 for RNA*later*-containing stabilizer tube), and quick (n = 14 only needed 5 - 10 minutes to use the kit). The resulting RNA was shown to be of high quality (mean RIN = 7.8) with most samples above a RIN of 6 and yields above 500 ng, benchmarks often used for most common sequencing technologies. homeRNAmax has also been successfully implemented in an ongoing longitudinal clinical study.

3.5 References

(1) Kwan, P. K. W.; Cross, G. B.; Naftalin, C. M.; Ahidjo, B. A.; Mok, C. K.; Fanusi, F.; Permata Sari, I.; Chia, S. C.; Kumar, S. K.; Alagha, R.; Tham, S. M.; Archuleta, S.; Sessions, O. M.; Hibberd, M. L.; Paton, N. I. A Blood RNA Transcriptome Signature for COVID-19. *BMC Med. Genomics* **2021**, 14 (1), 155. https://doi.org/10.1186/s12920-021-01006-w.

(2) Prokop, J. W.; Hartog, N. L.; Chesla, D.; Faber, W.; Love, C. P.; Karam, R.; Abualkheir, N.; Feldmann, B.; Teng, L.; McBride, T.; Leimanis, M. L.; English, B. K.; Holsworth, A.; Frisch, A.; Bauss, J.; Kalpage, N.; Derbedrossian, A.; Pinti, R. M.; Hale, N.; Mills, J.; Eby, A.; VanSickle, E. A.; Pageau, S. C.; Shankar, R.; Chen, B.; Carcillo, J. A.; Sanfilippo, D.; Olivero, R.; Bupp, C. P.; Rajasekaran, S. High-Density Blood Transcriptomics

Reveals Precision Immune Signatures of SARS-CoV-2 Infection in Hospitalized Individuals. *Front. Immunol*. **2021**, 12. https://doi.org/10.3389/fimmu.2021.694243.

(3) Menezes, S. M.; Jamoulle, M.; Carletto, M. P.; Moens, L.; Meyts, I.; Maes, P.; Weyenbergh, J. V. Blood Transcriptomic Analyses Reveal Persistent SARS-CoV-2 RNA and Candidate Biomarkers in Post-COVID-19 Condition. *Lancet Microbe* **2024**, 5 (8). https://doi.org/10.1016/S2666-5247(24)00055-7.

(4) Frésard, L.; Smail, C.; Ferraro, N. M.; Teran, N. A.; Li, X.; Smith, K. S.; Bonner, D.; Kernohan, K. D.; Marwaha, S.; Zappala, Z.; Balliu, B.; Davis, J. R.; Liu, B.; Prybol, C. J.; Kohler, J. N.; Zastrow, D. B.; Reuter, C. M.; Fisk, D. G.; Grove, M. E.; Davidson, J. M.; Hartley, T.; Joshi, R.; Strober, B. J.; Utiramerur, S.; Lind, L.; Ingelsson, E.; Battle, A.; Bejerano, G.; Jonathan A. Bernstein; Ashley, E. A.; Boycott, K. M.; Merker, J. D.; Wheeler, M. T.; Montgomery, S. B. Identification of Rare-Disease Genes Using Blood Transcriptome Sequencing and Large Control Cohorts. *Nat. Med.* **2019**, 25 (6), 911–919. https://doi.org/10.1038/s41591-019-0457-8.

(5) Ming, W.; Xie, H.; Hu, Z.; Chen, Y.; Zhu, Y.; Bai, Y.; Liu, H.; Sun, X.; Liu, Y.; Gu, W. Two Distinct Subtypes Revealed in Blood Transcriptome of Breast Cancer Patients With an Unsupervised Analysis. *Front. Oncol.* **2019**, 9. https://doi.org/10.3389/fonc.2019.00985.

(6) Fuller, G.; Njune Mouapi, K.; Joung, S.; Shufelt, C.; van den Broek, I.; Lopez, M.; Robinson, A.; dhawan, S.; Mastali, M.; Spiegel, B.; Bairey Merz, N.; Van Eyk, J. E. Feasibility of Patient-Centric Remote Dried Blood Sampling: The Prediction, Risk, and Evaluation of Major Adverse Cardiac Events (PRE-MACE) Study. *Biodemography Soc. Biol.* **2020**, 65 (4), 313–322. https://doi.org/10.1080/19485565.2020.1765735.

(7) Li, K.; Naviaux, J. C.; Monk, J. M.; Wang, L.; Naviaux, R. K. Improved Dried Blood Spot-Based Metabolomics: A Targeted, Broad-Spectrum, Single-Injection Method. *Metabolites* **2020**, 10 (3), 82. https://doi.org/10.3390/metabo10030082.

(8) Lim, M. D. Dried Blood Spots for Global Health Diagnostics and Surveillance: Opportunities and Challenges. *Am. J. Trop. Med. Hyg.* **2018**, 99 (2), 256–265. https://doi.org/10.4269/ajtmh.17-0889.

(9) Baillargeon, K. R.; Mace, C. R. Microsampling Tools for Collecting, Processing, and Storing Blood at the Point-of-Care. *Bioeng. Transl. Med.* **2023**, 8 (2), e10476. https://doi.org/10.1002/btm2.10476.

(10) Fredolini, C.; Dodig-Crnković, T.; Bendes, A.; Dahl, L.; Dale, M.; Albrecht, V.; Mattsson, C.; Thomas, C. E.; Torinsson Naluai, Å.; Gisslen, M.; Beck, O.; Roxhed, N.; Schwenk, J. M. Proteome Profiling of Home-Sampled Dried Blood Spots Reveals Proteins of SARS-CoV-2 Infections. *Commun. Med.* **2024**, 4 (1), 55. https://doi.org/10.1038/s43856-024-00480-4.

(11) Wikström, F.; Olsson, C.; Palm, B.; Roxhed, N.; Backlund, L.; Schalling, M.; Beck, O. Determination of Lithium Concentration in Capillary Blood Using Volumetric Dried Blood Spots. *J. Pharm. Biomed. Anal.* **2023**, 227, 115269. https://doi.org/10.1016/j.jpba.2023.115269.

(12) Lenk, G.; Ullah, S.; Stemme, G.; Beck, O.; Roxhed, N. Evaluation of a Volumetric Dried Blood Spot Card Using a Gravimetric Method and a Bioanalytical Method with Capillary Blood from 44 Volunteers. *Anal. Chem.* **2019**, 91 (9), 5558–5565. https://doi.org/10.1021/acs.analchem.8b02905.

(13) Baillargeon, K. R.; Brooks, J. C.; Miljanic, P. R.; Mace, C. R. Patterned Dried Blood Spot Cards for the Improved Sampling of Whole Blood. *ACS Meas. Sci. Au* **2022**, 2 (1), 31–38. https://doi.org/10.1021/acsmeasuresciau.1c00031.

(14) Heneghan, N.; Fu, J.; Pritchard, J.; Payton, M.; Allen, R. W. The Effect of Environmental Conditions on the Rate of RNA Degradation in Dried Blood Stains. *Forensic Sci. Int. Genet.* **2021**, 51, 102456. https://doi.org/10.1016/j.fsigen.2020.102456.

(15) Li, J.; Ulloa, G. M.; Mayor, P.; Santolalla Robles, M. L.; Greenwood, A. D. Nucleic Acid Degradation after Long-Term Dried Blood Spot Storage. *Mol. Ecol. Resour.* **2024**, 24 (6), e13979. https://doi.org/10.1111/1755-0998.13979.

(16) Haack, A. J.; Lim, F. Y.; Kennedy, D. S.; Day, J. H.; Adams, K. N.; Lee, J. J.; Berthier, E.; Theberge, A. B. homeRNA: A Self-Sampling Kit for the Collection of Peripheral Blood and Stabilization of RNA. *Anal. Chem.* **2021**, 93 (39), 13196–13203. https://doi.org/10.1021/acs.analchem.1c02008.

(17) Lim, F. Y.; Kim, S.-Y.; Kulkarni, K. N.; Blazevic, R. L.; Kimball, L. E.; Lea, H. G.; Haack, A. J.; Gower, M. S.; Stevens-Ayers, T.; Starita, L. M.; Boeckh, M.; Hyrien, O.; Schiffer, J. T.; Theberge, A. B.; Waghmare, A. High-

Frequency Home Self-Collection of Capillary Blood Correlates IFI27 Expression Kinetics with SARS-CoV-2 Viral Clearance. *J. Clin. Invest.* **2023**, 133 (23). https://doi.org/10.1172/JCI173715.

(18) Lim, F. Y.; Lea, H. G.; Dostie, A. M.; Kim, S.-Y.; van Neel, T. L.; Hassan, G. W.; Takezawa, M. G.; Starita, L. M.; Adams, K. N.; Boeckh, M.; Schiffer, J. T.; Hyrien, O.; Waghmare, A.; Berthier, E.; Theberge, A. B. HomeRNA Self-Blood Collection Enables High-Frequency Temporal Profiling of Presymptomatic Host Immune Kinetics to Respiratory Viral Infection: A Prospective Cohort Study. *eBioMedicine* **2025**, 112, 105531. https://doi.org/10.1016/j.ebiom.2024.105531.

(19) Haack, A. J.; Brown, L. G.; Zeng, Y.; Khan, T.; Robertson, I. H.; Kennedy, D. S.; Adams, K. N.; MacDonald, J. W.; Bammler, T. K.; Stefanovic, F.; Moloney, K.; Stolarczuk, J. E.; Takezawa, M. G.; Alizai, M. Y.; Hassan, G. W.; Lim, F. Y.; Chaussabel, D.; Walker, E. G.; Errett, N. A.; Berthier, E.; Theberge, A. B. A Flexible and Responsive Remote Study Design to Assess Gene Expression Changes During Wildfire Smoke Exposure with homeRNA, an At-Home Blood Sampling Kit. medRxiv October 14, 2025, p 2025.10.11.25337783. https://doi.org/10.1101/2025.10.11.25337783.

(20) Brown, L. G.; Wei, X.; Milton, L. A.; Alizai, M. Y.; MacDonald, J. W.; Bammler, T. K.; Zeng, Y.; Robertson, I.; Adams, K. N.; Toh, Y.-C.; Chaussabel, D.; Berthier, E.; Haack, A. J.; Theberge, A. B. From Home to Transcriptome: Comparing the Transcriptomic Profile of Induced Immune Response via Lipopolysaccharide Stimulation in homeRNA and Venous Blood. *Anal. Chem.* **2026**. https://doi.org/10.1021/acs.analchem.5c06040.

(21) Brown, L. G.; Haack, A. J.; Kennedy, D. S.; Adams, K. N.; Stolarczuk, J. E.; Takezawa, M. G.; Berthier, E.; Thongpang, S.; Lim, F. Y.; Chaussabel, D.; Garand, M.; Theberge, A. B. At-Home Blood Collection and Stabilization in High Temperature Climates Using homeRNA. *Front. Digit. Health* **2022**, 4, 903153. https://doi.org/10.3389/fdgth.2022.903153.

(22) Stefanovic, F.; Brown, L. G.; MacDonald, J.; Bammler, T.; Rinchai, D.; Nguyen, S.; Zeng, Y.; Shinkawa, V.; Adams, K.; Chaussabel, D.; Berthier, E.; Haack, A. J.; Theberge, A. B. Your Blood Is Out for Delivery: Considerations of Shipping Time and Temperature on Degradation of RNA from Stabilized Whole Blood. *Anal. Chem.* **2025**, 97 (3), 1635–1644. https://doi.org/10.1021/acs.analchem.4c04591.

(23) Stefanovic, F.; Robertson, I.; Moloney, K.; Edelson, J.; Nguyen, S.; Shinkawa, V.; Uchimura, K.; Lin, A.; Le, L.; Tokihiro, J. C.; Takezawa, M. G.; Phan, D.; Schiffer, J. T.; Boeckh, M.; Adams, K. N.; Waghmare, A.; Errett, N. A.; Berthier, E.; Lim, F. Y.; Theberge, A. B. Research In Your Mailbox: Remote Blood Self-Sampling Enables Participation of Underserved Populations in Longitudinal Studies. medRxiv February 6, 2026, p 2026.02.05.26345688. https://doi.org/10.64898/2026.02.05.26345688.

(24) Mueller, O.; Lightfoot, S.; Schroeder, A. RNA Integrity Number (RIN)–Standardization of RNA Quality Control., **2016**. https://www.agilent.com/cs/library/applications/5989-1165EN.pdf.

(25) Schroeder, A.; Mueller, O.; Stocker, S.; Salowsky, R.; Leiber, M.; Gassmann, M.; Lightfoot, S.; Menzel, W.; Granzow, M.; Ragg, T. The RIN: An RNA Integrity Number for Assigning Integrity Values to RNA Measurements. *BMC Mol. Biol*. **2006**, 7 (1), 3. https://doi.org/10.1186/1471-2199-7-3.

(26) Gallego Romero, I.; Pai, A. A.; Tung, J.; Gilad, Y. RNA-Seq: Impact of RNA Degradation on Transcript Quantification. BMC Biol. 2014, 12 (1), 42. https://doi.org/10.1186/1741-7007-12-42.

(27) Jahn, C. E.; Charkowski, A. O.; Willis, D. K. Evaluation of Isolation Methods and RNA Integrity for Bacterial RNA Quantitation. *J. Microbiol. Methods* **2008**, 75 (2), 318–324. https://doi.org/10.1016/j.mimet.2008.07.004.

(28) RNA-seq Sample Guidelines - The Huck Institutes (en-US). https://www.huck.psu.edu/core-facilities/genomics-core-facility/sample-recommendations/rna-seq-sample-guidelines (accessed 2025-07-21).

(29) Haack, A. J.; Zheng, Y.; Brown, L. G.; Stefanovic, F.; Jeacopello, I.; Adams, K.; Kennedy, D. S.; Stolarczuk, J. E.; Takezawa, M. G.; Hassan, G. W.; MacDonald, J.; Bammler, T.; Edelson, J.; Lin, A.; Lim, F. Y.; Chaussabel, D.; Walker, E. G.; Errett, N. A.; Berthier, E.; Theberge, A. B. Longitudinal Transcriptomic Studies Using homeRNA: A Framework For Studying the Effects of the Exposome and Wildfire Smoke Using Self-Sampled and Stabilized Blood, **2024**.

(30) Ozsolak, F.; Milos, P. M. RNA Sequencing: Advances, Challenges and Opportunities. *Nat. Rev. Genet.* **2011**, 12 (2), 87–98. https://doi.org/10.1038/nrg2934.

(31) Schuierer, S.; Carbone, W.; Knehr, J.; Petitjean, V.; Fernandez, A.; Sultan, M.; Roma, G. A Comprehensive Assessment of RNA-Seq Protocols for Degraded and Low-Quantity Samples. *BMC Genomics* **2017**, 18 (1), 442. https://doi.org/10.1186/s12864-017-3827-y.

(32) Jang, J. S.; Holicky, E.; Lau, J.; McDonough, S.; Mutawe, M.; Koster, M. J.; Warrington, K. J.; Cuninngham, J. M. Application of the 3′ mRNA-Seq Using Unique Molecular Identifiers in Highly Degraded RNA Derived from Formalin-Fixed, Paraffin-Embedded Tissue. *BMC Genomics* **2021**, 22 (1), 759. https://doi.org/10.1186/s12864-021-08068-1.

Chapter 4 │ Research In Your Mailbox: Remote Blood Sampling Enables Longitudinal Studies in Underserved Groups[3]

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Structured Abstract

Importance: When leveraged for longitudinal studies, remote sampling with transcriptomic readouts is a powerful tool for studying the host immune response. Remote study flexibility circumvents common barriers to research participation including length of commute, transportation, and scheduling, thereby expanding access to clinical research.

Objective: We investigated the effectiveness of a remote design for reaching women from underrepresented, underserved, and underreported (U3) populations. We sought to recruit individuals who qualify as underrepresented in clinical research, who are located in rural areas, or who come from disadvantaged backgrounds per the NIH definition.

Design: U3 women positive for COVID-19 were tracked over the course of 6 months. Participants self-collected nasal and homeRNA-stabilized blood samples. homeRNA is a platform for remote self-collection of blood samples with subsequent RNA stabilization. Five samplings were completed in 25 days (month 1). A subset of

[3] *This chapter contains collaborative work. FS, FYL, JTS, MB, AW, EB, and ABT contributed to study design. FS, IR, JCT, FYL, KM, and NAE formulated the survey questions. FS and IR developed the REDCap. FS and JE acted as study coordinators. FS and IR managed preparation of sampling materials. FS, KU, LL, and AL contributed to preparation of homeRNA kits and study supplies. FS, IR, and VS shipped study materials to participants. FS and SN received and process blood samples. FS made study ads. FS and MGT managed ads. FS and JE prepared correspondence scripts. FS and DP managed study website. KNA and IHR oversaw the IRB approval process, overall human subjects regulatory design, and compliance in study implementation. FS generated the figures and led the writing efforts for this chapter.*

participants likely to develop post-acute sequelae of COVID-19 (PASC) and their age-matched controls were selected for extended sampling (month 3). All participants were resurveyed at months 4, 5, and 6 about their symptoms.

Setting: This was a fully remote study.

Participants: U3 women aged 18+ who tested positive for SARS-CoV-2 were considered eligible. Forty participants were fully enrolled in the study, and 39 completed all study components.

Main Outcomes and Measures: We hypothesized that increased flexibility of a remote study would bolster participation of underrepresented populations in clinical research. To test this hypothesis, we collected usability data and general experience by self-report using electronically administered surveys.

Results: Survey responses show high satisfaction in the study, where all participants who completed the study (*n* = 39/39) indicated they would be willing to participate in a similar study again, with most (*n* = 32/39) indicating a willingness to participate for up to 4 years with around 15 samples collected per year.

Conclusions and Relevance: The high retention (*n* = 39/40) and satisfaction of participants in this study indicates the utility of a remote study design for longitudinal research. We also find that study topic, flexibility of sampling, and positive interactions with the study team are important factors for participant recruitment and retention.

4.1 Introduction

Remote research has the potential to remove obstacles to participation that have resulted in an underrepresentation of certain groups of people. Specifically, the remote study model allows for greater flexibility with sampling time and location, which facilitates participation of individuals with challenging schedules, those who are caregivers, as well as those who have limited access to transportation.[1,2] While some barriers to participation such as awareness of ongoing research and mistrust in science and medicine[3] cannot be easily overcome, online recruitment and the ability to participate from home reduce the discomfort of going to a medical facility.[4] Bolstering the participation of underreported groups, researchers can build more robust datasets and develop medical treatment and interventions that are effective for everyone.[5]

Moreover, remote studies have the potential to afford opportunities for sampling at relevant times and frequencies, especially with sensitive molecular signatures associated with transient events, such as wildfire smoke exposure, infectious disease, and response to medical treatment and interventions. By eliminating the need for in-person visits, remote research also limits risk of infection for both the participant and the research staff. In the wake of the COVID-19 pandemic, self-sampling allowed continuation of work despite closures of large research centers.[6,7] Remote research also allows for a single study team to reach populations across the entire country under one Institutional Review Board (IRB) protocol.

Longitudinal sampling with high temporal frequency is a powerful tool for elucidating underlying mechanisms of disease progression.[8,9] To this end, our lab previously developed an at-home blood sampling technology called homeRNA. The homeRNA platform allows participants to self-collect their blood and stabilize the RNA.[10] We have already demonstrated the utility of homeRNA in remote longitudinal studies in respiratory infections and have been able to sequence and interpret data from the resulting stabilized blood samples.[10–15]

In the work herein, we present a homeRNA-based longitudinal study tracking the progression of the host response to the SARS-CoV-2 virus in women defined as underrepresented, underserved, and underreported (U3) according to the NIH definition. We chose post-acute sequelae of COVID-19 (PASC), more commonly known as long COVID, as a case study for implementing homeRNA in U3 populations participating in longitudinal studies.

PASC is described as a condition with symptoms resulting from the COVID-19 infection and lasting for weeks, months, or even years following original infection.[16] Since the onset of the COVID-19 pandemic, studies have been published proposing mechanistic and health implications of PASC.[17–20] While significant work has been done to better understand this condition, at the time of writing, a standard definition is still evolving.[21–23]

To contribute to the emerging classification of infection blood biomarkers of PASC, we recruited a cohort of 40 COVID-19 positive women and tracked their symptoms over the course of 6 months. During the first month of infection, the participants collected blood samples with homeRNA, as well as nasal swabs. Given that we previously demonstrated the ability to detect and monitor pre-symptomatic signatures of COVID-19, one of the

main objectives of the present study design was to capture early infection blood biomarkers to better understand potential pathways that are involved in the progression to PASC.[12,14]

Here, we assess elements of longitudinal studies and the impacts of specific study design choices on accessibility and overall study experience. Since the low uptake of novel and effective interventions is well documented (at times lower than 50%)[24], we have opted to formulate our surveys in such a way that they can be analyzed by implementation scientist in the future. For this work, we formulated the closing surveys with the Consolidated Framework for Implementation Research (CFIR), which is a well-established method for validating implementation of novel interventions within the medical field.[25] Our results provide insight into effective strategies for high participant satisfaction, offering a roadmap for future study design enabling effective recruitment and retention of U3 participants.

4.2 Methods

4.2.1 Inclusion and Exclusion Criteria

Participants were recruited under a protocol approved by the University of Washington Institutional Review Board study number STUDY00016154. To meet the inclusion criteria subject had to be (1) aged 18 and older, (2) self-reported positive COVID-19 test result within 7 days of screen date, and (3) a woman of understudied, underrepresented, and underreported populations (U3). U3 is defined by NIH to include African American or Black, Hispanic or Latino, American Indian or Alaska Native, Asian American, Native Hawaiian and Other Pacific Islander populations, socioeconomically disadvantaged populations (see **Supplement 1** for NIH Disadvantaged Background criteria), underserved rural populations, and sexual and gender minorities. Individuals who (1) were unable to give consent, (2) were pregnant individuals by self-report, (3) were categorized as other protected populations (children, prisoners), or (4) did not complete the initial enrollment survey within 48 hours of consent were excluded from the study. These criteria were guided by the stated research goals of our funding source, the U3 Supplement mechanism of NIH Office of Research on Women's Health.

4.2.2 Survey Design

All surveys were built and collected using the University of Washington (UW) secure ITHS REDCap platform. These included: (1) screening surveys, (2) informed consent (ICF), (3) health history, (4) shipping information, (5) sampling surveys, (6) closing surveys, and (7) monthly follow-up surveys. The survey questions were formulated using questions from previous studies[12–14], the PhenX directory, and the Consolidated Framework for Implementation Research (CFIR).

Recruiting and Study Initiation

Participants were recruited through advertising between March and June of 2023. This was primarily done through Facebook and the help of an advertising service, WATE TV (for a sample ad and study timeline, see **Figures e1 and e2**). Participants who responded to the ads were screened for the inclusion and exclusion criteria and were balanced for age, race/ethnicity, and state of residence in an effort to capture a diverse cohort that closely corresponds to the demographics of the United States. After the screening process, prospective participants were sent the informed consent form (ICF). The purpose of this study as communicated to the participants in the ICF read: "You are being asked to volunteer for a pilot research study to help us understand more about the association between early immune response during infection and development of long COVID (also known as post-acute sequelae of COVID-19 (PASC) in underrepresented, understudied, and underreported women. In this study, you will collect blood and nasal samples from yourself." Once the ICF was signed, the participants were then promptly contacted by phone to discuss the outline of the study, expectations, and allow for any questions. Upon completing the phone call, the participants were asked to complete an extensive health history and complete shipping and pickup information for the collected samples. Once the participants were fully enrolled, they were sent a study kit (see *Study Kit Components* section for details). Upon receiving their study kit, participants were instructed to collect their first blood and nasal samples as soon as possible.

4.2.3 Study Design

The study was divided into three portions: (1) month 1 sampling, (2) extended sampling for select participants, and (3) follow-up symptom surveys and is summarized in Figure 4.1. During the first month of the study, participants collected a set of samples every 5 days. At each time point (1 - 5), participants collected and stabilized a blood sample (see *Blood Collection* section for details) and a nasal swab (see *Nasal Swab Collection* section for details). At time points and 1 and 5 (first and last samples of the month 1 sampling) participants also collected one additional unstabilized blood sample. Following sampling completion, participants filled out a survey about their COVID-19 symptoms and the sampling. The samples were then packaged and sent back to the lab (see *Sample Shipping* section for details) where they were kept at -20°C until further processing (see *Sample Processing* section for details).

For the extended sampling, a total of 15 participants were chosen due to resource limitations. We opted to select a greater number of PASC-predicted individuals to maximize likelihood of capturing PASC, since it is possible for PASC-predicted participants to recover fully. Due to the longitudinal design of our study, data from PASC+ participants can be informative even in the absence of corresponding controls. Longitudinal sampling allows us to follow gene expression within a participant over time, rather than focusing on comparison between cases and controls at a single time point. This will be explored in further detail in subsequent publications examining the gene expression data resulting from this work.

Following the month 1 sampling, participants were assessed for likelihood for developing PASC based on their self-reported medical history and persistence of COVID-19 symptoms. This assessment was based on the PASC literature available at the time, where body mass index (BMI), pre-existing conditions (asthma, constipation, reflux, rheumatoid arthritis, seasonal allergies, and depression/anxiety), persistence of symptoms at one month (fatigue, headache, difficulty breathing, alterations in taste/smell, hoarse voice, muscle pain, and malaise), and more than 5 symptoms at time of onset made individuals more likely to develop PASC.[16,19,22,26] Participants who had more than 2 lingering symptoms, more than 5 reported symptoms at onset, and had at least one pre-existing condition or a BMI greater than 26 were considered for the PASC-predicted cohort. From this pool, participants

were examined on a case-by-case basis, and 11 were selected as most likely to develop PASC. Any participant with ambiguities or low probability of developing PASC (e.g., a participant with only one pre-existing condition, healthy BMI, and who was vaccinated for COVID-19) were not selected. The pool of potential control participants was made by filtering for participants who met at least one of the following: no lingering symptoms, fewer than 5 symptoms at onset, no pre-existing conditions considered significant for PASC, or a high number of vaccine boosters (2+). To enable close correspondence between the control and PASC-predicted participants, control participants were enrolled based on roughly matching at least one of the PASC-predicted participants in age and BMI. A total of 5 controls were selected. The extended sampling (n = 16) was set to start roughly 2 weeks after a participant's 5th sample and consisted of an additional 5 sampling time points (6 - 10) spaced 5 days apart with stabilized blood draws and nasal swabs (but not the additional, unstabilized blood draws). After each sampling, the participants completed sample surveys, and after time point 10 they completed an additional closing survey. All participants (n = 39) were also surveyed for their overall wellbeing and symptoms at months 4, 5, and 6.

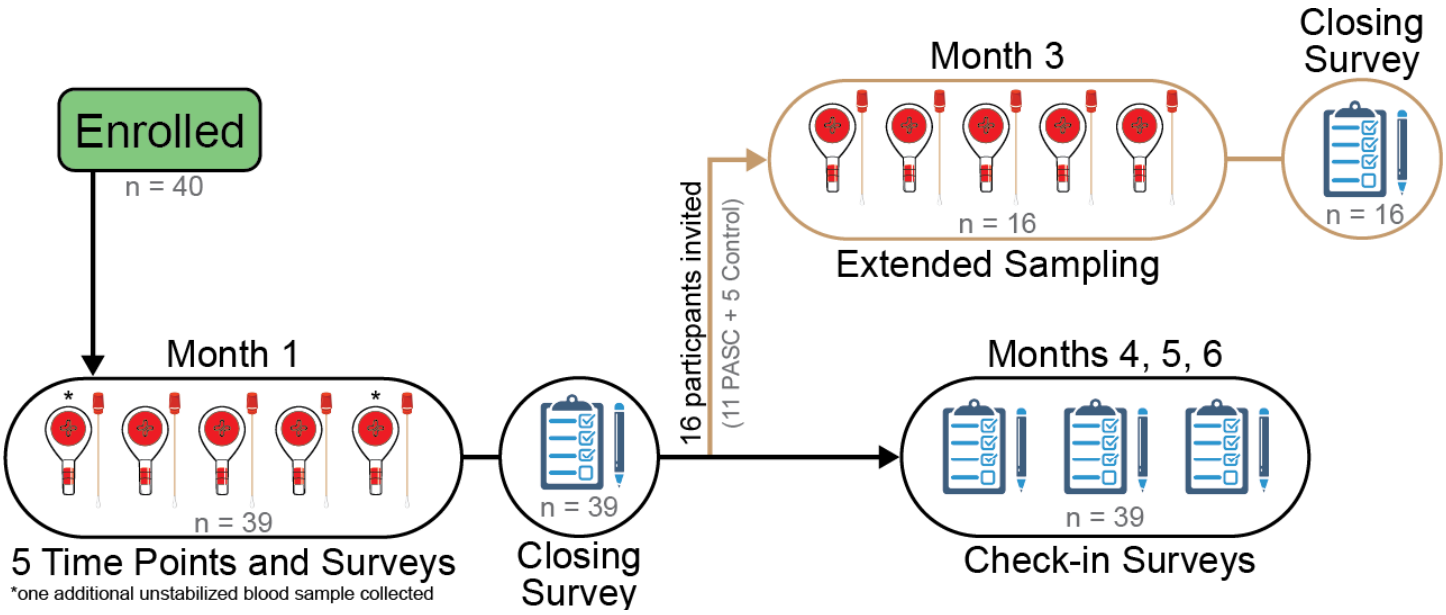


Figure 4.1. Outline of the study timeline. After enrollment, participants were sent a study kit with materials needed for completing the first five sample time points over the course of the first month of their infection. Over the course of the first month, participants collected a total of 7 blood samples and 5 nasal swabs. Participant

symptoms were tracked and those who were mostly likely to develop PASC, along with age- and BMI-matched controls, were invited to the extended sampling which consisted of an additional 5 blood samples and 5 nasal swabs during month 3. Each time point was accompanied by a sampling survey with details about sampling time, blood volume collected, and experience using the kit. All participants were resurveyed for symptoms at months 4, 5, and 6. At time samples 5 and 10, participants were also given a closing survey with questions about overall study experience.

4.2.4 Study Kit Components

The study kit included all the materials necessary for sampling and consisted of (1) individualized schedule for sampling and welcome letter, (2) copies of instructions for use (IFUs) for the blood collection and nasal swabs, (3) kits with sampling materials for time points 1 - 5 (or 6 - 10 for extended sampling), (4) 2 Tasso-SST kits (note: not included in kit for extended sampling), (5) ThermoPro TP49 temperature and humidity monitor, (6) backup materials, and (7) 5 pre-labeled UPS Labpaks. For a view of the fully prepared package, see Figure C3.

Each time point sampling box included materials needed to complete the blood draw, stabilization, and nasal swab collection. For blood draw and stabilization this included a Tasso-SST, homeRNA stabilizer tube filled with RNA*later*, Medline Instant Hot Pack Medium, a self-adhesive bandage, alcohol swab, and a 50 mL Falcon conical tube. The nasal swab collection components included one Sterile PurFlock Ultra Flocked Swab, Regular Tip, 6 in. Polystyrene Shaft and one Copan Diagnostics Universal Transport Medium (UTM-RT™) in Screw-Cap Tube.

The backup materials included 2 additional duplicates of each component needed for sampling in case of issues with the original materials.

4.2.5 Blood Collection and Stabilization

Blood collection and stabilization were performed using the standard homeRNA protocol.[10] The additional blood samples at timepoints 1 and 5 that are not stabilized were collected according to the Tasso-SST manufacturer's instructions. The collected samples were then placed in a 95kPa biohazard bag.

4.2.6 Nasal Swabs

Nasal swabs were collected by swirling the swab inside each nostril for 15 seconds. The swabs were then placed in the UTM-RT and snapped off. The UTM-RT tubes were then secured with the screw-top cap and added to the same 95kPa biohazard bag.

4.2.7 Sample Shipping

Once all the samples were collected and placed in the 95kPa biohazard bag, the biohazard bag was sealed and placed in the original time point box. The box containing the samples was then placed into a paid and pre-labeled UPS LabPak. The LabPak was left outside the following morning for courier pickup which was scheduled by the study team.

4.2.8 Sampling Surveys

Following each sampling, participants completed sample surveys. Here, they were asked about their ongoing symptoms and questions about the sampling, including sampling time, blood collected, ease of use, etc.

4.2.9 Closing Surveys

The closing surveys were administered both at sampling time point 5 and time point 10. The original version of the surveys at timepoint 5 was administered to the first 10 participants but was then reformulated using the Consolidated Framework for Implementation Research (CFIR), a determinants framework commonly used in healthcare settings to explore barriers and facilitators to the adoption of evidence-based interventions.[27]

Participants who filled out the original version were resurveyed with the updated closing survey within two months. The original responses were preserved but are not presented here for easy comparison between all participants. To characterize the usability of the homeRNA platform in U3 populations, we focused on the Innovation Domain and put special emphasis on the Innovation Evidence-Base, Innovation Relative Advantage, and Innovation Design constructs. These were selected to explore the perceived effectiveness of homeRNA, the ease of use compared to in-person clinic visits, and the overall experience with the kit usage. The closing survey also includes questions from the Harvard Flourish Scale to assess the well-being of our participants.[28]

4.2.10 Study Compensation

Study compensation was issued each time participants completed a survey. For time points that included sample collections, participants earned $20 each time, for a total of $100 for month 1 sampling and an additional $100 for extended sampling. Monthly check-in surveys were compensated at $10 each for a total of $30 per participant. The study compensation was issued in the form of digital Tango gift cards. Additionally, each study kit included a University of Washington branded mug as a souvenir for participating in the study.

4.2.11 Sample Processing

When ready for sample processing, the samples were taken out of the -20°C freezers and transferred into the biosafety cabinet (BSC). The samples were carefully removed and the outside containers were sterilized first with ethanol then with trifectant spray. The stabilized blood samples and nasal swab samples were inventoried and transferred to -80°C until future processing. The unstabilized blood samples were centrifuged for 10 minutes at 15,000 RCF. Roughly 50 µL of the supernatant was removed and transferred into Screw Cap Micro Tubes (RNAse/DNAse free) and stored at -80°C for future analysis.

4.2.12 Survey Analysis

Sampling and closing surveys were used for the usability analysis of the study design and the homeRNA platform. The data was transferred from REDCap into R. The data manipulation (consisting of data sorting, organization,

and descriptive statistics) was handled using the tydiverse library and data visualization was done with ggplot, GraphPad's Prism, and SankeyMATIC.

4.3 Results

In this study, we tracked participant symptoms for 6 months after the initial onset of symptoms, taking care to recruit participants as close to their first positive COVID-19 test as possible. In the process of recruiting, we received a total of 334 complete responses, of which 65 met all inclusion criteria to participate in our study and were invited to participate (Figure 4.2A). A total of 44 participants completed the consent process, of which 40 completed at least one sampling and were considered to be fully enrolled (for details on blood collection time and volume, see Figure C4). Most of these remaining participants (98%, $n = 39/40$) completed all sampling timepoints, surveys, and follow-ups, with one participant being withdrawn (lost to followup) from the study after completing collection of the first three samples. We were able to recruit participants from 19 states (Figure 4.2B, Table 4.1) with a diverse racial, ethnic, and age distribution (Figure 4.2C, Table 1), most of whom (80%, Figure 4.1C) are categorized as having a disadvantaged background per the NIH).

Considering PASC predictive factors outlined in the *Methods* section, a subset of 11 individuals were predicted to be likely to develop PASC. These participants, along with a smaller subset of 5 controls who were age- and BMI- matched as closely as possible to the 11 PASC predicted participants, were invited to participate in an additional set of 5 sampling timepoints over the third month of their infection.

All participants who completed the original 5 timepoints, as well as those who participated in the extended sampling ($n = 39$ total) were surveyed at months 4, 5, and 6 from initial symptom onset and all 39 participants completed these follow-up surveys.

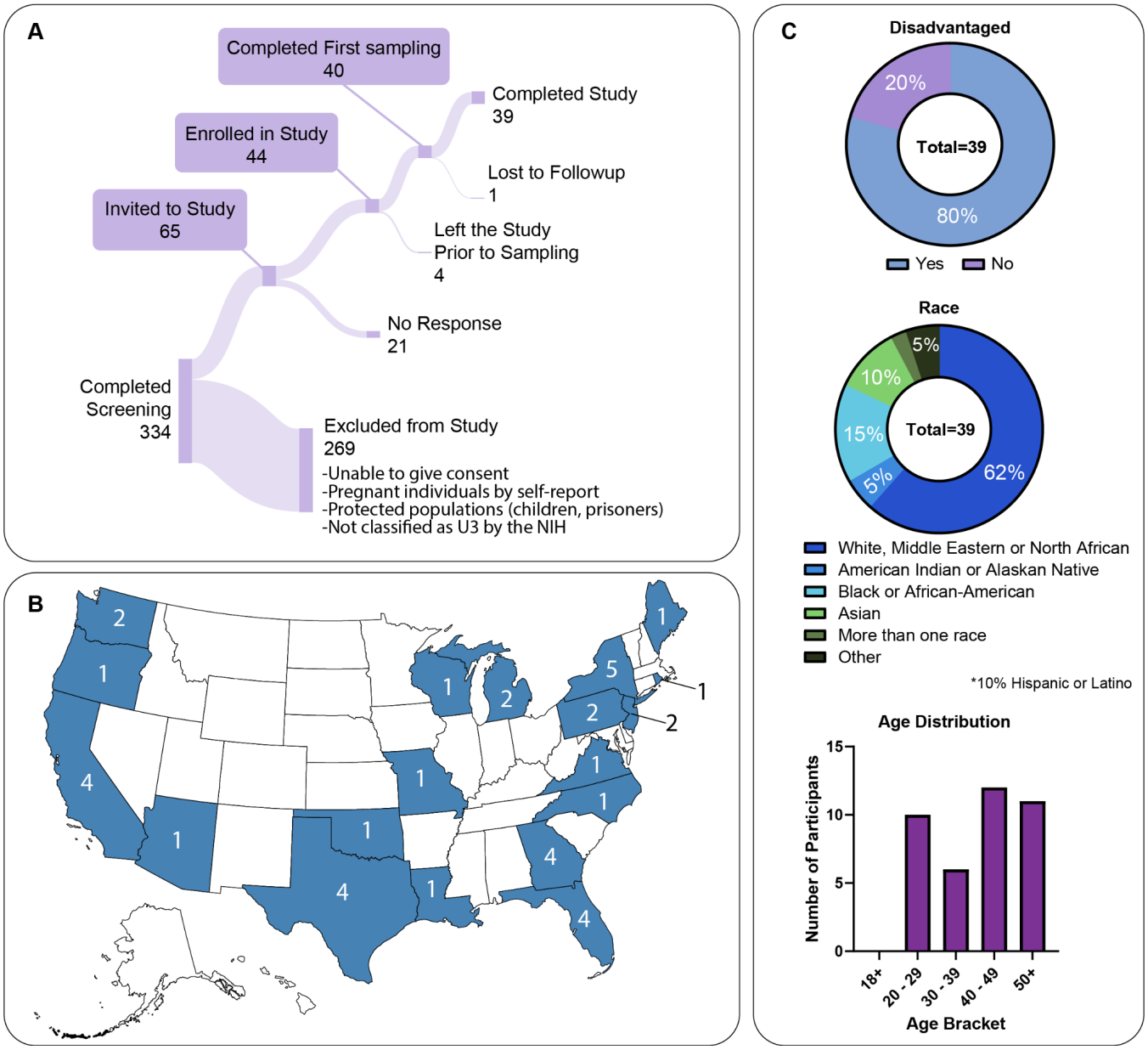


Figure 4.2. Outline of participant recruitment and demographics. (A) Sankey diagram depicting screening, enrollment, and retention in the study, (B) map of the United States with numbers of participants from each state, and (C) demographics of study participants including disadvantaged background per NIH, race, and age bracket.

Table 4.1. Participant demographics

Characteristic	All Participants (n = 39)	Extended Study participants (n = 16) PASC Predicted (n = 11)	Non-PASC Control (n = 5)
Sex[1]			
Male	0	0	0
Female	39 (100%)	11 (100%)	5 (100%)
Age (years)[1]	43 (14)	44 (13)	58 (10)
Gender			
Woman	37 (95%)	11 (100%)	5 (100%)
Man	0 (0%)	0 (0%)	0 (0%)
Transgender	0 (0%)	0 (0%)	0 (0%)
Nonbinary	2 (5%)	0 (0%)	0 (0%)
Race[1]			
White, Middle Eastern or North African	24 (62%)	5 (45%)	3 (60%)
American Indian or Alaskan Native	2 (5%)	1 (9%)	1 (20%)
Black or African-American	6 (15%)	2 (18%)	0 (0%)
Asian	4 (10%)	2 (18%)	1 (0%)
More than one race	1 (3%)	1 (9%)	2 (0%)
Other	2 (5%)	0 (0%)	1 (20%)
Ethnicity[1]			
Hispanic	6 (15%)	1 (9%)	1 (20%)
Non-Hispanic	33 (85%)	10 (91%)	4 (80%)
COVID-19 Vaccination Status[1]			
Vaccinated	34 (87%)	9 (82%)	4 (80%)
Not Vaccinated	5 (13%)	2 (18%)	1 (20%)
Number of COVID Infections (including present infection)[2]	2 (0.86)	2 (1)	1 (0)
BMI[2]	30 (7.2)	30 (7.1)	30 (4.8)

[1]n (%), [2]mean (SD)

The closing survey at timepoint 5 included questions about the ease of use of the homeRNA platform, as well as general study experience. High participant satisfaction was illustrated (Figure 4.3) by the willingness of the cohort to participate in future research, with all (n = 39/39) participants indicating that they would be willing to participate in this same study or a similar study again, with 95% (n = 37/39) of participants agreeing or strongly agreeing that they enjoyed participating in this study. A high percentage (92%, n = 36/39) of participants either agreed or strongly agreed that they perceived participation in a remote study to be easier than a study that required in-person visits. Most participants (82%, n = 32/39) indicated they would be willing to participate for up to 4 years in a similar study with 15 samples collected per year.

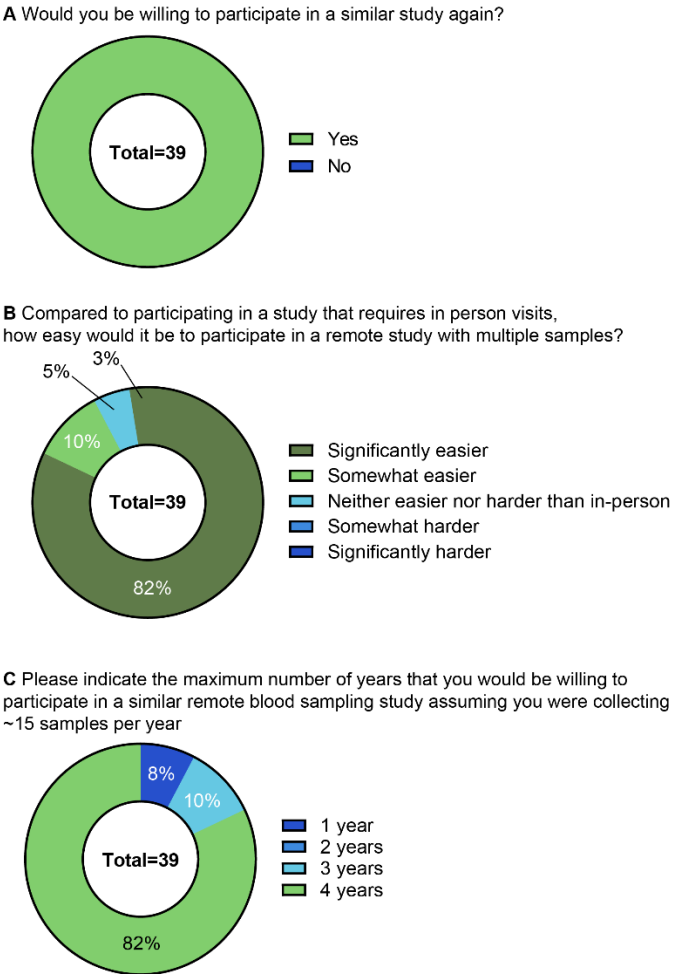


Figure 4.3. Summary of closing survey responses. All participants (n = 39/39) indicated that they would participate in a similar study again (A). Most participants (92%) indicated that a remote study would be easier or significantly easier than an in-person one. Most participants (82%) also indicated that they would be willing to participate in a remote study with 15 samples collected per year up to four years, which was the longest option available.

Beyond the flexibility of the remote design, study experience hinges on the sampling materials. Overwhelmingly, participants found the kit components easy to use, with the majority (>90%) indicating that they

either agreed or strongly agreed with each of the statements outlined in Figure 4.4, with only one participant disagreeing for a single component (Tasso-SST instructions). The participants similarly reported enjoying participating in the study with 77% (n = 30/39) strongly agreeing and 18% agreeing (n = 7/39). Furthermore, our design included a phone call prior to enrollment to provide participants with an opportunity to connect with a study coordinator, ask questions about the study, and voice any concerns. A high percentage (82%, n = 32/39) reported that they strongly agreed that the study phone call was helpful, with an additional 10% (n = 4/49) indicating that they agreed.

Sampling flexibility may be a strong component of the participants' positive perception of the study. This was reflected in the high percentage (92%, n = 36/39) of participants who either agreed or strongly agreed that they perceived participation in a remote study to be easier than a study that required in-person visits (Figure 4.3B).

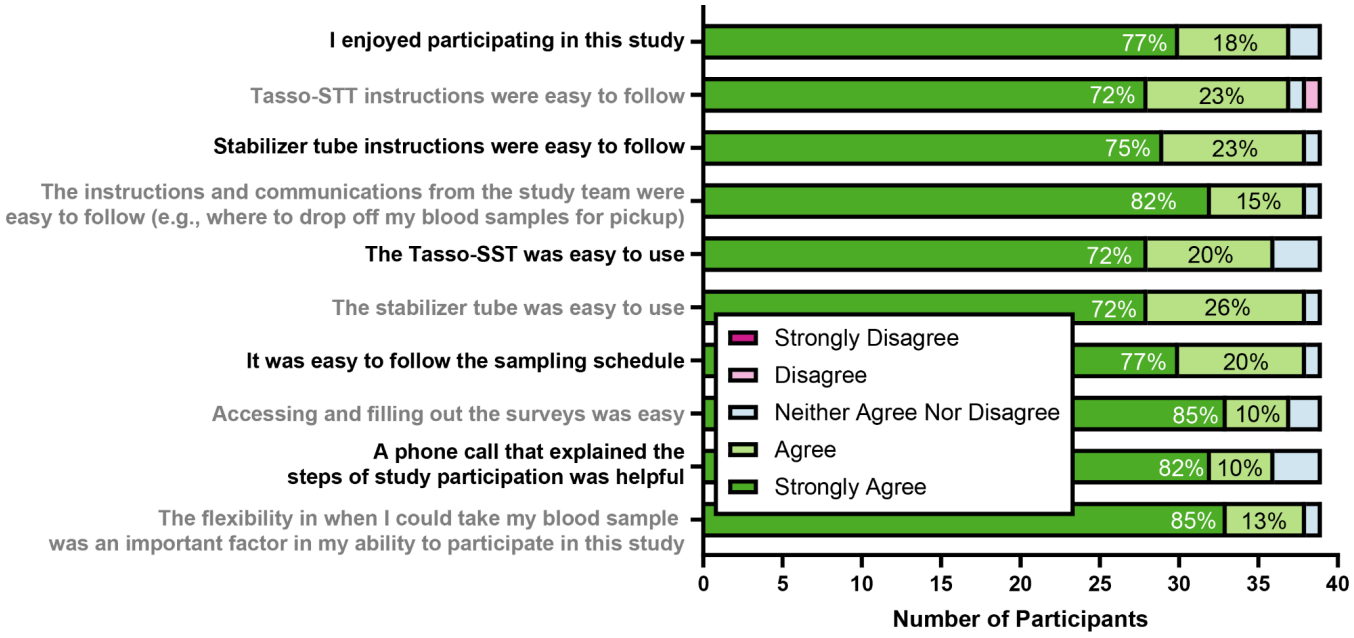


Figure 4.4. Summary of participant study experience. Each bar on the graph represents one question from the closing survey, with each of the sections corresponding to level of agreement for each statement. Overall, the majority of the participants (>90%) agreed or strongly agreed that they enjoyed participating in the study and

that each of the components of the homeRNA kit was easy to use. Only one person reported finding the Tasso-SST difficult to use.

An additional consideration for study design includes participant motivation. Our participants were asked to indicate all factors they considered when joining the study. The strongest motivator in this cohort was the research topic, with 85% indicating an interest in adding to the body of knowledge on the health impacts of COVID-19 as a reason for joining the study, followed closely by flexibility of a remote study (69%). Participants were also asked about their motivation for continuing in the study. They similarly indicated an interest in adding to the body of knowledge on the health impacts of COVID-19 (87%), flexibility of a remote study (67%), and positive interactions with the study team (59%) as motivators for their continued participation. Total number of responses can be found in Figure C5. It is also worth noting that this work was conducted at a time when interest in COVID research was waning nationally and free COVID tests were being discontinued.

The sampling flexibility was an important factor in participants' ability to be in the study (98%), and the initial phone call was helpful (92%). The sampling flexibility (time of day, location) overcame the reported barriers to in-person study participation (Figure C6), and the initial study phone call established rapport between the participants and study coordinators.

For participants who were selected for the extended sampling, another closing survey was administered at time point 10. The answers from these questions were analyzed using the same methods and can be found in Figure C7. The only notable changes were that at time point 10, no participants rated any of the kit components as difficult to use, and one person changed their answer for how long they would be willing to do a similar study from 3 years (survey at time point 5) to 4 years (survey at time point 10).

4.4 Discussion

In recent years, there has been an effort to determine factors and design considerations for conducting more inclusive clinical studies.[3,5,29–32] Some data have been collected on populations that qualify as U3 by the NIH definition and this has been an important step in balancing recruitment in clinical studies. Our work

contributes to these efforts by conducting a longitudinal study with complex molecular readouts and surveying our U3 cohort for motivation, study experience, and willingness to participate in future studies. The existing literature and our past experience conducting remote studies indicate that logistical barriers such as transportation, commute length, and scheduling prevent participation in research. One approach to overcoming these barriers is to shift the sample collection from a centralized clinic, research institute, or blood collection site to the participants' homes. The homeRNA platform, an at-home blood self-collection and RNA stabilization kit, enables this shift for longitudinal studies with transcriptomic readouts.

The successful implementation of the homeRNA platform in U3 populations is indicated by the high percentage (100%, n = 39/39) of participants who reported they would participate in a similar study again (Figure 4.3A). Participants also overwhelmingly reported that they enjoyed participating in the study and that homeRNA was easy to use (Figure 4.4) and that the study flexibility was an important contributor to their motivation for finishing the study. Further, we note that we had a greater number of calls from participants at study launch than with later recruits, which we attribute to the addition of more detailed instructions in the sampling reminder emails. In other words, we found that clear and detailed instructions, even when redundant, helped the participants understand the study procedures.

Furthermore, one of the more striking results of this work was the participant willingness to participate long-term in a remote clinical study (Figure 4.3C), with 82% (n = 32/39) indicating they would be willing to participate for up to 4 years in a similar study with 15 samples collected per year. This was the longest time period that participants were able to select in their surveys, suggesting that they may be willing to participate in even longer studies. In fact, in a follow-up study many participants from this cohort indicated that they would be willing to contribute to a study for “as long as necessary.” These survey data point to the success of the remote model in reaching U3 populations and suggest that remote studies can be valuable tools for longer-term longitudinal studies.

4.5 Limitations

While we were able to gather comprehensive data about participant symptoms, predicting likelihood of long-term symptoms was a major challenge. At the time of the study, there was limited and evolving information about the definition of PASC and the symptoms associated with it. A limitation of the study presented here is that we have not yet analyzed the nasal swabs to confirm COVID-19 infection status and that we do not know if there were any concurrent respiratory infections that could be contributing to the reported symptoms. As these samples are analyzed, our data will become more useful in understanding symptom progression. Moreover, the quality of the sequencing data from blood RNA will rely heavily on how well the samples were stabilized by the participants. Sample processing is currently underway and the preliminary results show good RNA quality and yields.

Furthermore, the closing survey was reformulated, and a subset of the first ten participants were asked to retake the survey up to two weeks after their original response. The time between their fifth sampling and the updated surveys could have impacted participant recollection of their experience in the study. However, for a question like "Based your experience in this study, would you participate in a similar remote or at-home study in the future?" (original closing survey) which was reformulated as "Would you be willing to participate in this study or a similar study again?" (updated closing survey), all ten participants answered "Yes" in both versions of the survey. While we do not report any meaningful changes in the responses between these two versions of the closing surveys, future studies would benefit from formulating the final versions of the survey prior to study launch to eliminate this potential confounding variable. Additionally, while the surveys were formulated with CFIR, the results are not organized based on the framework's constructs.

In terms of study logistics, one limitation of our design was recruitment. Since we primarily advertised through social media, individuals who would otherwise be eligible but did not know about our study would not be recruited. In other studies that focus on reaching U3 populations, it may be useful to adopt a hybrid approach where advertising is done both online and with physical media including flyers, posters, and newspapers.

Finally, it is important to note that while the remote, homeRNA-based study model overcomes logistical barriers to participation, more work needs to be done to overcome the larger cultural and systemic issues that have resulted in the underrepresentation of certain populations in clinical research.

4.6 Conclusion

The homeRNA-based remote longitudinal study model was successful in a cohort of COVID-19+ U3 women. Survey responses indicated high satisfaction with study experience with excellent retention (98%, n = 39/40) over 6 months, despite the complexity and burden of the study. This success was attributed to a combination of the flexibility of remote sampling, communication with the study team, and ease of use of the sampling kit components. As such, we conclude that the remote study model outlined here can be a powerful tool for reaching participants from U3 populations who might otherwise be unlikely to participate in scientific or clinical studies. In ongoing work, we are further characterizing this cohort and their motivations through additional surveys and interviews to better understand the factors that contributed to the excellent participant retention and engagement. Ultimately, we hope that this body of work will serve as a roadmap for designing flexible and inclusive longitudinal studies with remote biospecimen collection.

4.7 References

1. Robiner WN, Yozwiak JA, Bearman DL, Strand TD, Strasburg KR. Barriers to clinical research participation in a diabetes randomized clinical trial. *Social Science & Medicine*. 2009;68(6):1069-1074. doi:10.1016/j.socscimed.2008.12.025

2. Marcantonio ER, Aneja J, Jones RN, et al. Maximizing Clinical Research Participation in Vulnerable Older Persons: Identification of Barriers and Motivators. *Journal of the American Geriatrics Society*. 2008;56(8):1522-1527. doi:10.1111/j.1532-5415.2008.01829.x

3. Luebbert R, Perez A. Barriers to Clinical Research Participation Among African Americans. *J Transcult Nurs*. 2016;27(5):456-463. doi:10.1177/1043659615575578

4. Hensen B, Mackworth-Young CRS, Simwinga M, et al. Remote data collection for public health research in a COVID-19 era: ethical implications, challenges and opportunities. *Health Policy Plan*. 2021;36(3):360-368. doi:10.1093/heapol/czaa158

5. Coakley M, Fadiran EO, Parrish LJ, Griffith RA, Weiss E, Carter C. Dialogues on diversifying clinical trials: successful strategies for engaging women and minorities in clinical trials. *J Womens Health (Larchmt)*. 2012;21(7):713-716. doi:10.1089/jwh.2012.3733

6. Nomali M, Mehrdad N, Heidari ME, et al. Challenges and solutions in clinical research during the COVID-19 pandemic: A narrative review. *Health Science Reports*. 2023;6(8):e1482. doi:10.1002/hsr2.1482

7. Loucks TL, Tyson C, Dorr D, et al. Clinical research during the COVID-19 pandemic: The role of virtual visits and digital approaches. *Journal of Clinical and Translational Science*. 2021;5(1):e102. doi:10.1017/cts.2021.19

8. Chaussabel D, Pulendran B. A vision and a prescription for big data–enabled medicine. *Nat Immunol*. 2015;16(5):435-439. doi:10.1038/ni.3151

9. Rinchai D, Deola S, Zoppoli G, et al. High–temporal resolution profiling reveals distinct immune trajectories following the first and second doses of COVID-19 mRNA vaccines. *Science Advances*. 2022;8(45):eabp9961. doi:10.1126/sciadv.abp9961

10. Haack AJ, Lim FY, Kennedy DS, et al. homeRNA: A Self-Sampling Kit for the Collection of Peripheral Blood and Stabilization of RNA. *Anal Chem*. 2021;93(39):13196-13203. doi:10.1021/acs.analchem.1c02008

11. Stefanovic F, Brown LG, MacDonald J, et al. Your Blood is Out for Delivery: Considerations of Shipping Time and Temperature on Degradation of RNA from Stabilized Whole Blood. *Anal Chem*. 2025;97(3):1635-1644. doi:10.1021/acs.analchem.4c04591

12. Lim FY, Kim SY, Kulkarni KN, et al. High-frequency home self-collection of capillary blood correlates *IFI27* expression kinetics with SARS-CoV-2 viral clearance. *J Clin Invest*. 2023;133(23). doi:10.1172/JCI173715

13. Haack AJ, Brown LG, Zeng Y, et al. A Flexible and Responsive Remote Study Design to Assess Gene Expression Changes During Wildfire Smoke Exposure with homeRNA, an At-home Blood Sampling Kit. *medRxiv*. Preprint posted online October 14, 2025:2025.10.11.25337783. doi:10.1101/2025.10.11.25337783

14. Lim FY, Lea HG, Dostie AM, et al. homeRNA self-blood collection enables high-frequency temporal profiling of presymptomatic host immune kinetics to respiratory viral infection: a prospective cohort study. *eBioMedicine*. 2025;112. doi:10.1016/j.ebiom.2024.105531

15. Brown LG, Haack AJ, Kennedy DS, et al. At-home blood collection and stabilization in high temperature climates using homeRNA. *Front Digit Health*. 2022;4:903153. doi:10.3389/fdgth.2022.903153

16. Sudre CH, Murray B, Varsavsky T, et al. Attributes and predictors of long COVID. *Nat Med*. 2021;27(4):626-631. doi:10.1038/s41591-021-01292-y

17. Raman B, Bluemke DA, Lüscher TF, Neubauer S. Long COVID: post-acute sequelae of COVID-19 with a cardiovascular focus. *Eur Heart J*. 2022;43(11):1157-1172. doi:10.1093/eurheartj/ehac031

18. Parotto M, Gyöngyösi M, Howe K, et al. Post-acute sequelae of COVID-19: understanding and addressing the burden of multisystem manifestations. *The Lancet Respiratory Medicine*. 2023;11(8):739-754. doi:10.1016/S2213-2600(23)00239-4

19. Al-Aly Z, Xie Y, Bowe B. High-dimensional characterization of post-acute sequelae of COVID-19. *Nature*. 2021;594(7862):259-264. doi:10.1038/s41586-021-03553-9

20. Thaweethai T, Donohue SE, Martin JN, et al. Long COVID trajectories in the prospectively followed RECOVER-Adult US cohort. *Nat Commun*. 2025;16(1):9557. doi:10.1038/s41467-025-65239-4

21. Wisk LE, L'Hommedieu M, Diaz Roldan K, et al. Variability in Long COVID Definitions and Validation of Published Prevalence Rates. *JAMA Netw Open*. 2025;8(8):e2526506. doi:10.1001/jamanetworkopen.2025.26506

22. Bowe B, Xie Y, Al-Aly Z. Postacute sequelae of COVID-19 at 2 years. *Nat Med*. 2023;29(9):2347-2357. doi:10.1038/s41591-023-02521-2

23. Cai M, Xie Y, Topol EJ, Al-Aly Z. Three-year outcomes of post-acute sequelae of COVID-19. *Nat Med*. 2024;30(6):1564-1573. doi:10.1038/s41591-024-02987-8

24. Bauer MS, Kirchner J. Implementation science: What is it and why should I care? Psychiatry Research. 2020;283:112376. doi:10.1016/j.psychres.2019.04.025

25. Breimaier HE, Heckemann B, Halfens RJG, Lohrmann C. The Consolidated Framework for Implementation Research (CFIR): a useful theoretical framework for guiding and evaluating a guideline implementation process in a hospital-based nursing practice. BMC Nurs. 2015;14(1):43. doi:10.1186/s12912-015-0088-4

26. Pre-existing conditions associated with post-acute sequelae of COVID-19 | Elsevier Enhanced Reader. doi:10.1016/j.jaut.2022.102991

27. Damschroder LJ, Reardon CM, Widerquist MAO, Lowery J. The updated Consolidated Framework for Implementation Research based on user feedback. *Implementation Science*. 2022;17(1):75. doi:10.1186/s13012-022-01245-0

28. Our Flourishing Measure | The Human Flourishing Program. Accessed October 17, 2025. https://hfh.fas.harvard.edu/measuring-flourishing

29. Wendler D, Kington R, Madans J, et al. Are Racial and Ethnic Minorities Less Willing to Participate in Health Research? *PLOS Medicine*. 2005;3(2):e19. doi:10.1371/journal.pmed.0030019

30. Girardi G, Longo M, Bremer AA. Social determinants of health in pregnant individuals from underrepresented, understudied, and underreported populations in the United States. *Int J Equity Health*. 2023;22(1):186. doi:10.1186/s12939-023-01963-x

31. Stewart J, Krows ML, Schaafsma TT, et al. Comparison of Racial, Ethnic, and Geographic Location Diversity of Participants Enrolled in Clinic-Based vs 2 Remote COVID-19 Clinical Trials. *JAMA Netw Open*. 2022;5(2):e2148325. doi:10.1001/jamanetworkopen.2021.48325

32. Garber M, and Arnold RM. Promoting the Participation of Minorities in Research. *The American Journal of Bioethics*. 2006;6(3):W14-W20. doi:10.1080/15265160600686331

Appendix

A. Appendix for Chapter 2

EXPERIMENTAL

Participant Recruitment and Demographics

Healthy adult volunteers (18 years or older) were recruited in Seattle, Washington via word of mouth under a protocol approved by the University of Washington Institutional Review Board study number STUDY00014133. Written informed consent was obtained from all participants.

Investigating RNA Integrity of RNA*later* Stabilized Blood Samples in Temperature Controlled Experiments

Investigating RNA Integrity of RNA*later* Stabilized Blood Samples in Temperature Controlled Experiments

Exposure to Longer Term (2, 4, and 8 days) High Temperatures (>37°C)

A venous blood draw was performed and promptly stabilized with RNA*later* at the manufacturer's recommended ratio (2.6 mL RNA*later* per 1 mL blood). For the first biological replicate, blood was acquired via venipuncture from Bloodworks and stabilized within 1-2 hours. For the second and third replicate, blood was collected via venipuncture in lab under study number STUDY00014133. After stabilization with RNA*later,* the stabilized blood was aliquoted in 1.8 mL samples to replicate the maximum volume of homeRNA (0.5 mL blood and 1.3 mL RNA*later*). After stabilization, each sample was stored according to the following conditions: (1) immediately incubated at set temperatures (25°C, 37°C, 40°C, 45°C, and 50°C) for 2, 4, and 8 days, (2) kept at 4°C for 24 hours then incubated at set temperatures (25°C, 37°C, 40°C, 45°C, 50°C) for 2, 4, and 8 days, and (3) kept at 25°C for 24 hours then incubated at set temperatures (25°C, 37°C, 40°C, 45°C, 50°C) for 2, 4, and 8 days. Each condition had a corresponding control: (1) frozen immediately at -20°C, (2) incubated at 4°C for 24 hours then frozen at -

20℃, (3) incubated at 25℃ for 24 h then frozen at -20℃. One sample at each temperature and condition was removed after 2, 4, and 8 days such that there was a single replicate for each temperature and condition on each day of removal. At these timepoints, the blood was moved from the incubators to the -20℃ freezer until ready for RNA isolation. For each condition, RNA from the stabilized blood samples was extracted based on timepoints, resulting in three total extraction batches based on day removed after high temperature exposure (day 2, 4, and 8) with six samples in each batch (condition control, 25℃, 37℃, 40℃, 45℃, and 50℃). The experiment was repeated two more times for a total of three replicates from two donors (replicate 2 and 3 from the same donor, with the blood drawn on separate days).

Exposure to Shorter Term (<2 days) High Temperatures (>37℃)

A venous blood draw was performed in lab under study number STUDY00014133 and promptly stabilized with RNA*later* at the manufacturer's recommended ratio (2.6 mL RNA*later* per 1 mL blood). The stabilized blood was then aliquoted to 1.8 mL samples. One sample was immediately frozen at -20℃ as the control. The rest of the samples were then treated according to the following conditions: (1) one sample kept at room temperature (25℃) overnight (16 h), then frozen (-20℃), (2) five samples kept at room temperature (25℃) overnight (16 h), moved to incubators of corresponding temperatures (25℃, 37℃, 40℃, 45℃, 50℃) for 6 h, then frozen (-20℃), (3) five samples kept at room temperature (25℃) overnight (16 h), moved to incubators of corresponding temperatures (25℃, 37℃, 40℃, 45℃, 50℃) for 6 h, moved back to room temp (25℃) for 24 h, then frozen (-20℃). This resulted in a total of one replicate for each temperature and condition combination. The stabilized blood samples were stored at -20℃ until ready for RNA isolation and assessment. All 12 samples from each replicate were extracted in one batch. The experiment was repeated two more times for a total of three replicates from three separate blood draws from one donor.

Effects of a Freeze-Thaw Cycle on RNA Stability

For this experiment, blood from a venous blood draw is stabilized using RNA*later* according to manufacturer instructions. The blood is then aliquoted into 9 samples which are separated into three conditions: (1) control which is immediately frozen, (2) room temperature (25°C) for 24 h (room temperature overnight, RTON) then frozen, and (3) freeze-thaw (FT) where the samples were frozen immediately after stabilization for 24 h, then kept at room temperature for 24 h before being frozen again. The samples were frozen at -20°C for the duration of the experiment then moved to -80°C until further processing. The experiment was performed two more times for a total of three replicates. Once the RNA was extracted the samples were tested for RNA integrity using the Agilent 2100 Bioanalyzer.

Investigating RNA Integrity of RNA*later* Stabilized Blood Samples in Real World Setting with homeRNA

*Shipping of RNA*later-*Stabilized Blood Across United States with Continuous Temperature Monitoring*

A venous blood draw was performed in the lab under study number STUDY00014133. The blood sample was aliquoted into 48 Tasso-SST tubes at 0.5 mL each. The blood was then stabilized by attaching the Tasso-SST tubes to a custom-designed stabilizer tube with 1.3 mL RNA*later* and shaken to mimic blood stabilization in homeRNA kits. The remaining blood was stabilized in bulk (2.6 mL RNA later per 1 mL blood), aliquoted into 1.8 mL samples, and immediately frozen at -20℃ as controls. The 48 homeRNA-stabilized samples were then placed in 50 mL conical tubes fitted with custom adapters as described in Haack et al. 2021.[9] The 50 mL tubes were then adhered together using tape in sets of three such that three technical replicates would be sent to each shipped location. Each set had an RC-5+ continuous temperature monitor (Elitech) directly attached with lab tape (see Figure A1A), with temperatures being recorded every 2 minutes. These constructs were then placed in a biohazard bag and into the box used for homeRNA kits (see Haack et al., 2021) prior to being packaged into a UPS LabPak. The full setup is shown in Figure A1. The complete packages were shipped to volunteers in 14 different states across the United States via UPS. The states included: Washington (WA), New Mexico (NM), North Carolina (NC), Minnesota (MN), Maine (ME), Massachusetts (MA), Kansas (KS), Illinois (IL), Georgia

(GA), Colorado (CO), California (CA), Arizona (AZ), Hawaii (HI), and Nebraska (NE). One additional package was kept in a 25℃ incubator (In-Lab/Lab). Upon delivery, the volunteers were instructed to keep the packages inside overnight at room temperature and adhere the return labels to the package. For pickup, most volunteers left the package outside on the front porch, two volunteers left the package indoors in a mailroom, and one volunteer directly handed the package to the UPS courier. Most samples were picked up within 1 – 2 days from the volunteer and shipped back to the lab. Shipping return times ranged from overnight to 4 days after pickup. Upon return to the lab, the packages were immediately placed into a -20℃ freezer. Once all the packages were returned, the samples that were kept in the lab in a 25℃ incubator were also frozen at -20℃. All samples were stored at -20℃ until ready for RNA isolation. RNA was extracted from all shipped homeRNA-stabilized blood samples in three extraction batches, with one technical replicate from each shipping location and in-lab baseline samples (immediately frozen and constant 25℃ sample), resulting in 16 samples extracted in each batch.

Investigating RNA Integrity of RNA*later* Stabilized Blood Samples in Real World Setting with homeRNA

Visualization of Temperature Over Time Across Shipping Locations

The data from the temperature probe directly attached to the samples is assumed to have readings closest to the "true" temperature the samples would experience in transit and only the data from this probe are visualized and used for downstream analysis. Since the temperature probes were not stopped until all packages arrived, the data needed to be filtered prior to graphing. The data were filtered using the R dplyr package such that the time did not exceed 200 hours (close to the maximum time for the last sample returned to lab) and was above 10°C given that the lowest temperature reading prior to being frozen was closer to 20°C for all samples. The temperature was then plotted against time (hours) to illustrate the variability in shipping process and temperature fluctuations.

RNA Isolation

For all samples, total cellular RNA was isolated using the Ribopure Blood RNA Isolation Kit (Thermo Fisher) according to manufacturer's protocol and eluted in two 50 µl aliquots. Isolated RNA was stored at -80°C until ready for further analysis.

Assessment of total cellular RNA integrity and yield

After isolating the RNA from the homeRNA-stabilized samples, RNA Integrity number (RIN) values were obtained on a Bioanalyzer 2100 (Agilent) from the first 50 µl elution. The Agilent 2100 Bioanalyzer uses proprietary, built-in software to calculate RIN values, which is based on the relative peak height and shape of the 18S and 28S rRNA fragments in the resulting electropherogram after separation. All samples were assessed using the RNA 6000 Nano Kit (Agilent).

For the in-lab temperature controlled experiments, RNA concentration was measured from the first 50 µl elution aliquot using a Cytation 5 Multi-Mode Reader (Agilent Biotek Instruments) with a Take3 Micro-Volume Plate at the wavelengths of 260, 280, and 320 nm. For the field experiments, RNA concentration was measured from the first 50 µl elution using a Qubit 4 Fluorometer using the RNA High Sensitivity (HS) Assay kit (Invitrogen).

RNA Sequencing and Analysis

3' mRNA-seq of Real-World Shipping Experiment

After isolation the samples were tested for RIN scores on the Agilent 2100 Bioanalyzer (see Assessment of total cellular RNA integrity and yield section above and Figure A8 for resulting electropherograms). A 100 ng of total RNA from 20 selected samples were sequenced by the Lexogen facility (Vienna, Austria) using 3' mRNA-sequencing technology (Lexogen QuantSeq 3' mRNA-seq V2 Library Prep Kit FWD with UDI) on an Illumina NextSeq 2000 instrument according to the manufacturers' standard instructions. The 16 samples from the third batch were selected for sequencing as the third batch had the greatest proportion of the median RIN values amongst the three replicates from each location. Additionally, all three replicates from the baseline sample (frozen

immediately after stabilization) and all three replicates from the location with the lowest RIN (NE, mean RIN of 5.5) were sent for sequencing. In total, 20 samples (16 from batch 3, 2 additional from NE, 2 additional from In-Lab) were sent for 3' mRNA sequencing. Prior to library preparation, all samples were treated with Ambion DNase I (Invitrogen) for 10 minutes at 37°C followed by heat inactivation with EDTA at 75C for 10 minutes. Libraries were prepared using 17 ng of RNA as input. Libraries were amplified for 18 cycles and were quality controlled on a Fragment Analyzer device using the DNF-474 HS NGS Fragment kit (1-6000 bp) (Agilent). The samples were sequenced to a read depth of 5 million reads.

Transcriptomic Analysis of Individual Gene Expression Counts

Summarized counts/genes were imported into R and analyzed using the limma-voom pipeline, which uses conventional weighted linear regression to model the data.[31,32] The matrix of gene counts was filtered to remove those genes with consistently low expression, and then converted to log counts/million counts, using estimated library sizes calculated using the trimmed mean of M-values (TMM) method.[33] The logCPM values have a dependence between the mean and variance which is estimated as part of the limma-voom pipeline and used to estimate observation-level weights. These weights are used as part of the weighted line regression to adjust for heteroskedasticity. We fit a model that includes time >30°C for each sample, the RIN (to adjust for RNA quality), and five surrogate variables computed using the Bioconductor sva package. The surrogate variables are intended to adjust for unobserved variability and have been shown to increase power to detect differences.[34] We then tested for any apparent changes in gene expression that correlate with exposure to high temperatures.

TABLES

Table S1: Summary of temperature probe data

Location	**RUCA**	**Time (h)**	**T_{avg} (°C)**	**T_{med} (°C)**	**T_{max} (°C)**	**T_{sd} (°C)**	**Hours above 30°C**

	[4]						
AZ	1	168	26.8	26.9	40	4.2	38.3
CA	1	72	26.5	25.4	44.4	4.9	11.1
CO	4	96	24.3	24.1	39.5	4.4	7.6
GA	1	72	24.8	24.3	41.9	4.4	7.4
HI	4	193	26.1	25.5	38.5	3.7	22.8
IL	1	72	23.7	22.6	38	1.9	4.5
In-Lab	1	200	25.5	25.5	25.7	0.4	0
KS	1	71	25.8	24.0	43.3	4.9	10.4
MA	1	72	26.4	26.5	40.9	3.5	8.2
ME	5	72	24.0	23.4	35.4	3.2	3.8
MN	1	71	25.4	23.3	39.3	5.6	14.4
NC	5	72	27.7	28	38.8	4.1	18
NE	10	168	28.5	27.2	45.1	5.5	55
NM	1	72	26.4	26.1	39.3	3.9	9.5
WA	1	71	23.8	23.1	36.6	3.1	3.7

[4] A RUCA value of 1 represents a metropolitan area where a value of 10 represents a rural area.

FIGURES

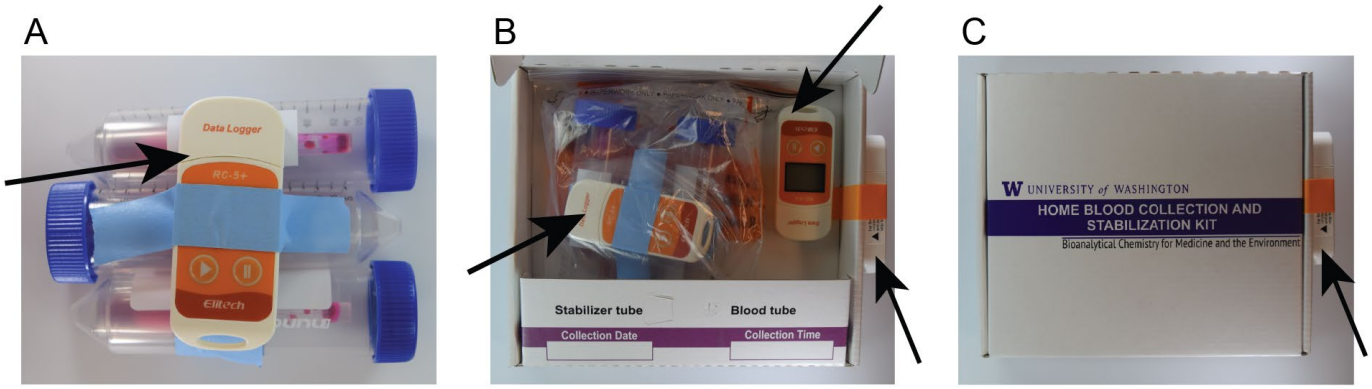


Figure A1. Shipping experiment continuous temperature probe setup. (A) Each homeRNA-stabilized sample is placed in a 50 mL conical tube with a custom insert to prevent excessive movement in the shipping process. Three such replicates are taped together and a temperature monitor (indicated by black arrows) is attached directly to this construct. (B) The samples are sealed in a biohazard bag and are then placed in the homeRNA box. Another temperature probe is placed inside of the box as a backup. (C) Then an additional temperature probe is attached to the outside as a final backup; the entire box is placed into a UPS LabPak and shipped to the participants. These samples are kept indoors overnight at which point a pre-printed return label (in a label pouch on the outside of the LabPak) is attached. The LabPak is placed on the volunteers' front porch or mailroom and picked up by UPS to be returned to the lab.

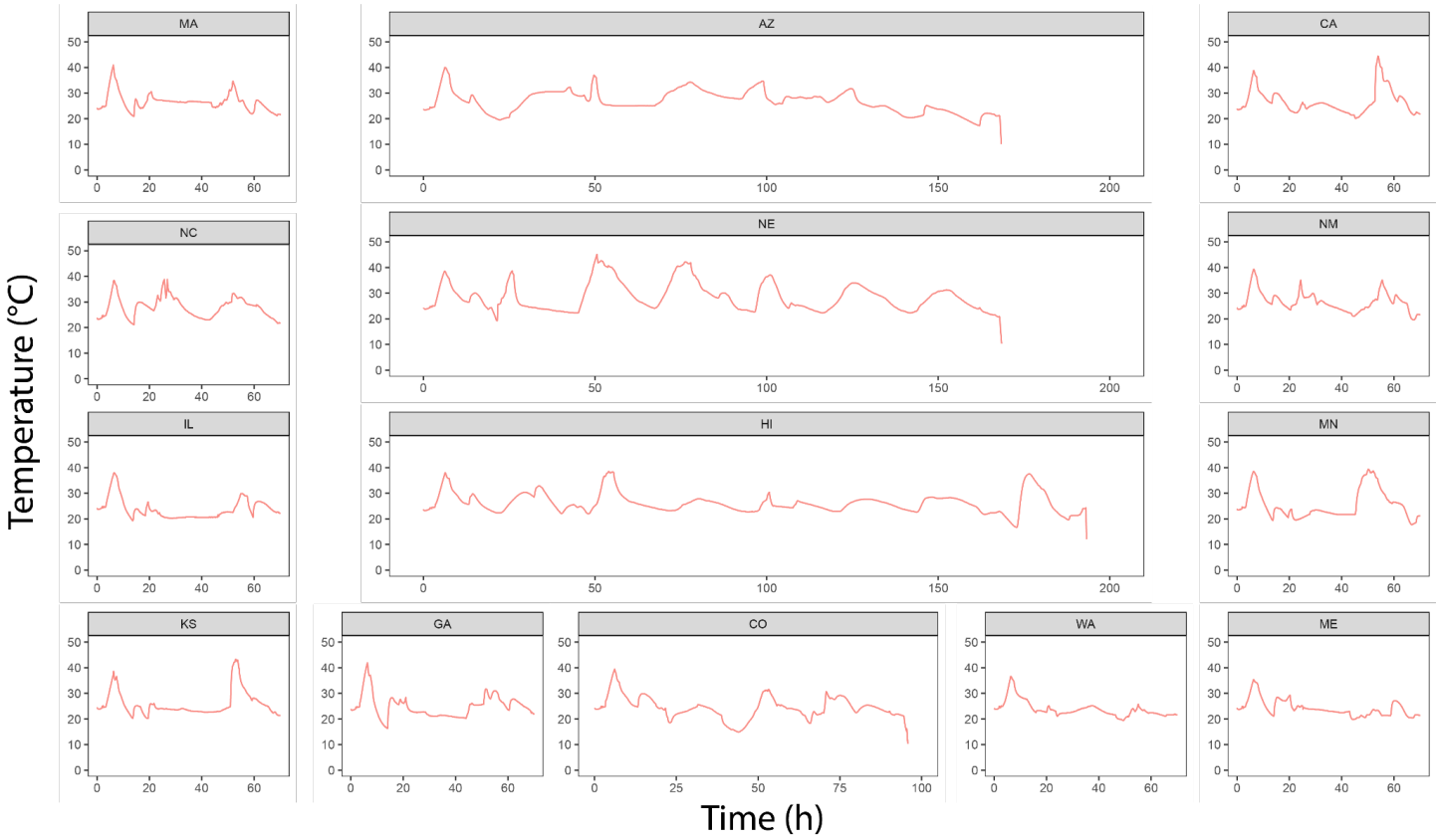


Figure A2. Visual summary of temperature probe data from each location. Before the packages were picked up by the courier service and shipped to the participant, all packages were placed in a UPS box located on the University of Washington campus. During this time in the UPS box, the temperature monitors in the packages showed a peak, with an average maximum temperature of 38.6°C (over 14 temperature probes) and reaching up to 41.9°C (one temperature probe).

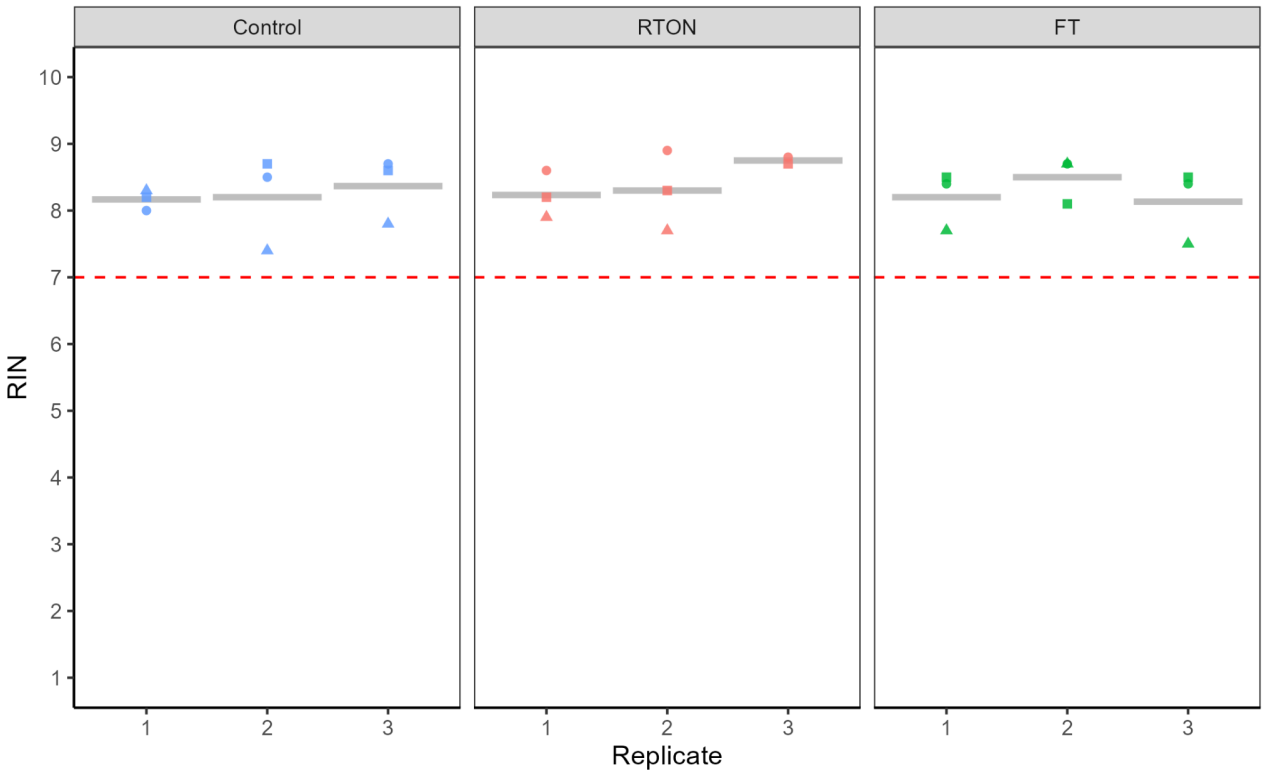


Figure A3. Effects of freeze-thaw on RNA integrity in homeRNA-stabilized blood samples. The resulting RINs are plotted here where each column represents the condition, with the gray crossbars indicating average values for each replicate. Overall, the data did not suggest that there were any adverse effects on RNA integrity as a result of a freeze-thaw cycle of homeRNA-stabilized blood samples.

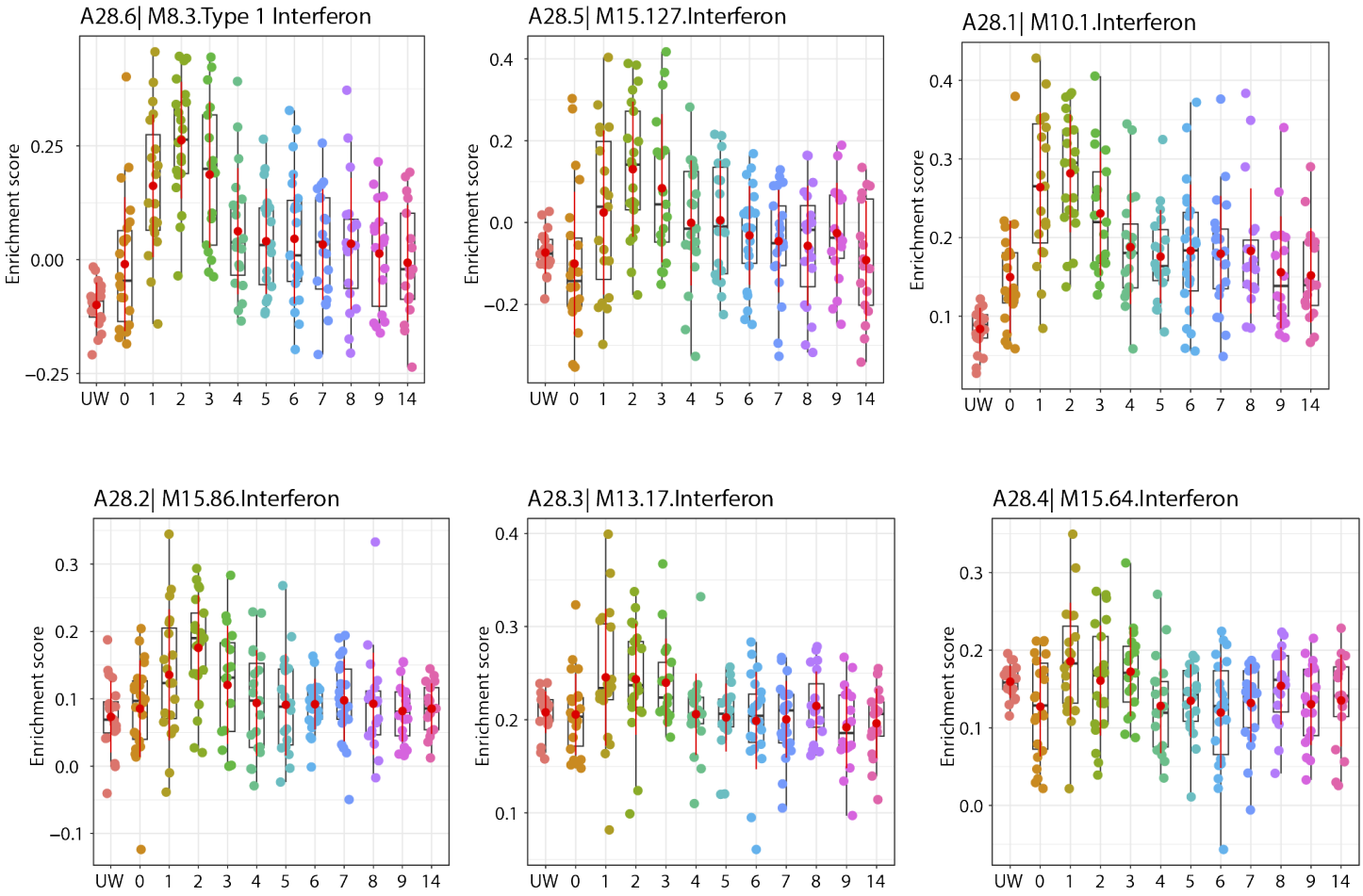


Figure A4: Analysis of Interferon Modules Enrichment Scores of Subjects Pre- and Post- Vaccination. The dot plots show the enrichment scores for six interferon response modules (M8.3, M10.1, M13.17, M15.64, M15.86, and M15.127) for the current study (UW) and at different days before and after administration of COVID-19 mRNA vaccine (COVAX study – day 0 = pre-vaccination sample). Following the UW (shipping) samples, the y-axis represents days of sampling from the COVAX study.

Supplementary discussion of figure A5

In addition to the BloodGen3 analysis, we also fit a model that includes the time spent above a temperature of 30°C, RIN, and 4 surrogate variables (generated using the Bioconductor sva package) to account for any unobserved technical variability.[34] After fitting this model, we selected genes with a false discovery rate (FDR) of <0.05, which estimates the maximum number of false positives in a set of significant results, as well as requiring at least a 1% change in expression. This resulted in only two genes (MMP9 and ADGRG3) that had a decrease in expression as a function of time spent at 30°C or higher (Figure A5). While both genes appear to be affected by storage at high temperatures, they also exhibit a notable amount of noise mainly due to the small sample size. Moreover, linear regression is not resistant to outliers; in our data, we propose that there are some (3 for MMP9 and 4 for ADGRG3) high-leverage points that are multiple orders of magnitude greater than the rest of the points and are not representative of the rest of the samples. This suggests that the two genes MMP9 and ADGRG3 potentially could be affected by shipping conditions, but future work with more sensitive methods could potentially capture differences in the transcriptome not detected here.

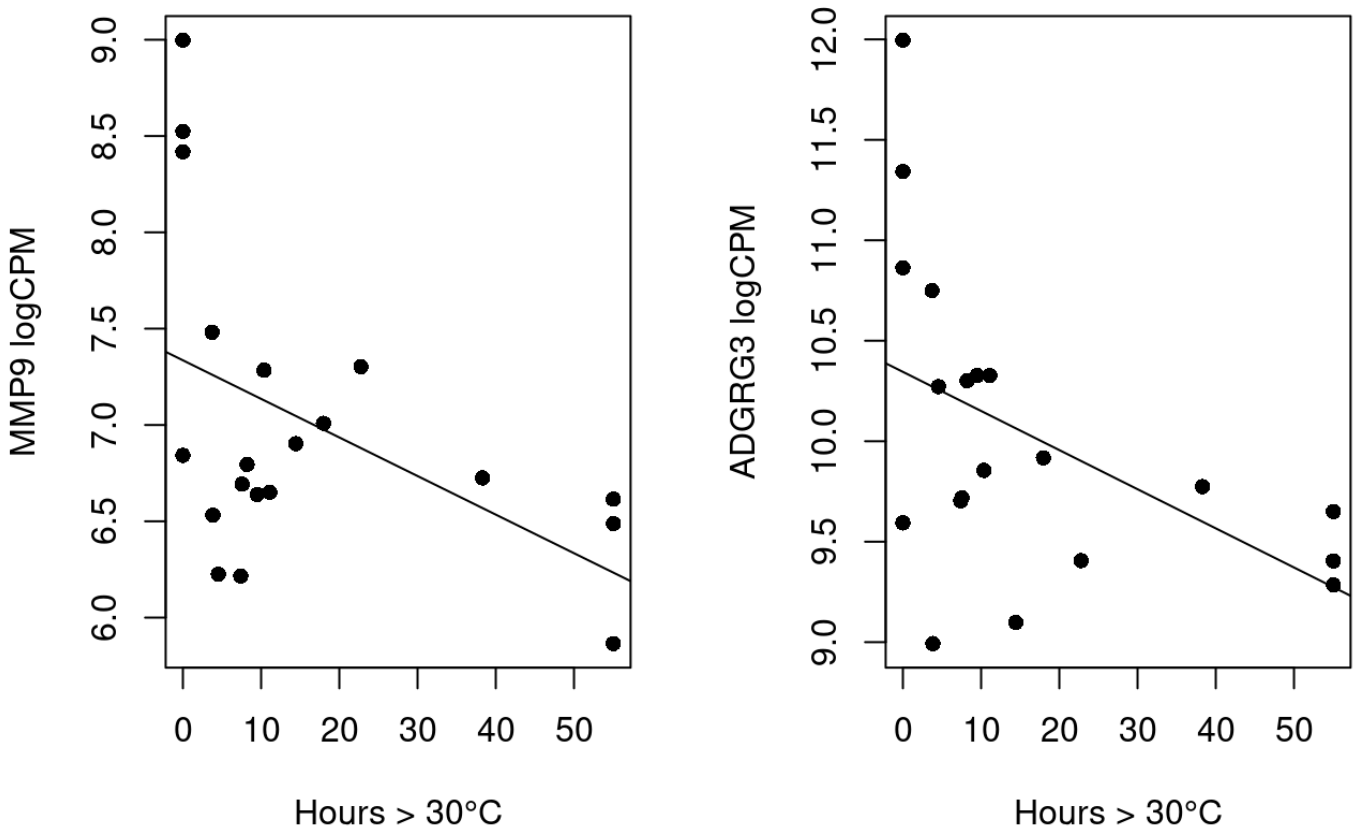


Figure A5. Expression levels of MMP9 and ADGRG3 as a function of time in excess of 30°C. The slope for both genes corresponds to an approximate 34% reduction in transcript for each 10 hours > 30°C.

Supplementary discussion of figures S6 and S7

Further, we fit linear regressions with “time in transit” or “time above 30°C” against RIN scores to better understand the effects these variables have on RNA degradation (Figures S6 and S7). All samples that spent below 100 hours in transit had RIN scores at or above 7. The samples that were in transit the longest were AZ (168 h), HI (193 h), and NE (168 h) and had mean RINs of 6.1, 6.2, and 5.5. Here, the data show that the time in transit alone is not representative of RIN since HI had a higher RIN score than NE even though it was in transit for longer. If we consider time spent over 30°C, AZ was exposed to temperatures above 30°C for only 38.3 hours, HI was exposed for 22.8 hours, while NE was exposed for a total of 55 hours. When the time spent above 30°C is considered, the corresponding RIN scores seem to follow a more sensible pattern with longer exposures leading to lower RIN values.

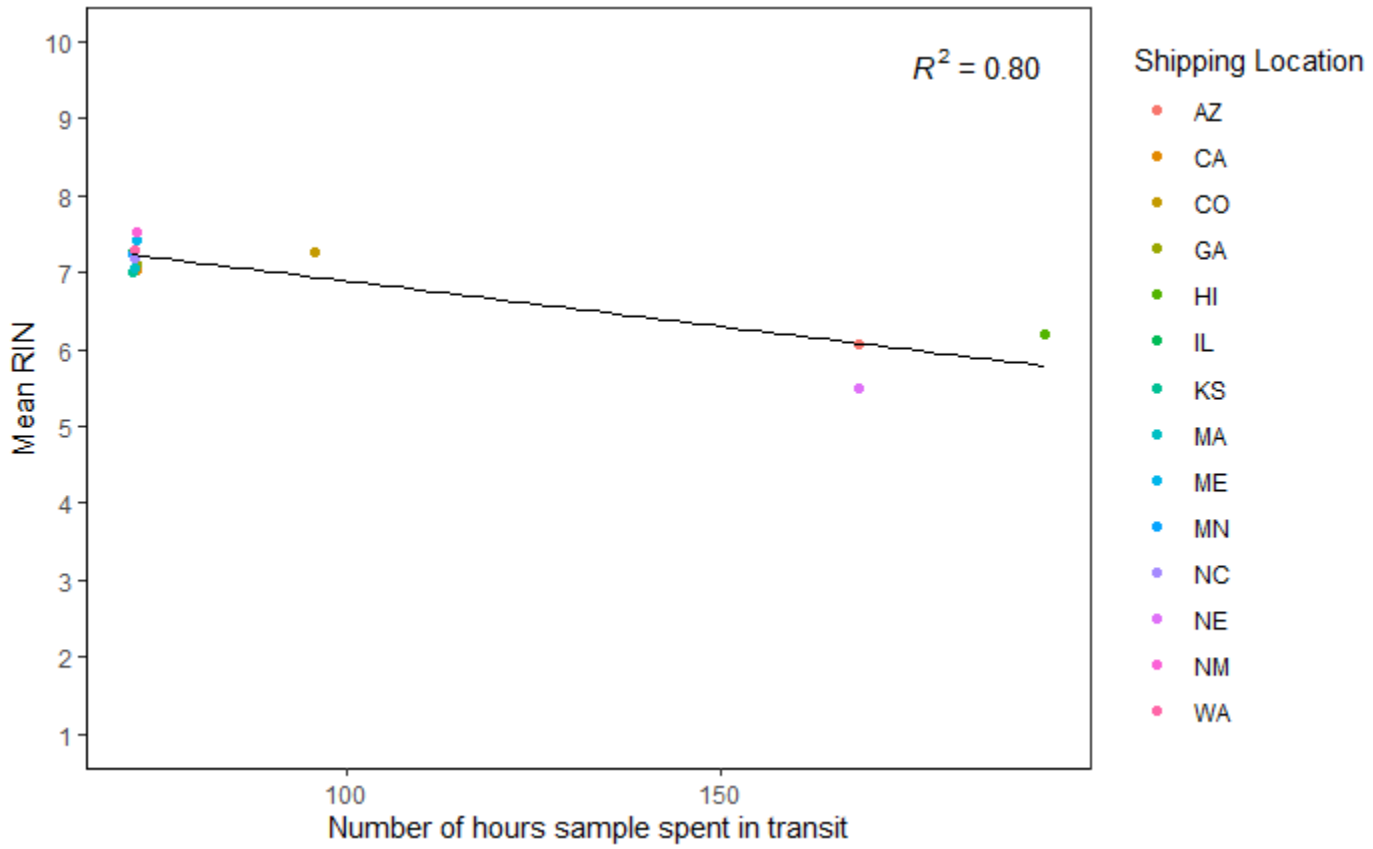


Figure A6. Effects of transit time on RNA integrity in homeRNA-stabilized blood samples. Mean RIN scores for each location are plotted against the times in transit for each location which are extrapolated from the temperature probe data visualized above. Though a trend of lower RINs with longer times in transit is observed, the R^2 value is only 0.57, indicating that there is not a strong correlation between the two variables suggesting that increased time in transit minimally affects the RNA integrity of homeRNA-stabilzied blood samples. Despite this trend, all RIN values are well within the acceptable range for 3' mRNA sequencing.

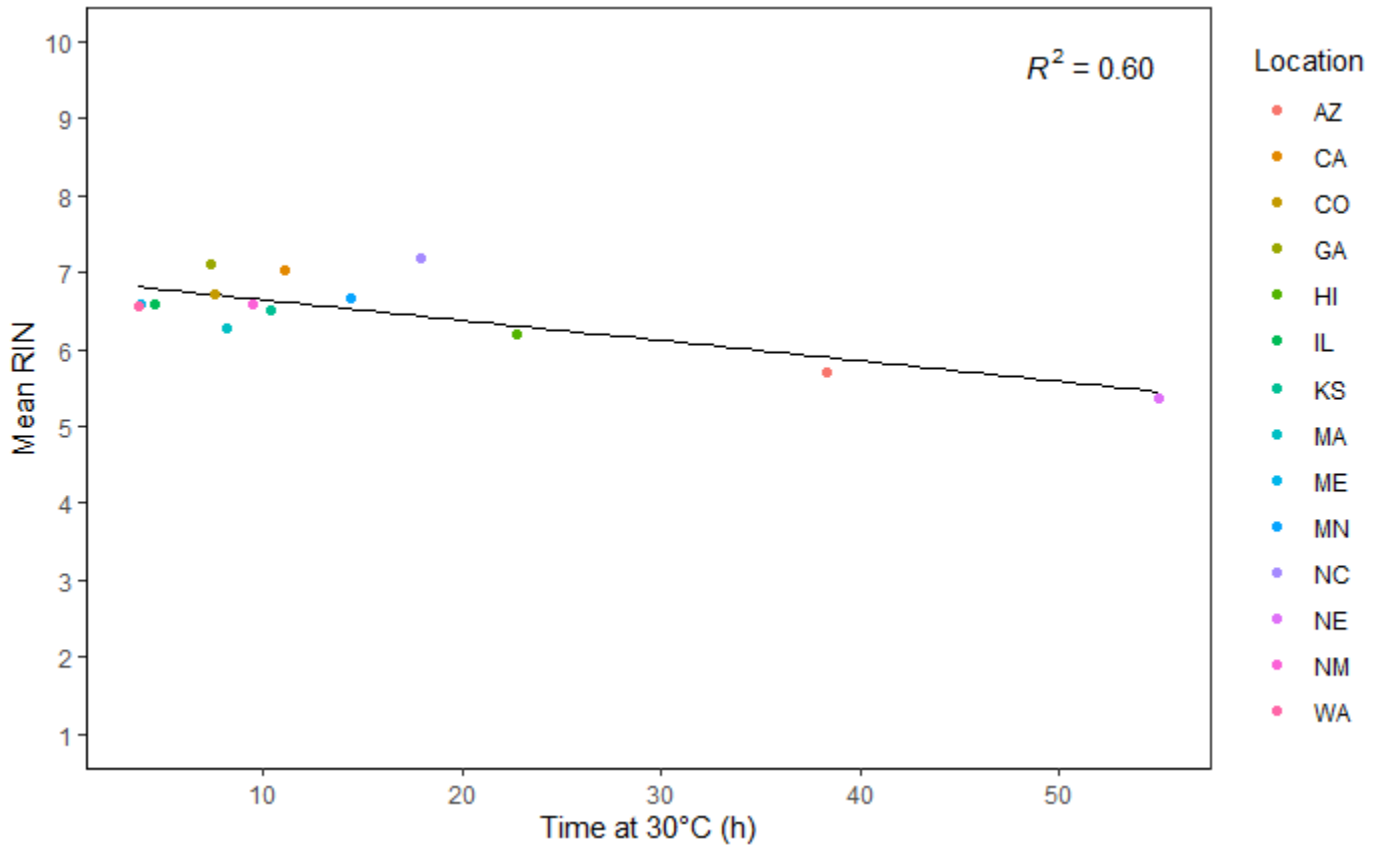


Figure A7. Effects of time spent above 30°C on RNA integrity in homeRNA-stabilized blood samples. Mean RIN scores for each location are plotted against the number of hours a sample experienced a temperature of 30°C or higher. Longer times spent at or above 30°C correspond to lower mean RIN values, however, there is not a strong correlation between the two variables ($R^2 = 0.60$), suggesting that longer times spent above 30°C minimally affect the RNA integrity of homeRNA-stabilized blood samples.

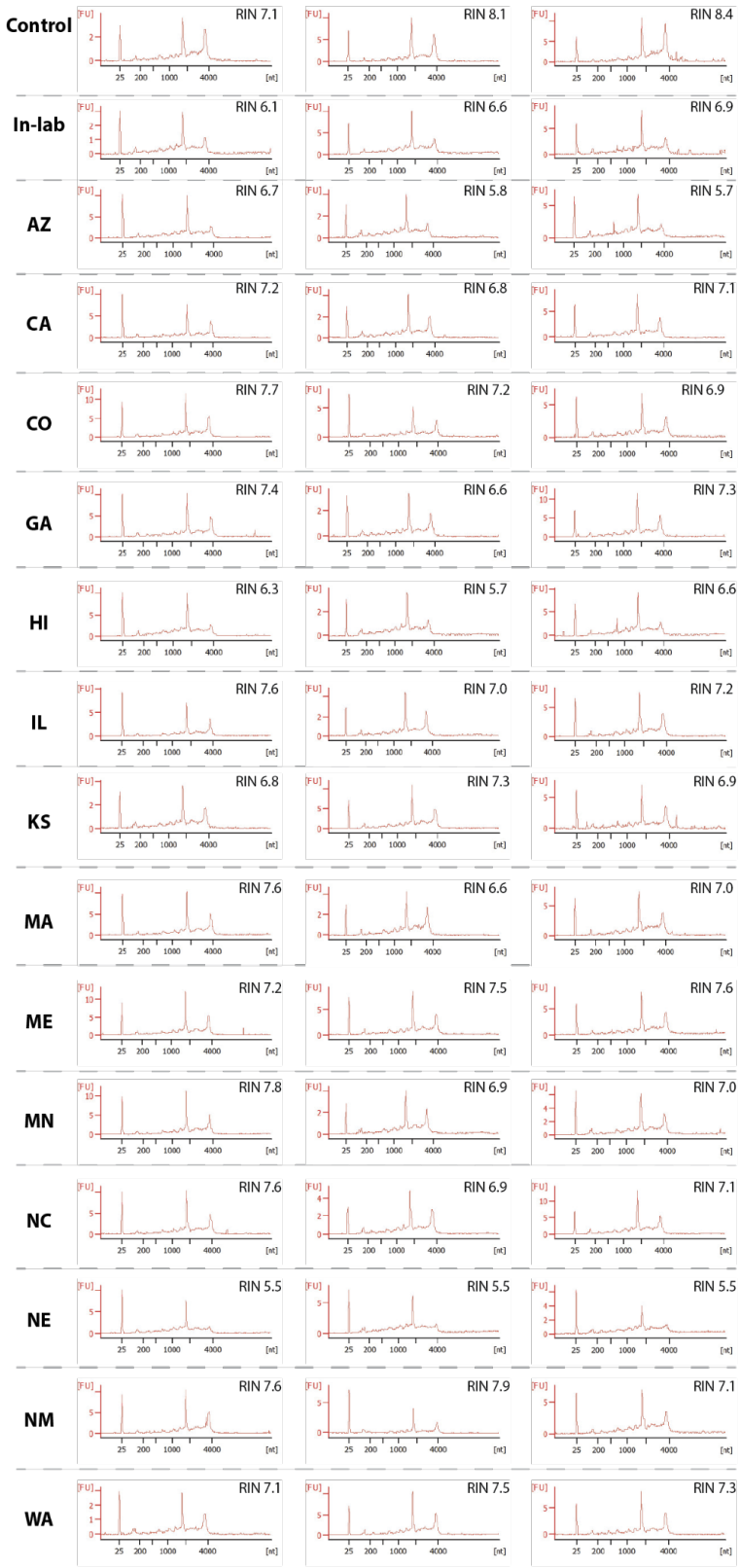
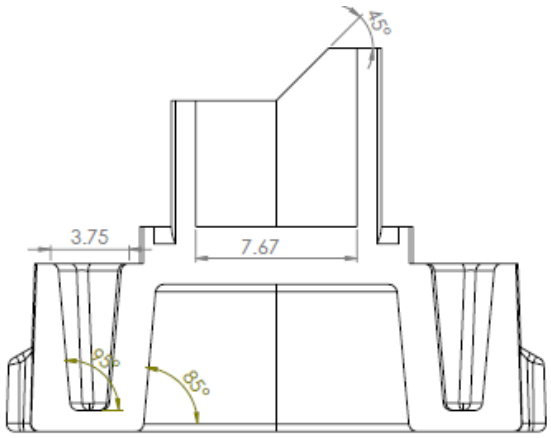


Figure A8. Electropherograms of isolated RNA from RNA*later*-stabilized blood samples shipped to 14 different US states. Electropherograms were obtained as raw data using a RNA 6000 Nano Kit on an Agilent 2100

bioanalyzer, which uses fluorescence to detect the marker (~25 nt), 18S rRNA fragments (~1900 nt), and 28S rRNA fragments (~3900 nt). The RIN algorithm then uses these peaks to assign a RIN value to the corresponding sample. Each state was shipped blood samples in triplicate (left, middle, and right columns are replicates 1, 2, and 3, respectively). Samples from the third replicate (right column) were sequenced with 3'mRNA-seq. All three replicates from the Control and Nebraska (NE) samples were also sequenced with 3'mRNA-seq.

B. Appendix for Chapter 2



SECTION C-C
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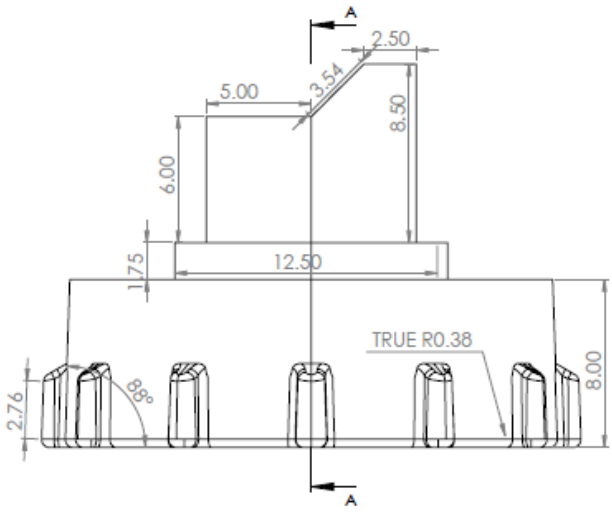

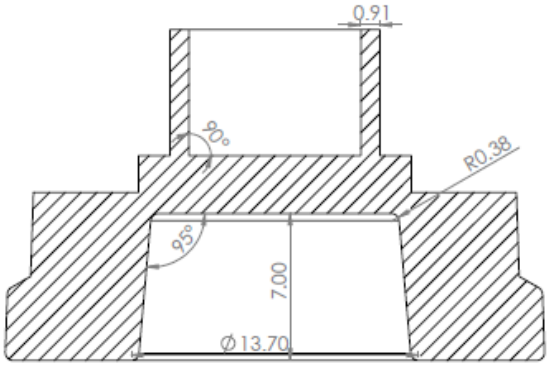

SECTION A-A
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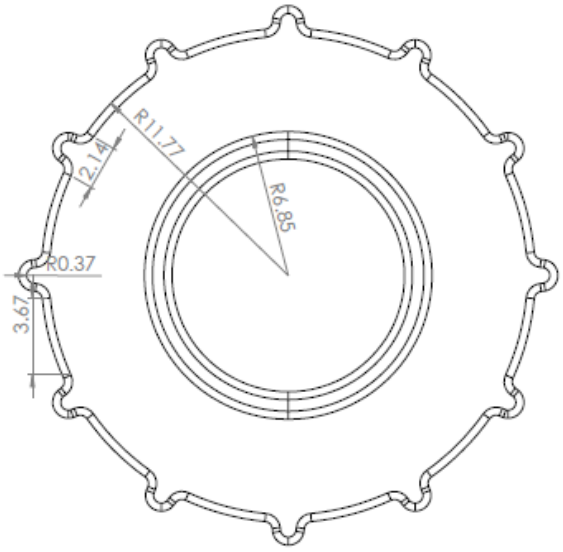

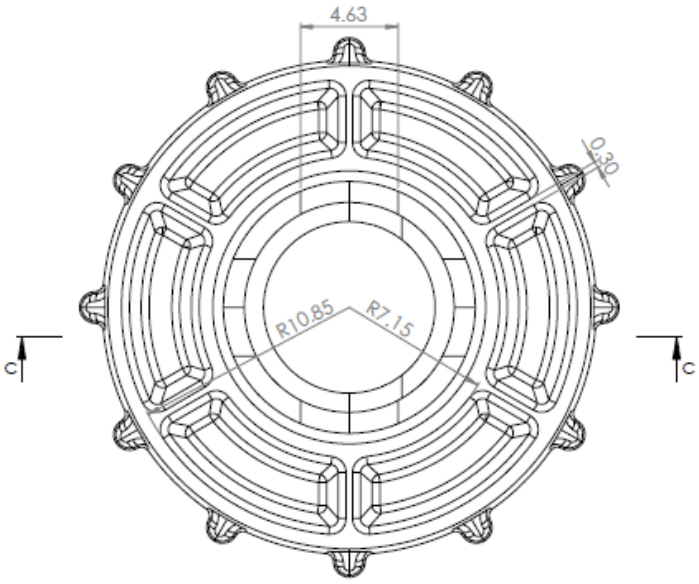

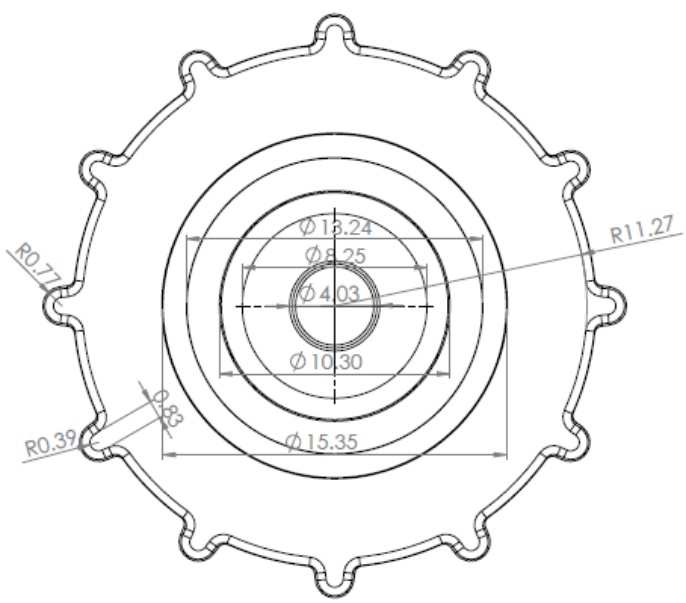

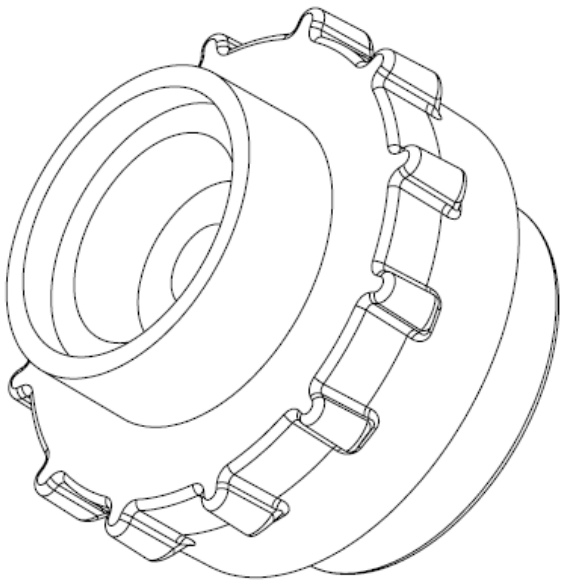

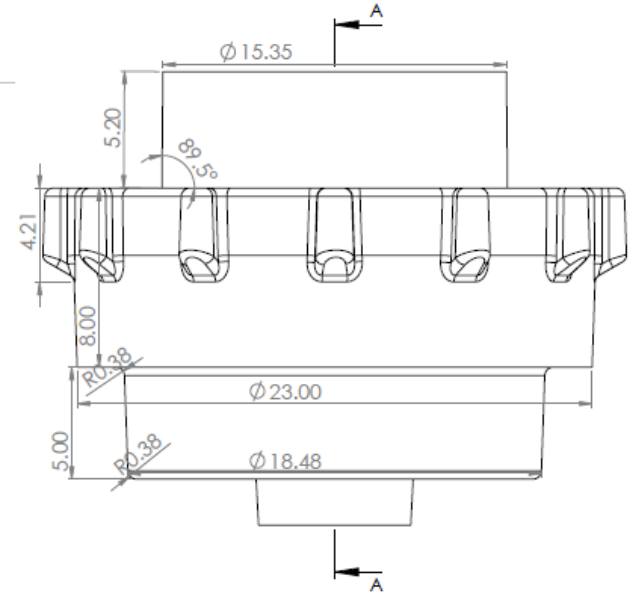

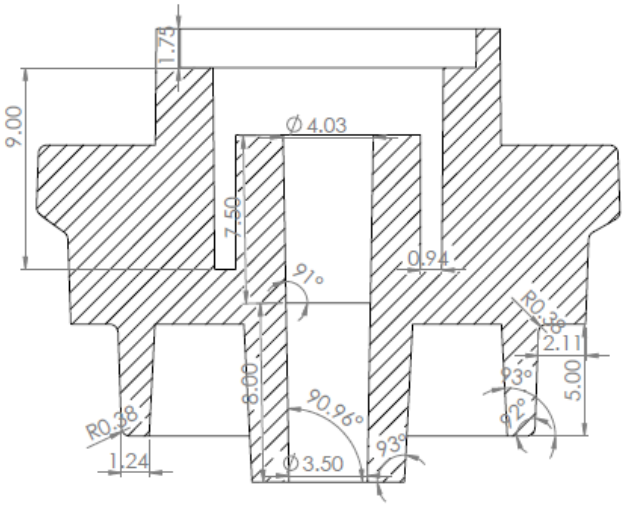

SECTION A-A
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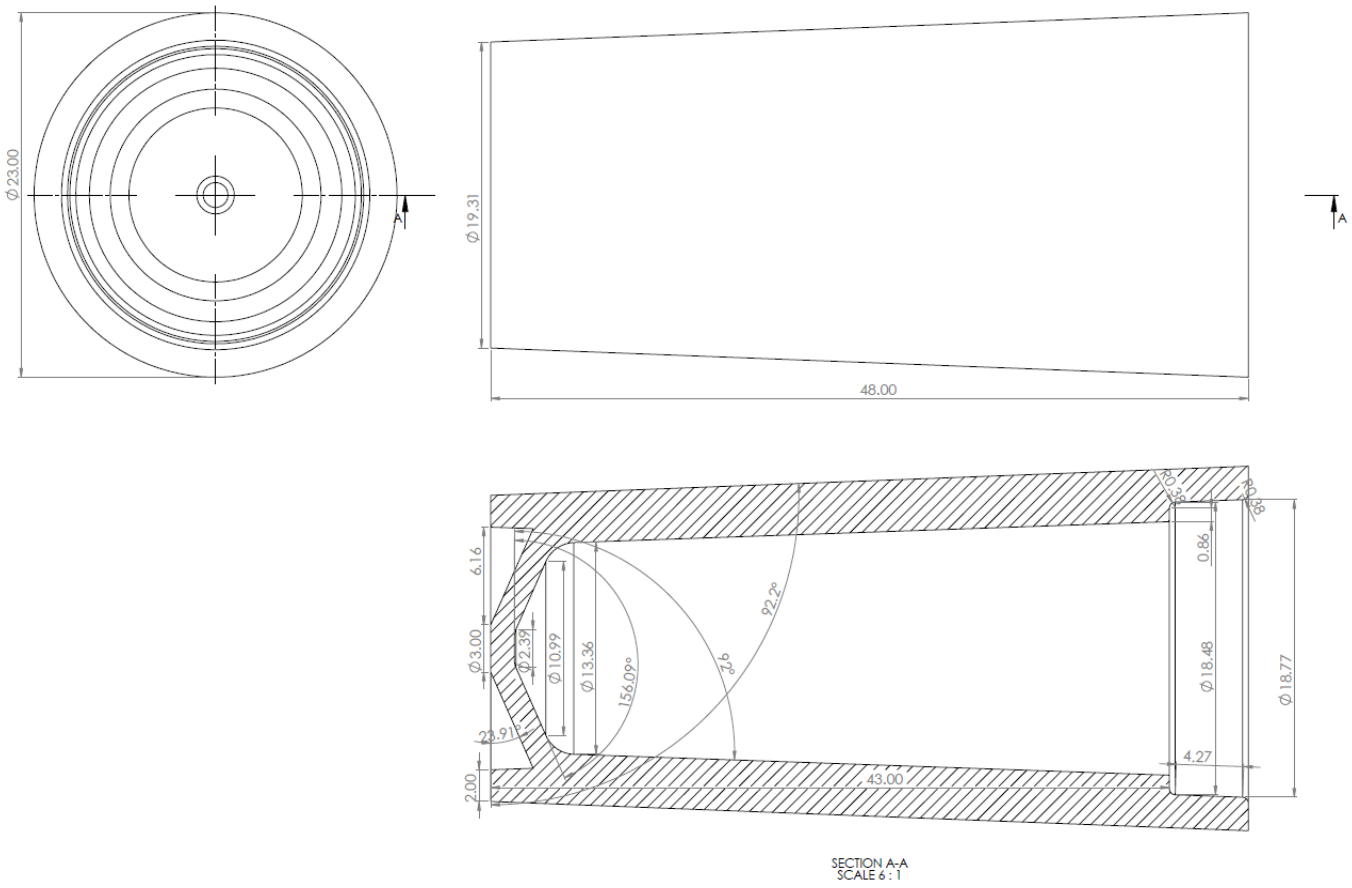


Figure B1. Engineering drawings of the homeRNAmax components. All dimensions are in millimeters.

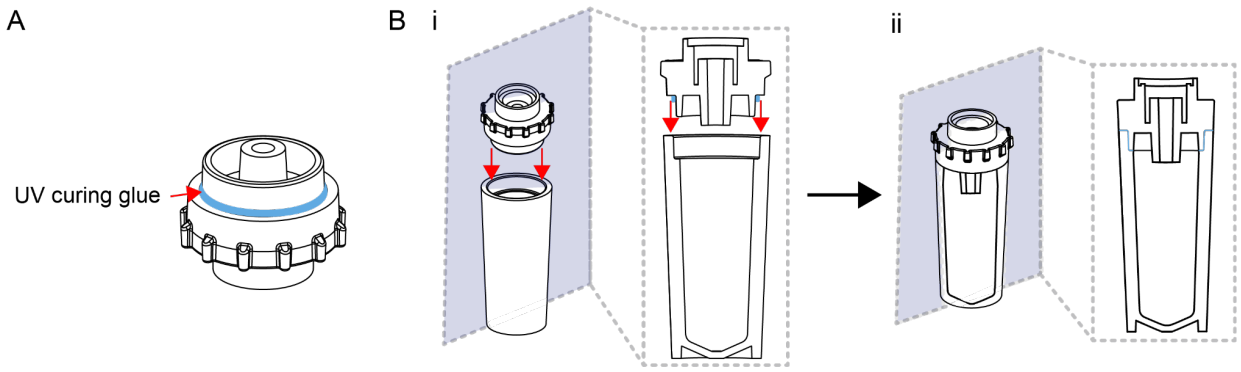


Figure B2. Fabrication of stabilizer tube. A) Location where UV curing glue (blue) is applied to the adapter piece. B) Mating of the adapter piece with the vial piece showing location of the glue before (i) and after (ii) inserting the adapter piece into the vial piece. Glue is then cured with a 405 nm UV lamp as described in the materials and methods.

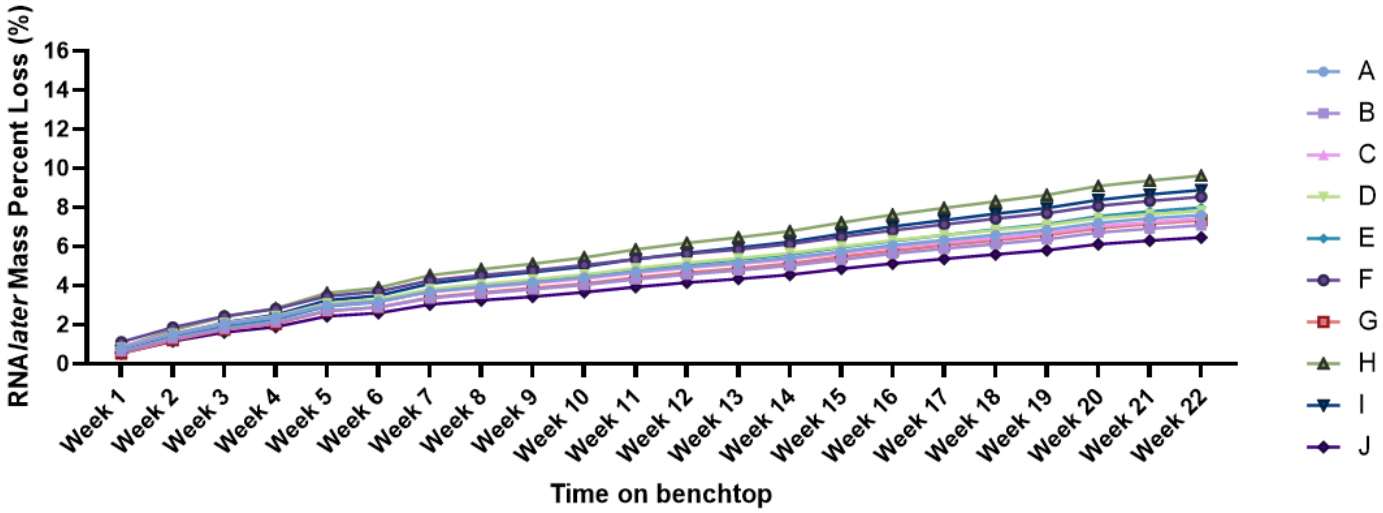


Figure B3. Summary of evaporation test data. homeRNAmax tubes were filled with RNA*later* and stored upright at room temperature on a lab bench. The mass was measured each week and a percent loss was calculated. These data show that evaporation is roughly linear with an approximate loss of between 0.5% and 1% (~0.020 - 0.039 grams) per week stored at room temperature.

PREPARE FOR COLLECTION

BEFORE YOU BEGIN

Point your phone's camera here to watch an instructional video:

Or type this URL:
https://tinyurl.com/ms5eu5f2

1. Remove cap from stabilizer tube by twisting it off and discard the cap. Write down the kit code.
Set the tube aside. You will use this in step 12.

2. Twist and remove cap from blood collection tube, and discard the cap.

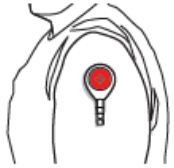
3. Remove Tasso from box. Connect blood collection tube to Tasso device, and press until snug.

4. Activate warmer by bending the silver disc. Knead to fully activate, and apply to upper arm for 2 minutes.

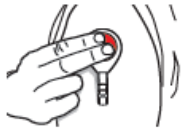
5. Clean area with alcohol wipe and allow to dry.

COLLECT BLOOD USING TASSO DEVICE

6. Remove clear plastic cover over the red button on the Tasso device.
Peel tab behind the device.

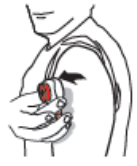


7. Stick device to upper arm with tube pointing down. Hang arm straight down at side.
Do not remove once it is on.

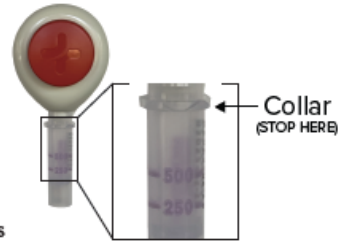


8. Press button all the way down ONCE and release.
You may not feel anything.

9. Start a 5 minute timer.
Keep arm at your side. You may not see blood right away. It can take 1-2 minutes for blood to flow. Use a mirror or phone camera as needed to watch blood flow.

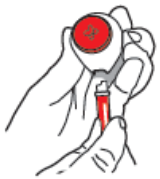


10. After 5 minutes OR when the blood reaches the collar of the tube, *whichever comes first*, remove the device from your arm.
Many people do not fill the tube to the collar.

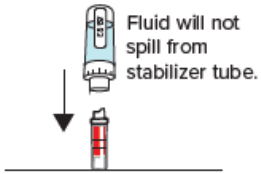


NOTE: If you have not collected any blood skip to step 15.

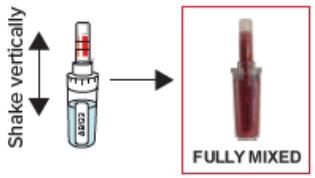
STABILIZE AND PACKAGE



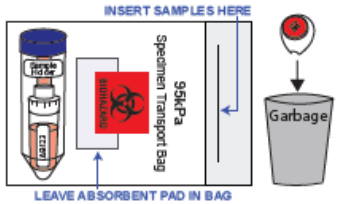
11. Remove tube by twisting slightly and pulling down.
This may take a bit of finger strength.



12. *With the blood collection tube on the bottom*, connect the stabilizer tube (from step 1) and blood tube together until you can't press any farther.



13. *With the stabilizer tube on the bottom*, shake up and down until the fluid in the top and bottom compartments is the same color. For most participants, this takes about 10 seconds.
If fluid is only in the bottom, it should be the same color throughout.
DO NOT DISCONNECT TUBES.



14. Place blood sample in sample holder, and put into the specimen bag. Place specimen bag inside the original box.
Remaining used materials, including the Tasso device and warmer, can be thrown away.

15. Refer to email instructions for filling out the Online Survey and for mailing your sample back to the lab.

Home Blood Collection Kit

INSTRUCTIONS FOR USE

For research use only

Version 2/18/25

KIT CONTENTS

Make sure your kit contains all components listed below

1) Tasso device

2) Stabilizer tube and blood collection tube

3) Alcohol wipe

4) Warmer

5) Bandage

6) Specimen bag

7) Sample holder

Thank you for participating in our study!

BEFORE YOU BEGIN

Wash your hands and gather the following materials not included in your kit:

- Pen or note-taking app
- Timer
- Mirror or smart phone camera with selfie mode

PRECAUTIONS RELATED TO TASSO BLOOD SAMPLING DEVICE

- For use only on a single participant. Discard the entire Tasso device after use.
- Single use only.
- Keep out of reach of children.
- Not for use on infant heels.
- Do not resterilize.
- For external use only.
- Do not use if device packaging has been opened or damaged.
- Use while seated as fainting may occur with any blood sampling procedure.
- Minor bruising, residual marks, or scarring may occur at the sample collection site. (Note: The occurrence and severity of these events depend on physiological characteristics and chosen anatomic site.)
- Multiple collections from the same anatomic location may increase risk of residual marks or scarring and may impact the healing process.
- Do not perform collections on an area that shows evidence of skin issues such as infection, inflammation, extensive scarring, or broken skin.
- Participants taking blood thinners may experience prolonged bleeding.
- The Tasso device contains sharps. Handle with care.

PRECAUTIONS RELATED TO STABILIZER TUBE

- If you come in direct contact with stabilizing fluid, wash your hands.
- Do not ingest stabilizing fluid.

INTENDED USE

The Tasso device is a single use blood collection device that is intended for the self collection of capillary blood from the upper arm of adults (18 years or older). The stabilizer tube contains liquid that is intended for stabilizing the collected blood. The home blood stabilizing kit is for academic research use.

STORAGE

Store at 15 - 30°C (60 - 80°F) in a dry place.

Figure B4. Instructions for use for the homeRNAmax kit. Instructional video file and Adobe editable .pdf are included as attachments in the Supplemental Information.

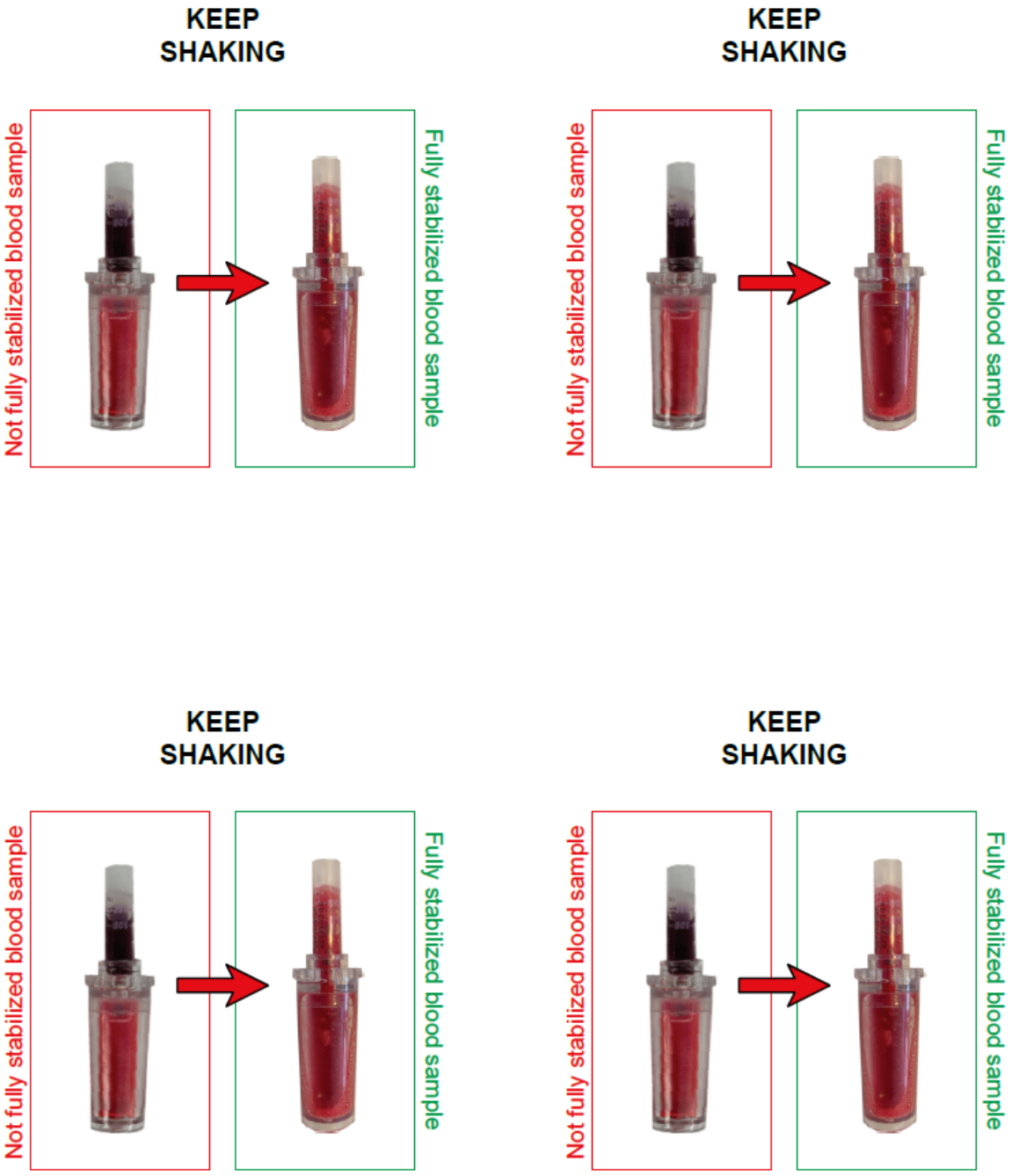


Figure B5. Stickers showing adequate stabilization. Stickers adhere to inside of kit box lid. Labels used are Avery 5168. Adobe editable .pdf is included as an attachment in the Supplementary Information.

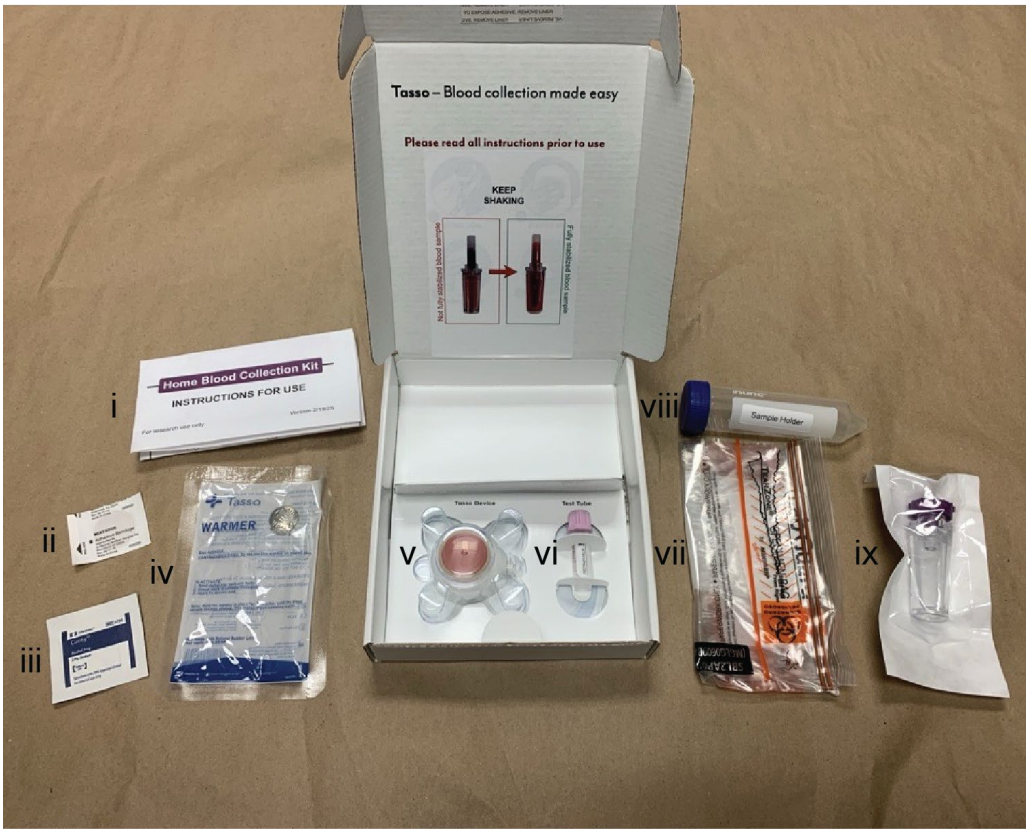


Figure B6. Components of the homeRNAmax kit. Kit components include i) instructions for use, ii) sterile bandage, iii) alcohol wipe, iv) warmer for warming the arm prior to application of the Tasso+ device, v) Tasso+ device, vi) BD Microtainer® blood tube, vii) sample return bag, viii) sample holder, and ix) stabilizer tube containing RNA*later*.

Table S1. Components of the homeRNAmax kit.

Kit component	**Manufacturer(s)**	**Quantity**
Sterile Tasso+ blood collection device	Tasso, Inc.	1
RNA stabilizer tube	Our lab	1
BD Microtainer® blood tube (K2EDTA coated)	BD	1
Instant heat pack (product number ACC0014)	Tasso, Inc.	1

Sterile alcohol wipe	Covidien	1
Sterile bandage	McKesson	1
Specimen transport bag with absorbent pad	Path-Tec	1
50 mL conical tube	BD	1
Instructions for use	Our lab (see page S6-S7 of SI)	1
Blood-stabilizer mixing instruction sticker	Our lab (see page S8 of SI)	1

Table S2. homeRNAmax does not leak upon inversion at the volumes tested.*

Volume of fluid in tube (mL)	Replicate	Height of fluid before inversion (mm)	Height of fluid above nozzle after inversion (mm)	Result
2.00	1	11.0	1.99	No leak
2.00	2	13.2	4.11	No leak
2.00	3	12.9	3.09	No leak
2.00	4	12.9	3.27	No leak
4.00	1	22.6	11.5	No leak
4.00	2	24.9	13.7	No leak
4.00	3	24.1	13.3	No leak
4.00	4	23.4	13.1	No leak
6.00	1	32.8	22.5	No leak
6.00	2	34.0	24.3	No leak
6.00	3	33.7	24.3	No leak
6.00	4	33.7	24.0	No leak
Filled (~6.70 mL)	1	35.8	26.1	No leak
Filled (~6.70 mL)	2	37.2	28.8	No leak
Filled (~6.70 mL)	3	36.3	27.0	No leak
Filled (~6.70 mL)	4	36.3	27.5	No leak

*Note: Experiment was performed in quadruplicate. Volume of fluid used in homeRNAmax for RNA stabilization applications is 3.60 mL. The room where the experiment was conducted ranges in temperature from 17 - 21 °C, and in humidity from 35 - 45%. Tubes were considered to have “leaked” if any amount of fluid left the tubes during the observation period, which did not happen in any of the cases.

Table S3. homeRNAmax RNA concentration, yield, and data on blood collection. The concentrations were obtained on the Qubit Flex and are multiplied by 50 µL (elution volume). For the final concentrations reported in the manuscript, the concentrations from elution 1 and elution 2 are added together.

Sample ID	Elution number	Concentration (ng/µL)	Yield (ng)	Time on arm (min.)	Blood level	Approximate blood volume (µL)
IGMJ	1	32.4	1620	>5	Level E	750 µL
	2	4.11	205.5			
AXUI	1	16.1	805	3-5	Level D	500 µL
	2	2.88	144			
FJZM	1	8.25	412.5	3-5	Level A	125 µL
	2	1.3	65			
EYWH	1	33.7	1685	3-5	Level D	500 µL
	2	3.48	174			
CUBY	1	29.3	1465	3-5	Level E	750 µL
	2	3.87	193.5			
BWNG	1	42.3	2115	3-5	Level F	1000 µL
	2	5.84	292			

MPWJ	1	87.8	4390	3-5	Level F	1000 µL
	2	9.45	472.5			
QLGG	1	9.85	492.5	3-5	Level F	1000 µL
	2	3.47	173.5			
VIVZ	1	2.15	107.5	3-5	Level E	750 µL
	2	0.866	43.3			
PLFF PLFF	1	36.7	1835	3-5	Level A	125 µL
	2	6.21	310.5			
SQFE	1	12	600	3-5	Level A	125 µL
	2	2.49	124.5			
MQRF	1	52.5	2625	3-5	Level E	750 µL
	2	9.69	484.5			
VHJF	1	53.5	2675	>5	Level F	1000 µL
	2	10	500			
POXS	1	17.9	895	3-5	Level C	375 µL
	2	4.62	231			
VVXN	1	26.1	1305	3-5	Level F	1000 µL

	2	5.09	254.5			
ZBVY	1	78.8	3940	<3	Level F	1000 µL
	2	9.67	483.5			
VYFO	1	36	1800	3-5	Level F	1000 µL
	2	9.64	482			
ZIBT	1	46.2	2310	3-5	Level F	1000 µL
	2	9.97	498.5			
PLFF-2	1	6.11	305.5	>5	Level E	750 µL
	2	4.34	217			

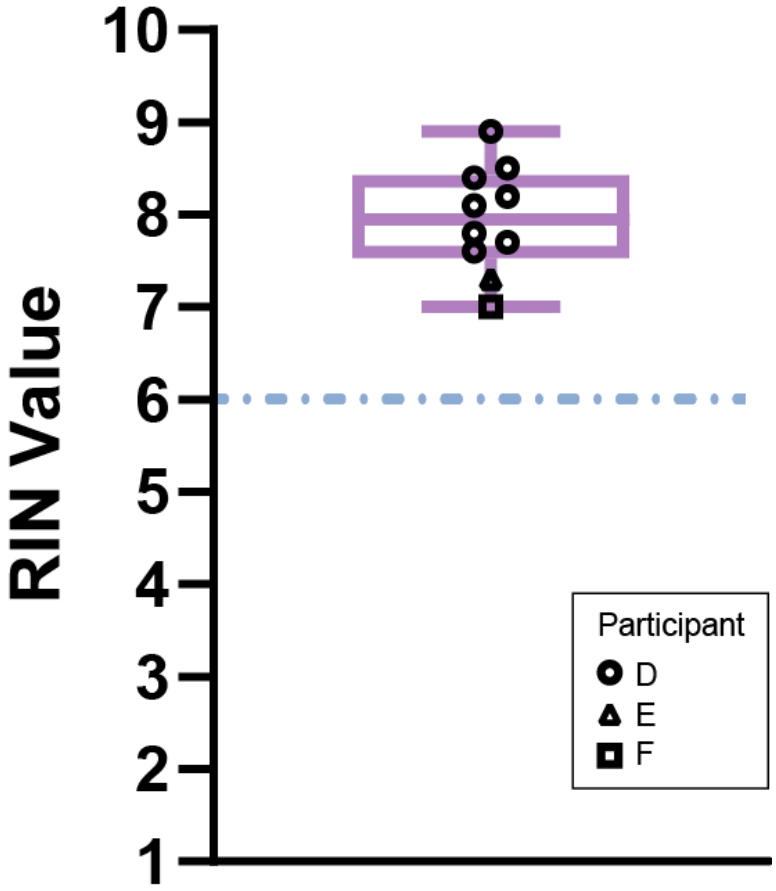
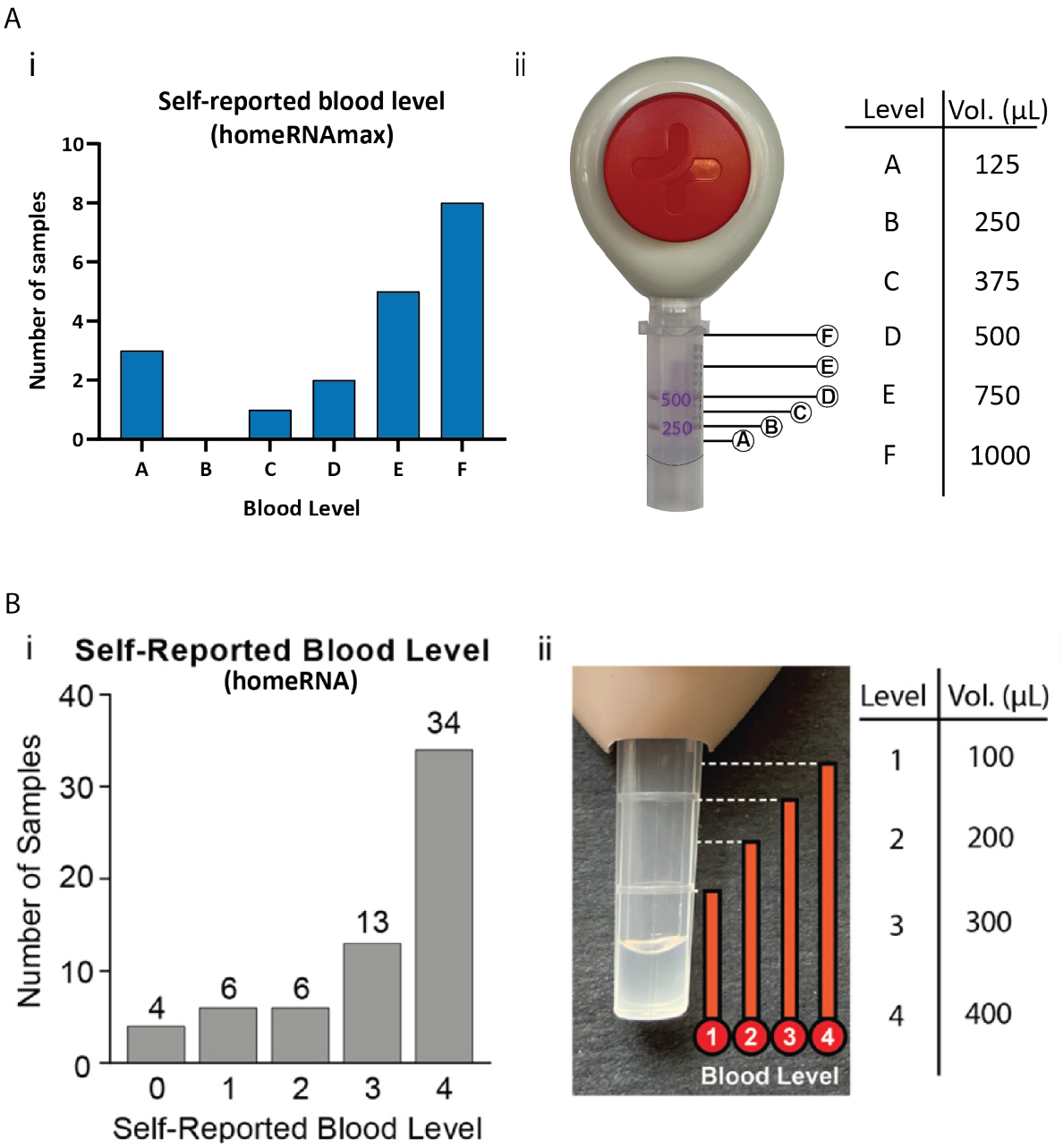


Figure B7. RNA integrity of remaining (n = 10/44) homeRNAmax-stabilized blood samples from a longitudinal clinical study. RIN values obtained using a Thermo Fisher TapeStation (RIN values obtained using a Agilent 2100 Bioanalyzer for n = 34/44 samples are shown in Figure 6). Shapes on the graph represent which samples came from which participants. The dashed line represents a RIN of 6, a common cutoff for RNA sequencing technologies.



Figures in panel B reproduced from Haack et al.

Figure B8. Comparison of homeRNAmax blood level (A, top) and original homeRNA[2] blood level (B, bottom) from participant-reported data following sampling. In the homeRNAmax, level B represents a volume of around 250 µL and level D represents a volume of 500 µL. The Images with annotated levels are included in the participant surveys as guidance for reporting collected blood level.

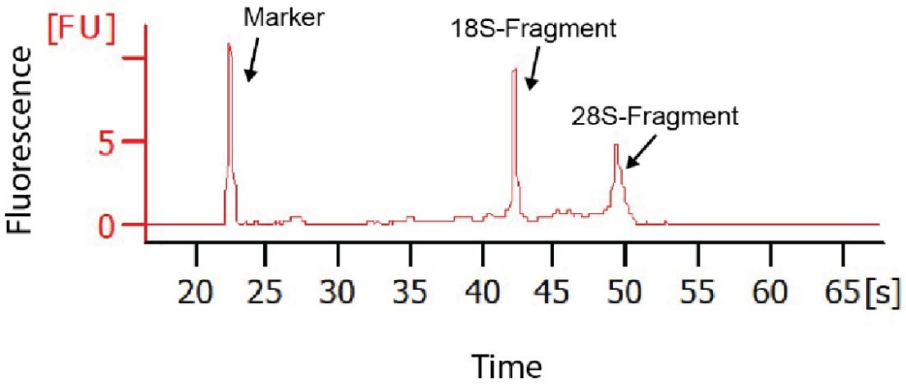


Figure B9. General Information on Bioanalyzer Profile. Electrophoretogram obtained from Sample B1 annotated with the marker and two other major peaks that help determine RIN including the 18-S fragment peak, and the 28-S fragment peak. More information on interpreting bioanalyzer data, including examples of electrophoretograms obtained from samples with various RIN values can be found in Schroeder 2006.[1] Figure and caption reproduced from Haack, A. J.; Lim, F. Y, et. al.[2]

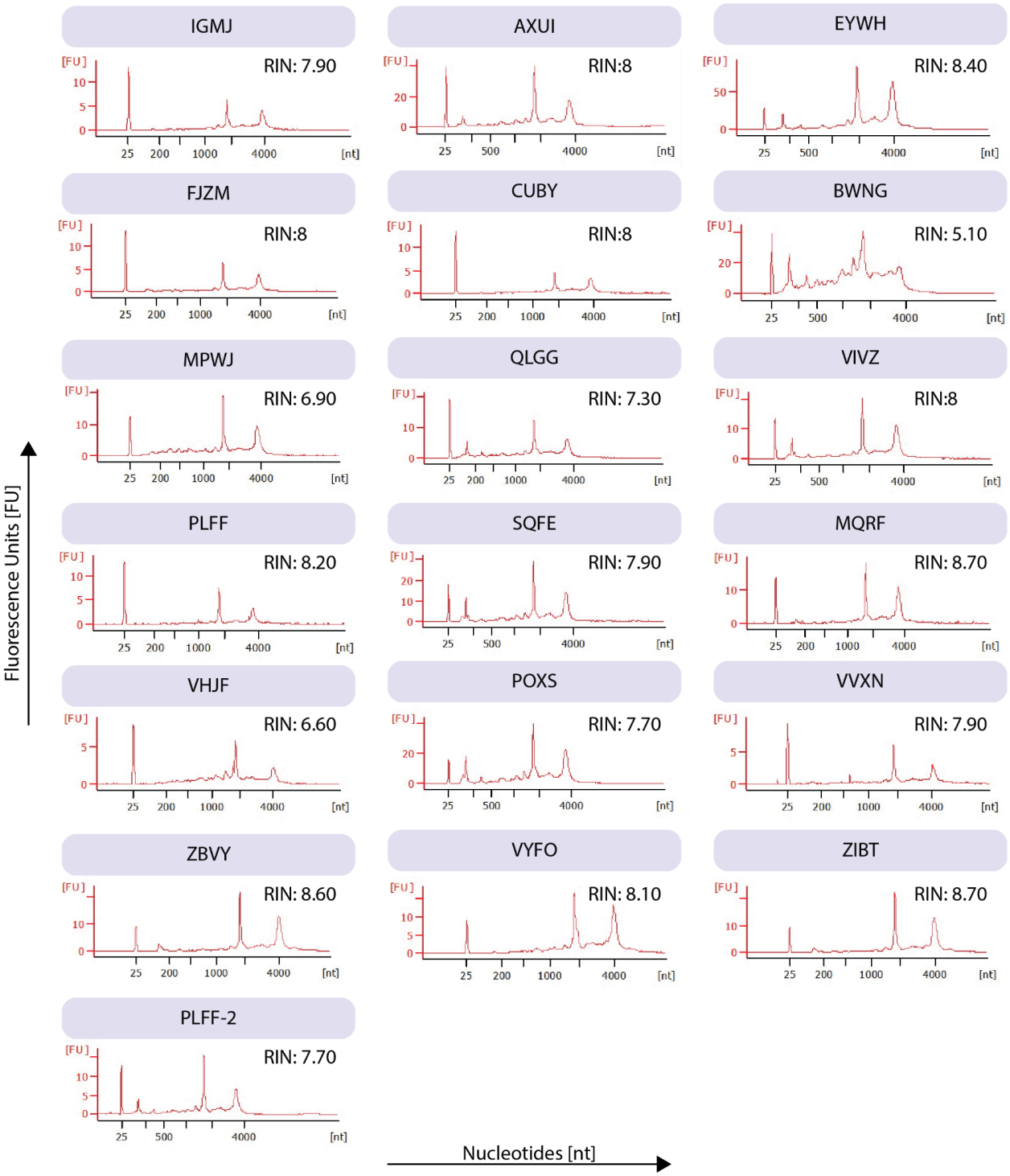


Figure B10. Agilent 2100 Bioanalyzer electropherograms for isolated RNA from homeRNAmax-stabilized samples. The samples were analyzed with Nano or Pico kits, depending on concentration. IGMJ, FJZM, CUBY, MPWJ, PLFF, MQRF, VHJF, VVXN, ZBVY, VYFO, and ZIBT were analyzed using the Nano kit, and AXUI, EYWH, BWNG, QLGG, VIVZ, SQFE, POXS, and PLFF-2 were analyzed using the Pico kit. For samples that

were analyzed multiple times, the lowest of the two, or middle value of three is used and reported here and in the manuscript.

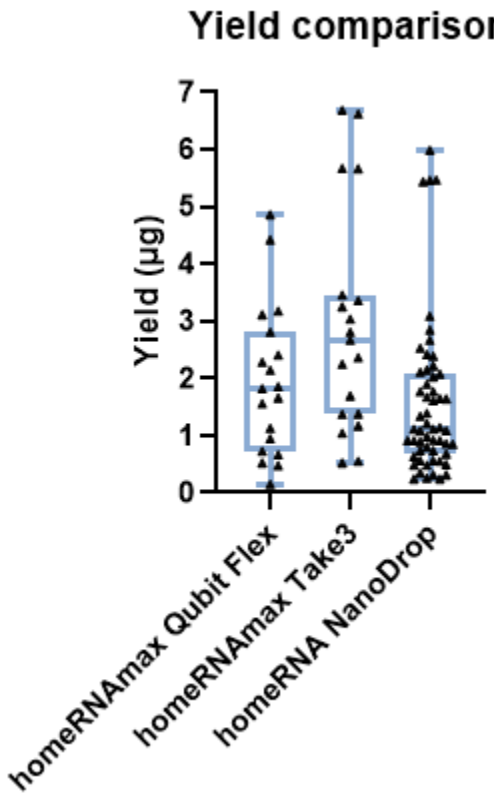


Figure B11. Comparison of RNA yield from present study (Qubit Flex, Cytation 5 Take3) and original homeRNA study[2] (NanoDrop). As expected, yields obtained from the Qubit Flex are slightly lower than those obtained from the Take 3. While an ideal comparison between the original homeRNA study and the present study is difficult because of the discrepancy in analytical instruments used, the Take3 and NanoDrop both use spectrophotometric methods (absorbance at 260 nm) to estimate RNA yield. When comparing these, it is clear that the distribution of the yields is similar between the homeRNA and homeRNAmax.

Appendix 1: Theoretical characterization of fluid retention in homeRNAmax stabilizer tube

In this study, we are analyzing the retention of a liquid in a homeRNAmax stabilizer tube tilted upside down (Fig. 1).

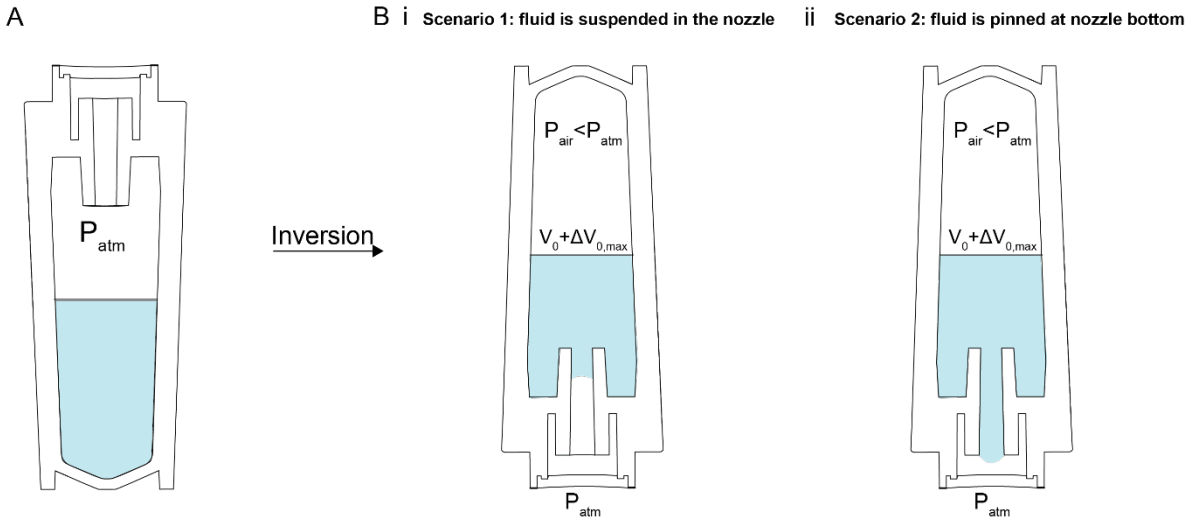


Figure B12. Schematic of stabilizer tube inversion. A) The tube before rotation. B) Schematic of an inverted stabilizer tube at equilibrium when i) fluid stops in the nozzle at equilibrium and ii) fluid is pinned to the nozzle opening at equilibrium. P_{air} is the pressure of the air in the stabilizer tube, P_{hyd} is the hydrostatic pressure of the fluid, P_{cap} is the capillary pressure at the interface between the fluid and the atmosphere, and P_{pin} is the pinning pressure.

Model

Scenario 1 (Fig. 1Bi): fluid is suspended in the nozzle

Inside the tube, we have the hydrostatic pressure (P_{hyd}), the pressure of the trapped air (P_{air}), and the capillary pressure in the nozzle (P_{cap}). The capillary pressure of the upper surface of the liquid in the tube is negligible since

the tube diameter is larger than the capillary length. Outside the tube, the pressure is the atmospheric pressure (P_{atm}) (101,500 Pa). The total pressure acting upon the meniscus suspended in the nozzle can be expressed by the following equation

$$P_{balance} = P_{hyd} + P_{cap} + P_{air} - P_{atm} \quad \text{(S1)}$$

The liquid is at equilibrium (immobile) when the pressure balance is zero

$$P_{hyd} + P_{cap} + P_{air} - P_{atm} = 0\ . \quad \text{(S2)}$$

The hydrostatic pressure P_{hyd} can be calculated using the following expression

$$P_{hyd} = \rho g\ H \quad \text{(S3)}$$

Where ρ is the density of the fluid (1000 kg/m^3), g is the acceleration due to gravity (9.8 m/s^2), and H is the height of the fluid. In this case the pressure at the liquid air interface at the nozzle opening is a capillary pressure in the nozzle P_{cap}

$$P_{cap} = \frac{2\gamma coscos\ \theta}{r_{noz}}. \quad \text{(S4)}$$

Where γ is the surface tension of the fluid (0.07 N/m), θ is the contact angle of the fluid on the nozzle material, and r_{noz} is the radius of the nozzle (1.75 mm). Note that P_{cap} is the same sign as P_{hyd}, meaning the capillary pressure does not prevent leakage in the tube.

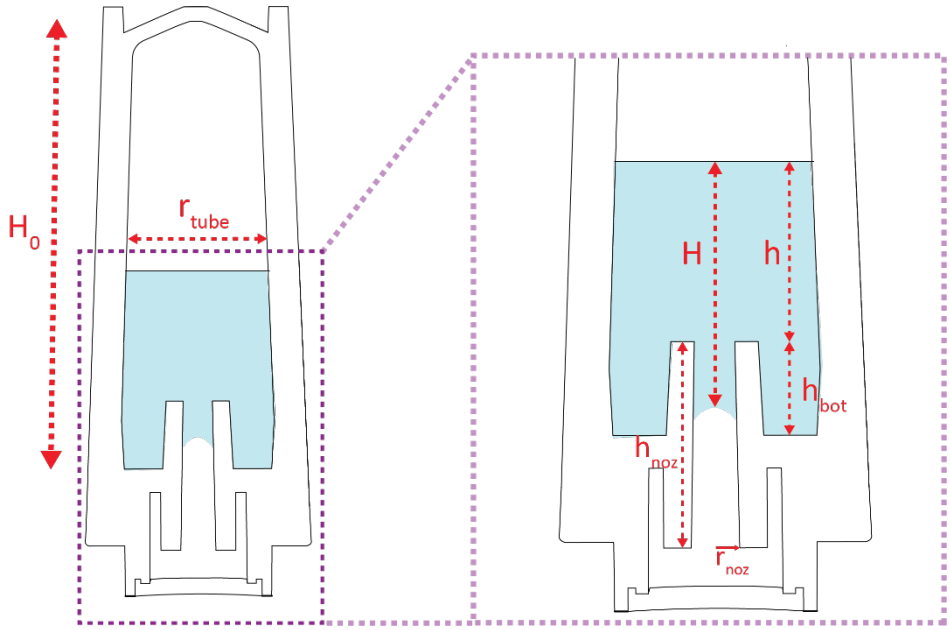


Figure B13. Schematic of stabilizer tube dimensions used in the theoretical model for Scenario 1.

By rearranging equation (S2), the pressure difference associated with the suspension of the fluid within the nozzle that is required to prevent leakage can be calculated.

$$P_{air} - P_{atm} = \Delta P_{air} = -\left(P_{hyd} + P_{cap}\right). \quad \text{(S5)}$$

It is worth noting that the precise pressure of the trapped air is difficult to determine. It depends on the way the tube has been tilted and on the initial volume in the tube in the upright position. It is likely that the liquid flows into the extremity of the nozzle during the tilt. The suspension of the fluid in the nozzle decreases the pressure of the trapped air, initially taken as P_{atm}. If we apply the Ideal Gas Law we find:

$$P_{air} \sim P_{atm} \frac{V_0}{V_{air}} \quad \text{(S6)}$$

Where V_0 is the initial volume of air (before tilting) and V_{air} is the volume of air after tilting. We can solve for the depression associated with the tilt by rearranging (S6):

$$\Delta P_{air} \sim P_{atm}\left(\frac{V_0}{V_{air}} - 1\right) \quad \text{(S7)}$$

Which can then be rearranged in terms of the change of volume:

$$\Delta P_{air} \sim -P_{atm}\left(\frac{\Delta V}{V_{air}}\right) \quad \text{(S8)}$$

The small amount of fluid that fills the nozzle would be sufficient to prevent leakage. Using water as an example, we can use equations (S3), (S4), and (S7) to approximate the order of magnitude of $\frac{\Delta V}{V_{air}}$ required to prevent leakage. If a stabilizer tube were filled with a height of water on the order of $10 - 10^2$ mm, the required pressure difference would be on the order of $10^2 - 10^3$ Pa. Since P_{atm} is on the order of 10^5 Pa, the difference in volume of the air (i.e., the volume of fluid that fills the nozzle) would only have to be on the order of 10^{-3}.

Scenario 2 (fig. 1Bii): fluid is pinned at nozzle bottom

In this scenario, rather than fluid suspending in the nozzle, a droplet forms at the nozzle tip, and whether the drop leaks or stays pinned is governed by the sign of the function

$$P_{balance} = P_{hyd} - P_{pin} + P_{air} - P_{atm} \quad . \quad \text{(S9)}$$

If $P_{balance} > 0$ leakage occurs. We shall analyze what occurs when $P_{balance} < 0$. The expression for P_{hyd} is the same as (S3), and, because the pendant droplet has a semi-spherical shape, P_{pin} can be calculated using the following expression

$$P_{pin} = \frac{-2\gamma}{r_{noz}}. \qquad \text{(S10)}$$

As for now, let us consider the value of the depression associated with the formation of the pendant drop. The volume of the pendant drop is

$$V_{drop} = \frac{2}{3\,\pi\, r_{noz}^3}. \qquad \text{(S11)}$$

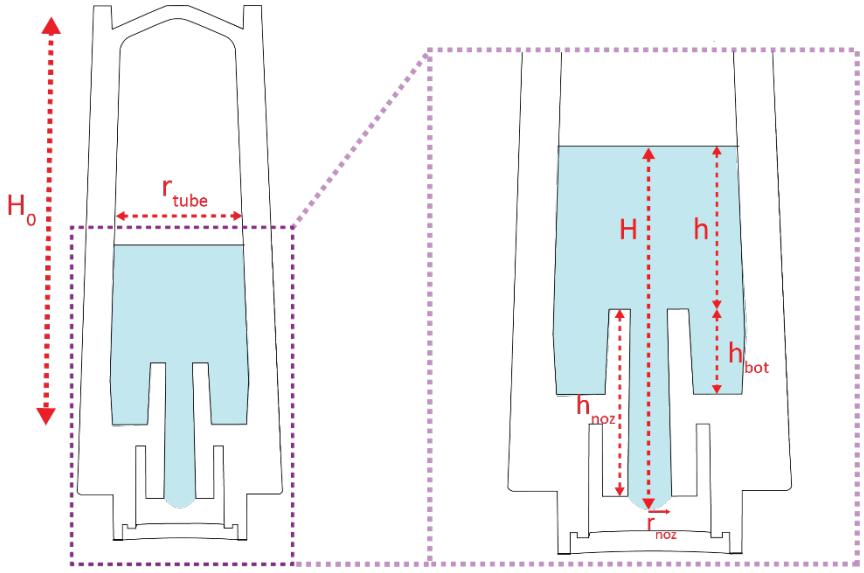


Figure B14. Schematic of stabilizer tube dimensions used in the theoretical model for Scenario 2. The variables are the same as in Scenario 1; they are repeated here for clarity.

So, the formation of the pendant drop decreases the pressure of the trapped air, initially taken as P_{atm}. If we apply the Ideal Gas Law

$$P_{air}\left(V_{air} + V_{drop}\right) = P_{atm}\, V_{air}\ . \qquad \text{(S12)}$$

Then the term $P_{air} - P_{atm}$ becomes

$$P_{air} - P_{atm} = -P_{atm}\ \frac{V_{drop}}{V_{air}+V_{drop}}\ . \qquad \text{(S13)}$$

So, the formation of the pendant drop creates a depression. If V_{air} varies from nearly zero—if the tube is initially completely filled with liquid—to approximately the volume $V_{air} \approx H_0\ \pi r_{tube}^2$, the quantity $P_{air} - P_{atm}$ varies as shown in figure 4.

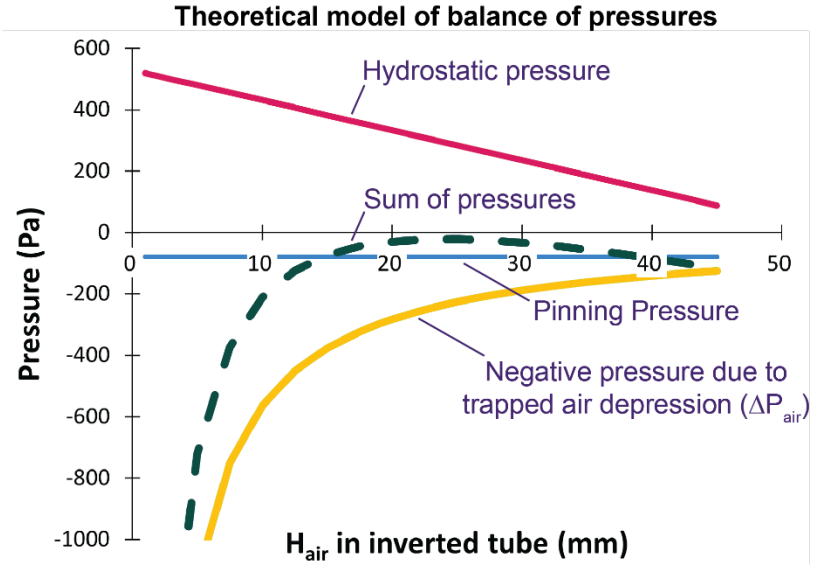


Figure B15. Theoretical prediction showing the sum of all pressures acting in the stabilizer tube (green) as a function of the height of the trapped air in the inverted stabilizer tube (H_{air}, mm), as well as the individual pressures (yellow, blue, and magenta).

When there is a small amount of liquid in the tube, the trapped air depression is small, and it is the pinning pressure that counterbalances the hydrostatic pressure. However, when the volume of liquid is large, the trapped air volume is small and the air depression becomes quite important. The pressure resultant $P_{balance}$ (green line in Fig. 4), has a maximum around H_{air}/15-25 mm.

Effect of a change of temperature on the equilibrium of the liquid in the tube

As noted at the end of *Theoretical model of the stabilizer tube* in the main text, some cases over timescales on the order of 10-20 minutes, additional meniscus movement has been observed after reaching equilibrium; we postulate that this movement is a result of effects related to temperature (e.g., evaporation, temperature changes over time). We have not observed any leakage as a result of these effects. Furthermore, in actual use cases, the tube is not left uncapped and inverted for longer than times on the order of seconds. The following section explores the effects of a temperature change.

Consider an inverted tube at equilibrium that undergoes a temperature change while inverted. The trapped air interface slightly moves upwards and the meniscus in the nozzle also moves upwards to reach another equilibrium state. Let us denote by the subscript 1 the first equilibrium state and subscript 2 the new equilibrium state.

The two equilibrium states are defined by

$$P_{air,1} + P_{hyd,1} + P_{cap} - P_{atm} = 0, \quad \text{(S14)}$$

And

$$P_{air,2} + P_{hyd,2} + P_{cap} - P_{atm} = 0, \quad \text{(S15)}$$

Then subtracting (S14) from (S15)

$$P_{air,2} - P_{air,1} = P_{hyd,1} - P_{hyd,2}. \quad \text{(S16)}$$

The first equilibrium hydrostatic pressure is determined by

$$P_{hyd,1} = \rho g(h + z), \quad \text{(S17)}$$

where h is the height of liquid above the upper end of the nozzle and z the height of liquid inside the nozzle. A decrease of pressure triggers the upwards motion of the meniscus of Dz, and a corresponding motion of the air/liquid surface Dh. Using the mass conservation equation

$$\Delta V = \pi r_{noz}^2 \Delta z = \pi r_{tub}^2 \Delta h. \quad \text{(S18)}$$

Then, the second equilibrium hydrostatic pressure is

$$P_{hyd,2} = P_{hyd,1} - \rho g \Delta z + \rho g \Delta h = P_{hyd,1} + \rho g \Delta h \left(1 - \frac{r_{tub}^2}{r_{noz}^2}\right). \quad \text{(S19)}$$

On the other hand, the trapped air volume decreases by

$$V_{air,2} = V_{air,1} - \pi r_{tub}^2 \Delta h \,. \quad \text{(S20)}$$

In terms of height

$$H_{air,2} = H_{air,1} - \Delta h \,. \quad \text{(S21)}$$

The new air pressure is then given by

$$\frac{P_{air,2}V_{air,2}}{T_2} = \frac{P_{air,1}V_{air,1}}{T_1}\,, \tag{S22}$$

so that

$$P_{air,2} = \frac{P_{air,1}V_{air,1}}{V_{air,2}}\,\frac{T_1}{T_2} = \frac{P_{air,1}V_{air,1}}{V_{air,1}-\pi r_{tub}^2\Delta h}\frac{T_1}{T_2} = \frac{P_{air,1}H_{air,1}}{H_{air,1}-\Delta h}\frac{T_1}{T_2}\,, \tag{S23}$$

Substitution of (S19) and (S20) in (S16) yields

$$P_{air,2} - P_{air,1} = P_{air,1}\left(\frac{H_{air,1}}{H_{air,1}-\Delta h}\,\frac{T_1}{T_2} - 1\right) = P_{hyd,1} - P_{hyd,2} = \rho g \Delta h \left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right). \tag{S24}$$

It is verified in (S24) that, if $T_2 = T_1$, then $\Delta h = 0$. Because the first equilibrium state is known, $H_{air,1}$ and $P_{air,1}$ are known. We then obtain an equation in Δh. Let us cast (S24) under the form

$$P_{air,1}\left(\frac{1}{1-\frac{\Delta h}{H_{air,1}}}\,\frac{T_1}{T_2} - 1\right) = \rho g\,\frac{\Delta h}{H_{air,1}}\,H_{air,1}\left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right), \tag{S25}$$

where $\frac{\varepsilon = \Delta h}{H_{air,1}}$ is now the unknown to determine. Then we obtain the quadratic equation

$$\frac{\rho g H_{air,1}}{P_{air,1}}\left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right)\varepsilon^2 + \left[1 - \frac{\rho g H_{air,1}}{P_{air,1}}\left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right)\right]\varepsilon + \frac{T_2}{T_1} - 1 = 0\,. \tag{S26}$$

Again, if $T_2 = T_1$, $\varepsilon = 0$ is solution, and $\Delta h = 0$. Note that $\frac{\rho g H_{air,1}}{P_{air,1}}$ is non-dimensional so that (13) is non-dimensional. An approximate solution can be obtained if the term in ε^2 is negligible, and

$$\varepsilon = \frac{\Delta h}{H_{air,1}} \approx \frac{\frac{T_2}{T_1} - 1}{\left[1 - \frac{\rho g H_{air,1}}{P_{air,1}}\left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right)\right]}. \quad \text{(S27)}$$

Let us consider $T_2 = 20\ C = 293\ K, T_1 = 28\ C = 301\ K, \frac{T_2}{T_1} \approx 0.97, \frac{r_{tub}}{r_{noz}} = 3, H_{air,1} = 20\ mm, P_{air,1} - P_{atm} = 300\ Pa$, so that $P_{air,1} = 9700\ Pa$. The term $\frac{\rho g H_{air,1}}{P_{air,1}}$ is equal to 2×10^{-3} approximately. Then $\varepsilon = \frac{\Delta h}{H_{air,1}} \approx \frac{\frac{T_2}{T_1} - 1}{\left[1 - \frac{\rho g H_{air,1}}{P_{air,1}}\left(\frac{r_{tub}^2}{r_{noz}^2} - 1\right)\right]} \approx 0.03$. Hence $\Delta h \approx 0.6\ mm$ and $\Delta z \approx 4.8\ mm$

References

[1] Schroeder, A.; Mueller, O.; Stocker, S.; Salowsky, R.; Leiber, M.; Gassmann, M.; Lightfoot, S.; Menzel, W.; Granzow, M.; Ragg, T., The RIN: an RNA integrity number for assigning integrity values to RNA measurements. BMC Mol. Biol 2006, 7 (3), 1-14.

[2] Haack, A. J.; Lim, F. Y.; Kennedy, D. S.; Day, J. H.; Adams, K. N.; Lee, J. J.; Berthier, E.; Theberge, A. B. homeRNA: A Self-Sampling Kit for the Collection of Peripheral Blood and Stabilization of RNA. *Anal. Chem.* **2021**, *93* (39), 13196–13203. https://doi.org/10.1021/acs.analchem.1c02008.

C. Appendix for Chapter 3

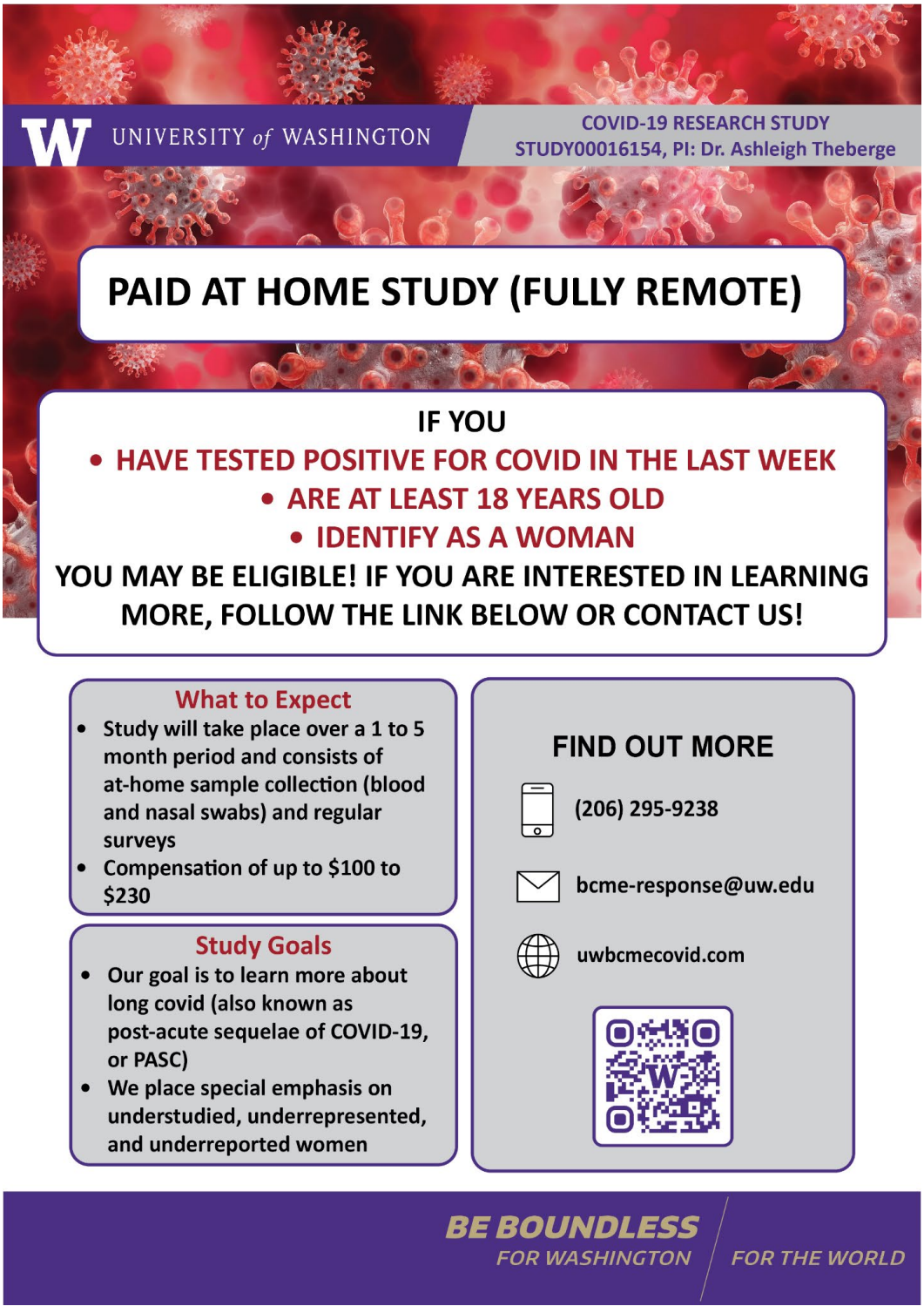


Figure C1. Example of a digital advertisement used for recruitment. Components that we have found important for our studies include time commitment, compensation, and location. Additional information such as inclusion criteria and study goals are useful additions provided that there is sufficient space.

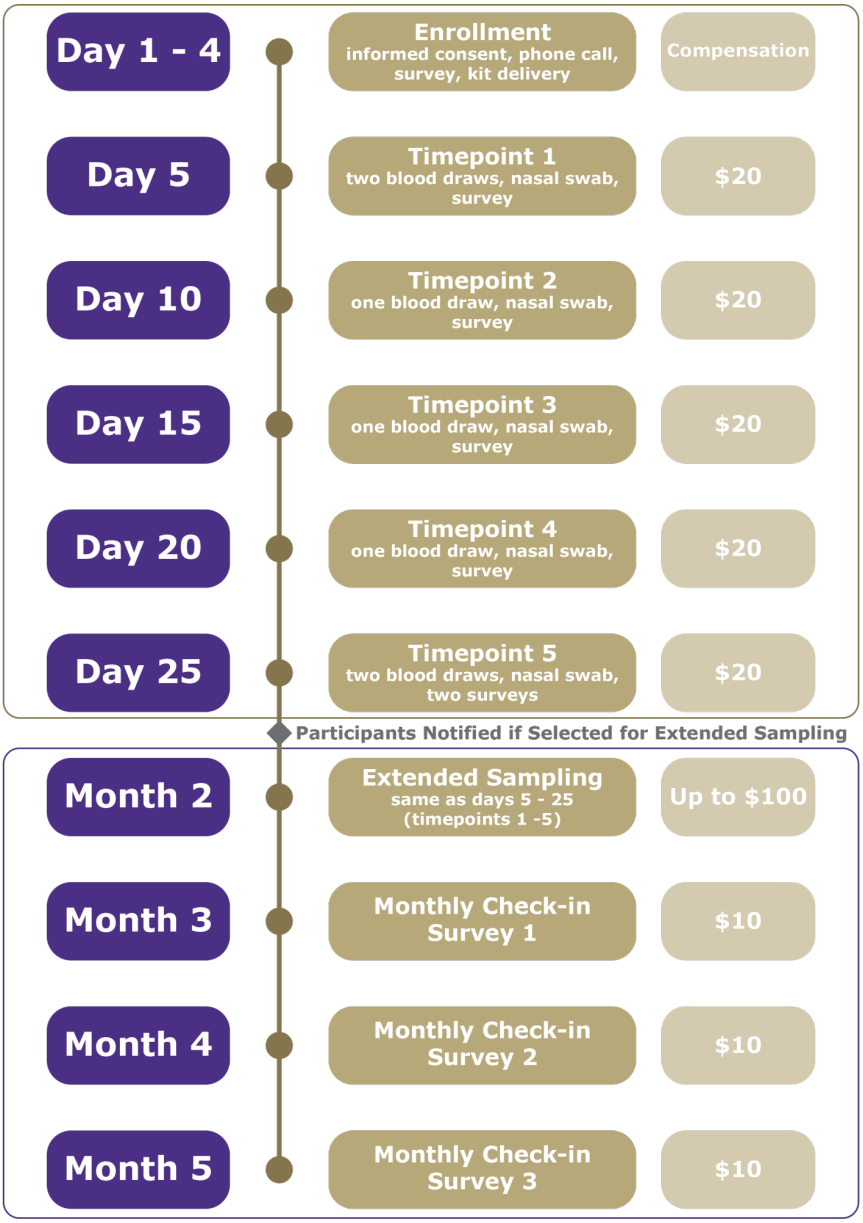


Figure C2. Study timeline provided to participants including breakdown of responsibilities at each time point and corresponding compensation.

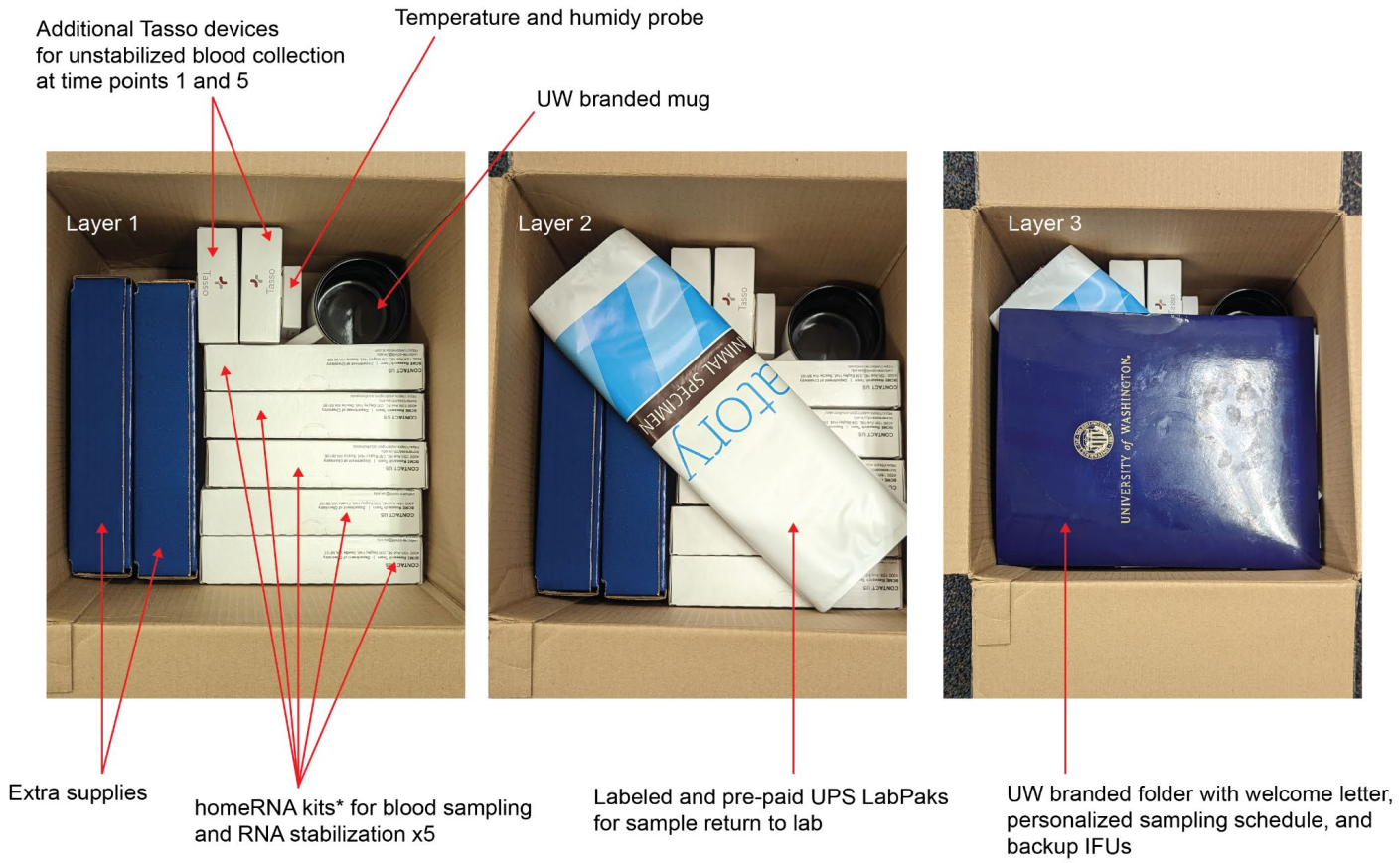
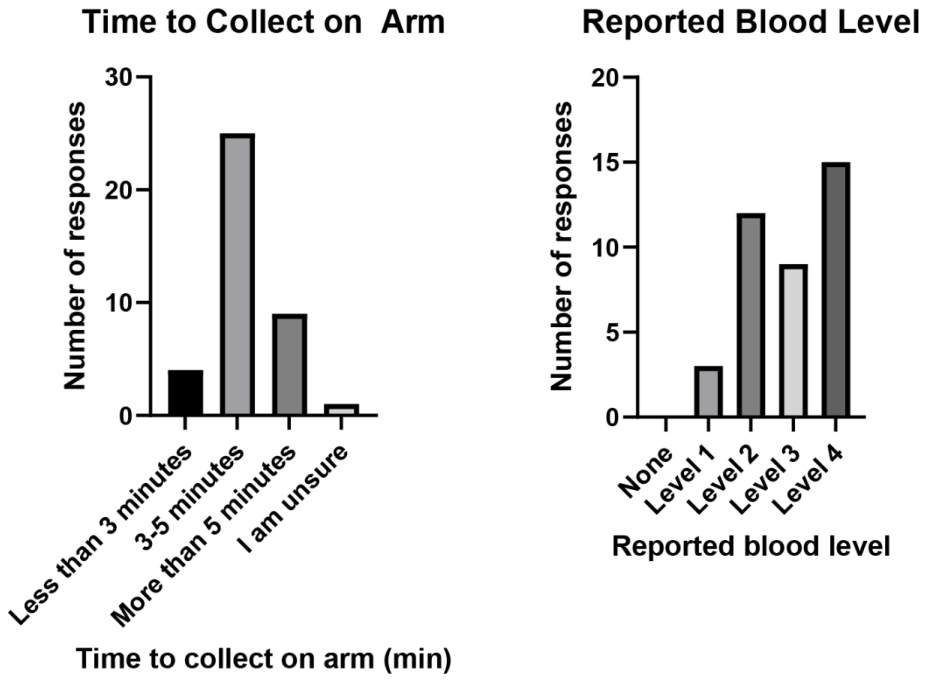


Figure C3. Study kit components. After enrollment in the study, participants were sent kits including all necessary study materials. This included 5 homeRNA kits (see Haack, Lim, et al. for details), 2 additional Tasso-SST devices for sampling without stabilization at time point 1 and 5, a temperature and humidity probe, a souvenir mug, 5 prepaid and labeled UPS LabPaks for sample return, and a folder including a welcome letter, personalized sampling timeline, and extra copies of instructions for use for each of the components.

A) First Collected Samples (n = 39)



B) All Collected Samples (n = 275)

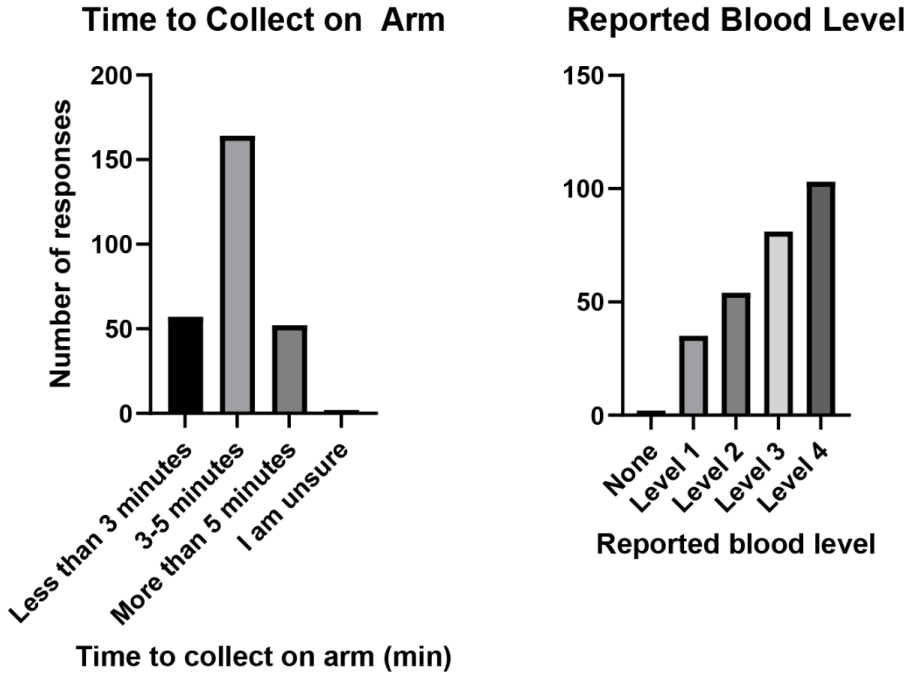


Figure C4. Summary of blood collection time and reported blood volume at first use (A) and across all samples (B). Overall, participants tended to spend roughly the same amount of time on blood collection throughout the study with fairly consistent blood volumes collected.

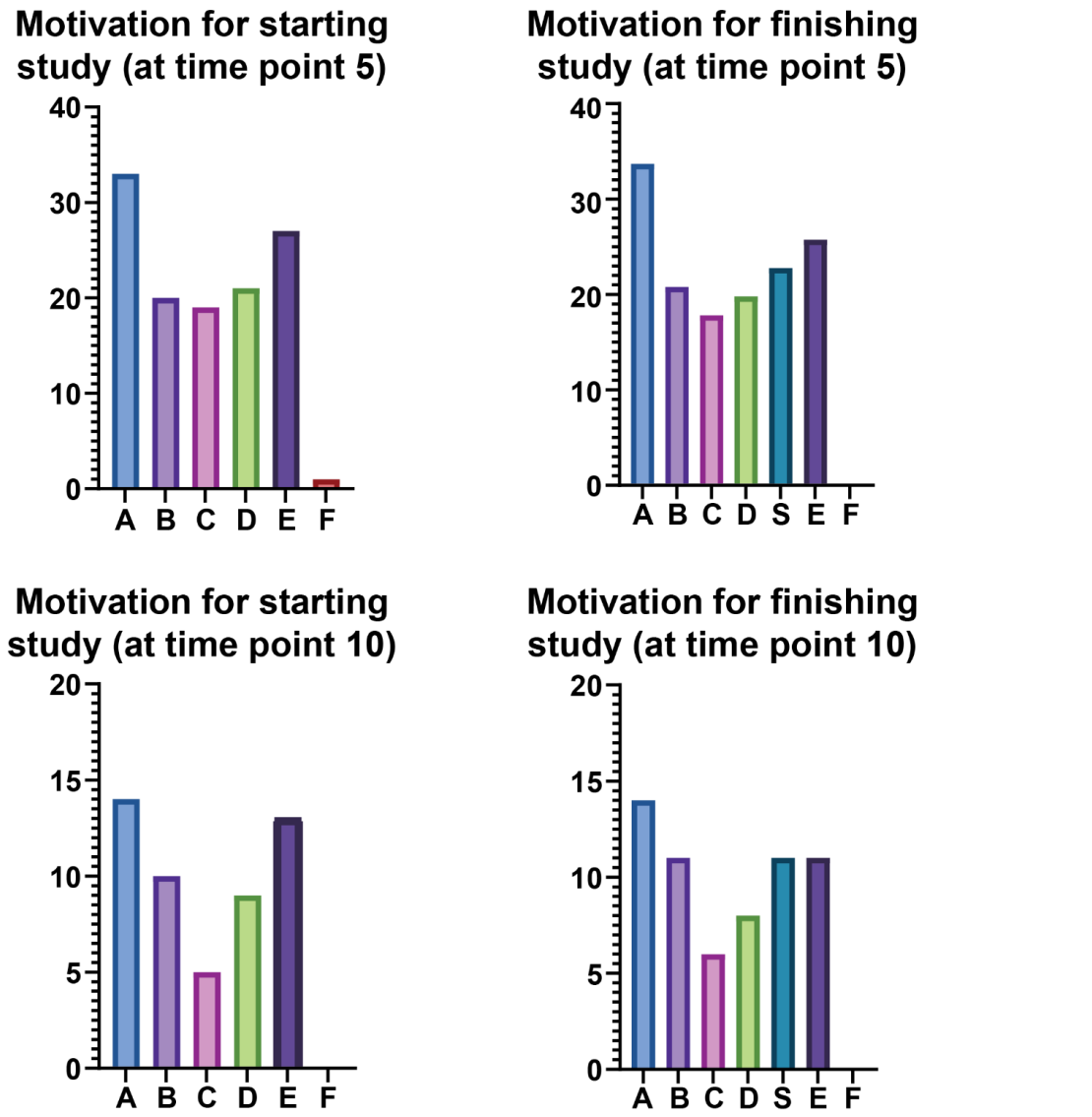


Figure C5. Summary of motivation for starting and continuing in study. The most important factors for starting in the study included interest in the topic (COVID-19) and flexibility of the remote study. In addition to these factors, participants reported positive interactions with the study team were a motivator for continuing in the study once enrolled. The answers of the whole cohort (n = 39) at time point 5 and the extended sampling participants at time point 10 did not differ meaningfully.

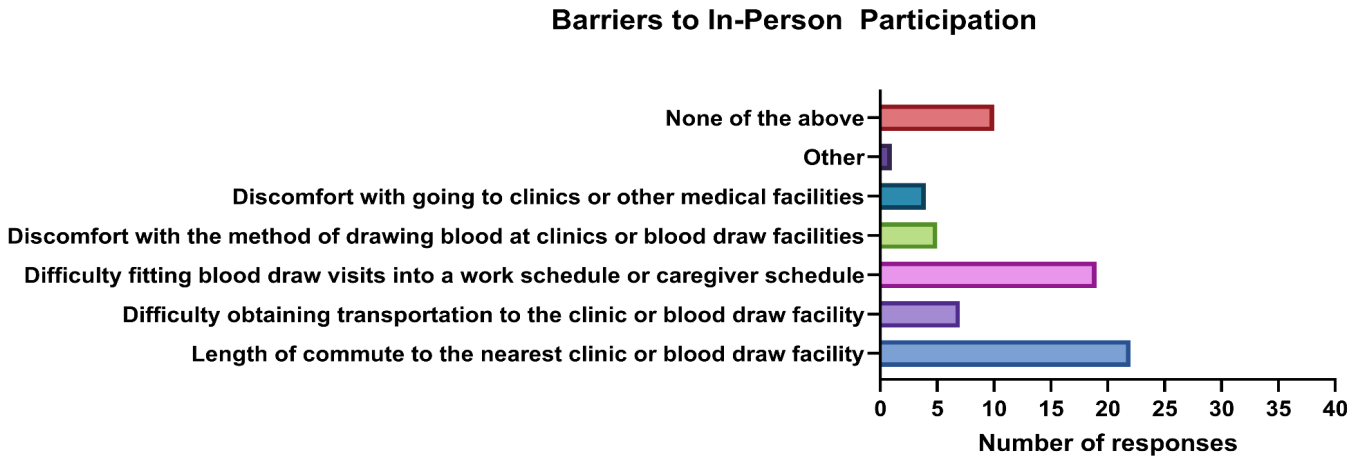


Figure C6. Summary of barriers to in-person participation.

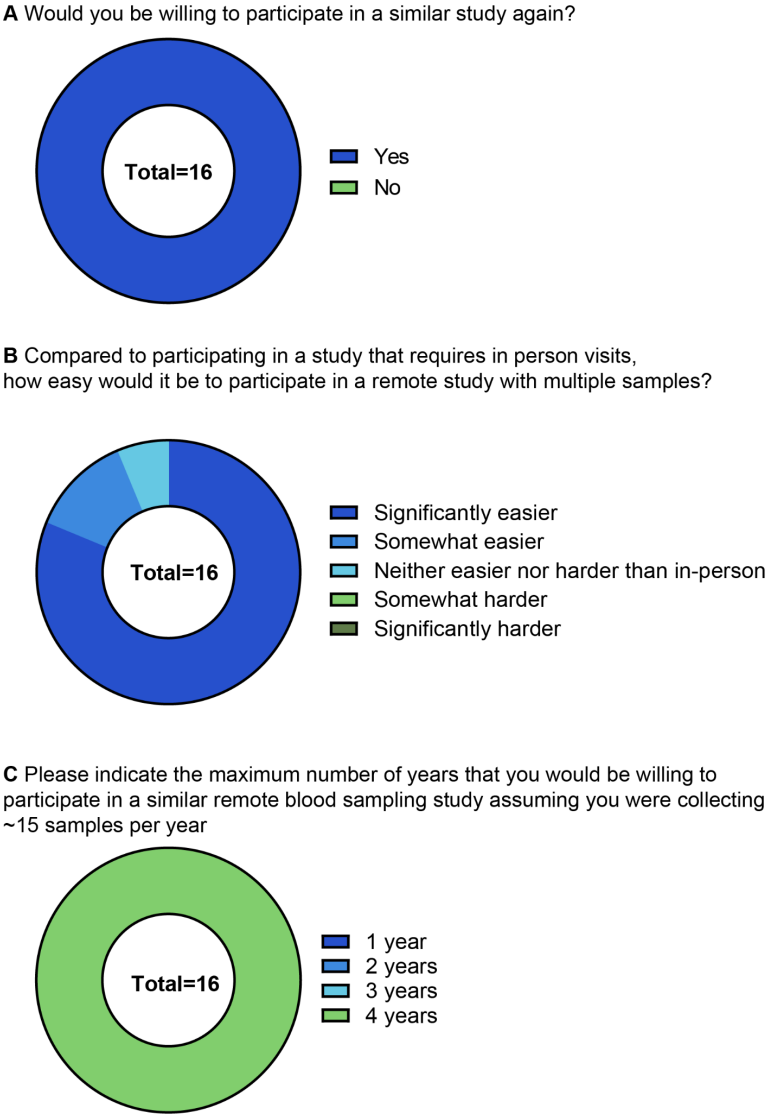


Figure C7. Summary of closing survey responses for extended sampling. All participants (n = 16/16) indicated that they would participate in a similar study again (A). Most participants (94%, n = 15/16) indicated that a remote study would be easier or significantly easier than an in-person one. All participants (n = 16/16) also indicated that they would be willing to participate in a remote study with 15 samples collected per year up to four years, which was the longest option available. Importantly, none of the extended study participants changed their answers negatively from their original responses to the closing survey at time point 5.

NIH Disadvantaged Background

The following text was copied from the NIH eRA commons on 9/26/2025 (https://www.era.nih.gov/commons/disadvantaged_def.htm)

Disadvantaged Background

An individual is considered to be from a disadvantaged background if he or she meets two or more of the following criteria:

- Were or currently are homeless, as defined by the McKinney-Vento Homeless Assistance Act (Definition: https://nche.ed.gov/mckinney-vento/);
- Were or currently are in the foster care system, as defined by the Administration for Children and Families (Definition: https://www.acf.hhs.gov/cb/focus-areas/foster-care);
- Were eligible for the Federal Free and Reduced Lunch Program for two or more years (Definition: https://www.fns.usda.gov/school-meals/income-eligibility-guidelines);
- Have/had no parents or legal guardians who completed a bachelor's degree (see https://nces.ed.gov/pubs2018/2018009.pdf);
- Were or currently are eligible for Federal Pell grants (Definition: https://studentaid.gov/understand-aid/types/grants/pell);
- Received support from the Special Supplemental Nutrition Program for Women, Infants and Children (WIC) as a parent or child (Definition: https://www.fns.usda.gov/wic/wic-eligibility-requirements).
- Grew up in one of the following areas: a) a U.S. rural area, as designated by the Health Resources and Services Administration (HRSA) Rural Health Grants Eligibility Analyzer (https://data.hrsa.gov/tools/rural-health), or b) a Centers for Medicare and Medicaid Services-designated Low-Income and Health Professional Shortage Areas (qualifying zipcodes are included in the file). Only one of the two possibilities in #7 can be used as a criterion for the disadvantaged background definition.