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Survey Field Methods for Expanded Biospecimen and Biomeasure Collection in NSHAP Wave 2

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Objectives. The National Social Life, Health, and Aging Project is a nationally representative, longitudinal survey of older adults. A main component is the collection of biomeasures to objectively assess physiological status relevant to psychosocial variables, aging conditions, and disease. Wave 2 added novel biomeasures, refined those collected in Wave 1, and provides a reference for the collection protocols and strategy common to the biomeasures. The effects of aging, gender, and their interaction are presented in the specific biomeasure papers included in this Special Issue.

Method. A transdisciplinary working group expanded the biomeasures collected to include physiological, genetic, anthropometric, functional, neuropsychological, and sensory measures, yielding 37 more than in Wave 1. All were designed for collection in respondents' homes by nonmedically trained field interviewers.

Results. Both repeated and novel biomeasures were successful. Those in Wave 1 were refined to improve quality, and ensure consistency for longitudinal analysis. Four new biospecimens yielded 27 novel measures. During the interview, 19 biomeasures were recorded covering anthropometric, functional, neuropsychological, and sensory measures and actigraphy provided data on activity and sleep.

Discussion. Improved field methods included in-home collection, temperature control, establishment of a central survey biomeasure laboratory, and shipping, all of which were crucial for successful collection by the field interviewers and accurate laboratory assay of the biomeasures (92.1% average co-operation rate and 97.3% average assay success rate). Developed for home interviews, these biomeasures are readily applicable to other surveys.

Key Words: Cardiovascular—Cytokines—Diabetes—Dried blood spots—In-home biomeasures—Steroids—Urinary measures.

BIOMEASURES encompass a broad range of physiological, anthropometric, functional, and sensory measurements. A fundamental component of The National Social Life, Health, and Aging Project (NSHAP) is the collection of biomeasures from respondents to objectively assess the estimation of incidence, prevalence, and severity of diseases and conditions in a community population; the detection of undiagnosed or subclinical conditions; and the provision of objective measures of health to use in conjunction with survey-based measures. With respect to NSHAP Wave 1 biomeasures, this article describes both continuity and refinements to NSHAP Wave 2 biomeasures, enabling longitudinal analyses, as well as novel biomeasures. Some biomeasures were collected during the interview with the result directly entered in Computer Assisted Personal Interview (CAPI) and others were assayed postinterview from collected biospecimens (see Tables 1 and 2).

For biological measures, reported values are dramatically affected by the conditions under which biospecimens are collected, transported, and assayed. Therefore, methods for acquiring biospecimens are an integral part of the science and data interpretation. NSHAP could not use clinical laboratory protocols because the biospecimens were collected in the respondents' homes by nonmedically trained field interviewers. Therefore, we adapted and created field methods for specimen collection and transportation that are well suited to home-based collection and yielded valid and reliable data.

In this article, we detail NSHAP's successful field methods. Data users can reference this article or summarize the field methods to satisfy the readers of medical, biopsychological, or clinical publications who require this level of detail to have confidence in the results and their replicability. In addition, as biomeasure collection in

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Table 1. Primary Applications of Wave 2 Biomeasures to Diseases, Aging Conditions, and Psychosocial Systems

			Multiple applications							
Biomeasures assayed from biospecimens	Wave(s) collected	Biospecimen	Diabetes and energetics	Cardiovascular function	Immune function	Kidney function	Biological stress	Psychological state	Reproductive function and sexuality	Social behavior
HbA1c	Waves 1–2	DBS	x							
Adiponectin	Wave 2	Plasma[a]	x							
Cholesterol	Wave 2	DBS	x	x						
HDL	Wave 2	DBS	x	x						
Apolipoprotein B	Wave 2	Plasma	x	x						
Hemoglobin	Waves 1–2	DBS	x	x						
VEGF	Wave 2	Plasma	x	x	x			x		
TNF-β	Wave 2	Plasma	x	x	x			x		
C-reactive protein	Waves 1–2	DBS	x	x	x	x				
IL-1β	Wave 2	Plasma	x	x	x	x	x	x		
IL-1rα	Wave 2	Plasma	x	x	x	x	x	x		
IL-6	Wave 2	Plasma	x	x	x	x	x	x		
TNF-α	Wave 2	Plasma	x	x	x	x	x	x		
MCP-1	Wave 2	Plasma		x	x					
TFG-α	Wave 2	Plasma		x	x					
Fibrinogen	Wave 2	Plasma		x						
IL-12	Wave 2	Plasma			x		x			
IL-2	Wave 2	Plasma			x					
sIL-2ra	Wave 2	Plasma			x					
IL-3	Wave 2	Plasma			x					
IL-4	Wave 2	Plasma			x					
IL-5	Wave 2	Plasma			x					
IL-10	Wave 2	Plasma			x					
IL-13	Wave 2	Plasma			x					
GM-CSF	Wave 2	Plasma			x					
IFN-γ	Wave 2	Plasma			x					
Epstein–Barr virus antibody	Waves 1–2	DBS			x					
NGAL (lipocalin-2)	Wave 2	Plasma				x				
Creatinine	Wave 2	Urine				x		x		x
Estradiol	Waves 1–2	Passive drool						x	x	x
Progesterone	Waves 1–2	Passive drool						x	x	x
Vaginal cytology	Waves 1–2	Vaginal swab						x	x	x
Bacterial vaginosis	Waves 1–2	Vaginal swab			x				x	
Yeast	Waves 1–2	Vaginal swab			x				x	
Testosterone	Waves 1–2	Passive drool					x	x	x	x
DHEA	Waves 1–2	Passive drool					x	x	x	x
Cortisol, basal, and reactive	Wave 2	Saliva sponge					x	x	x	x
Oxytocin	Wave 2	Urine						x		x
Vasopressin	Wave 2	Urine						x		x
Genotype	Wave 2	Oragene, DBS	x	x	x	x	x	x	x	x

Notes. DBS = dried blood spots; DHEA = dehydroepiandrosterone; GM-CSF = granulocyte-macrophage colony-stimulating factor; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; IL = interleukin; MCP = monocyte chemoattractant protein; NGAL = lipocalin-2/neutrophil gelatinase-associated lipocalin; TFG = transforming growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor.

[a]Plasma is obtained in the laboratory by spinning down the unclotted whole blood.

population-based studies continues to expand, it is important for researchers to share their detailed field methods with future survey researchers in order for the research community to establish industry standards for in-home collection and transport methods. Sharing methods also allows data users to better understand comparisons of reported biomeasure values across waves or across different surveys because different collection, storage, and analysis methods may explain different values and offer resolution.

Conceptual Criteria for Expanding Wave 2 Biomeasures

Building on the success of Wave 1, the Wave 2 biomeasures were collaboratively reviewed, refined, and expanded by the transdisciplinary NSHAP biomeasure working group (comprised of biopsychologists, physicians, biostatisticians, health services researchers, the NORC operations team and members of the McClintock Survey Biomeasures Laboratory [MSBL]). As in Wave 1, the group balanced, in an iterative process, the intellectual value of a specific biomeasure with

Table 2. Comparison of NSHAP Biomeasures Collected During Waves 1 and 2

Fluids and systems	Biological specimens and measures in interview	Wave(s) collected	Protocol comparison across Waves 1 and 2		
			In-home collection	Handling and shipping	Assay laboratory
Blood	Dried blood spots	Waves 1–2	Refined	Same	Changed
	Unclotted whole blood	Wave 2	New	New	New
Saliva	Saliva passive drool	Waves 1–2	Refined	Refined	Changed
	Saliva sponge: Salivette	Wave 2	New	New	New
	Saliva spit: Oragene	Wave 2	New	New	New
	Saliva spit: Orasure (HIV)	Wave 1	□	□	□
Genitourinary tract	Vaginal secretions: swab	Waves 1–2	Refined	Refined	Same
	HPV vaginal swab	Wave 1	□	□	□
	Urine	Wave 2	New	New	New
Anthropometrics	Height	Waves 1–2	Same	•	•
	Weight	Waves 1–2	Same	•	•
	Waist circumference	Waves 1–2	Same	•	•
	Hip circumference	Wave 2	New	•	•
Cardiovascular	Heart rate	Waves 1–2	Same	•	•
	Blood pressure	Waves 1–2	Same	•	•
	Preventricular contraction	Waves 1–2	Same	•	•
Frailty, activity, sleep	Timed walk	Waves 1–2	Refined	•	•
	Chair stands	Waves 1–2	Refined	•	•
	Actiwatch	Wave 2	New	•	•
Neuropsychology	Orientation	Waves 1–2	Refined	•	•
	Memory	Waves 1–2	Refined	•	•
	Attention	Waves 1–2	Refined	•	•
	Visuoconstruction	Wave 2	New	•	•
	Naming	Wave 2	New	•	•
	Language	Wave 2	New	•	•
	Executive function	Wave 2	New	•	•
	Abstraction	Wave 2	New	•	•
Sensory	Smell	Waves 1–2	Refined	•	•
	Taste	Wave 1	□	•	•
	Touch	Wave 1	□	•	•
	Vision	Wave 1	□	•	•

Notes. HPV = human papillomavirus; NSHAP = The National Social Life, Health, and Aging Project.
• Not applicable, data collected during interview.
□ Not repeated in Waves 1 and 2.

the challenges of collecting the biological measure or biospecimen in the home that would yield valid and reliable data with sufficient power to test key hypotheses.

Our specific research criteria were biomeasures that would (a) most fully characterize the most prevalent disease states, aging conditions, and health identified in Wave 1, (b) further test hypotheses about the physiological systems mediating the effects of psychosocial factors on health (and vice versa), and (c) measure fundamental biological mechanisms that are involved in multiple diseases, conditions, and psychosocial states. For example, it is well recognized that inflammation is a basic physiological process that underlies a wide range of diseases ranging from cardiovascular disease to arthritis to cancer. It is also well established that stressors can exacerbate inflammation while social networks can protect against the effects of stress. An integrative indicator of inflammation, C-reactive protein, was analyzed from dried blood spots (DBS) in both Waves 1 and 2. In Wave 2, we added the associated proinflammatory cytokines (see Tables 1 and 2). Data are publically available (NSHAP Wave 1: Waite, Linda J., Edward O. Laumann, Wendy Levinson, Stacy Tessler Lindau, and Colm A. O'Muircheartaigh. National Social Life, Health, and Aging Project (NSHAP): Wave 1. ICPSR20541-v6. Ann Arbor, MI: Inter-university Consortium for Political and Social Research [distributor], 2014-04-30. doi:10.3886/ICPSR20541.v6. NSHAP Wave 2: Waite, Linda J., Kathleen Cagney, William Dale, Elbert Huang, Edward O. Laumann, Martha K. McClintock, Colm A. O'Muircheartaigh, L. Phillip Schumm, and Benjamin Cornwell. National Social Life, Health, and Aging Project (NSHAP): Wave 2 and Partner Data Collection. ICPSR34921-v1. Ann Arbor, MI: Inter-university Consortium for Political and Social Research [distributor], 2014-04-29. doi:10.3886/ICPSR34921.v1.).

The measures were selected because they all serve multiple functions and have multiple applications in analysis of the NSHAP Wave 2 data (Table 1). The most prevalent diseases in Wave 1 were cardiovascular and diabetes, both associated with inflammation indicated by C-reactive protein (Vasilopoulos et al., forthcoming). To permit longitudinal

analysis, we retained these biomeasures in Wave 2, including C-reactive protein, an inflammatory marker, and also extended the measure with a battery of inflammatory cytokines and other molecules associated with these particular diseases. We hypothesized that the balance or pattern of cytokine production is the best indicator of inflammation, not just levels of one or two. Obesity, a known risk factor for these two diseases, was also prevalent in Wave 1, and we included molecules associated with lipid metabolism, such as adiponectin in Wave 2 (see Table 1). In addition, because impaired kidney function is a final common path for mortality, the panel included molecules that could potentially indicate preclinical levels of kidney impairment in our community-dwelling sample (See Table 1; Reyes et al., forthcoming).

Obtaining a panel of 22 analytes does not seem feasible for an in-home study collecting only blood spots, not venipuncture with multiple tubes of blood. Therefore, we refined our blood collection technique, still using lancets, and were able collect an additional five drops of whole unclotted blood. To maximize use of this small sample volume, we used a Bio-Plex system, driven by Luminex xMAP technology, at the University of Chicago Flow Cytometry Core Facility (detailed below). Our approach illlustrates how advances in biomedical assay techniques can be applied to field research where the volume of blood collected is small.

Some of these biomeasures also affect brain function, manifest in mental health and cognition, which may mediate the reciprocal relationships between social life, health, and disease during aging. Therefore, we selected those that also serve multiple functions across psychosocial systems (see Table 1). To this end, we also introduced a novel measure of stress: three salivary samples to assay free cortisol collected over the 2-hr interview, characterizing basal levels at the time of the interview, and also an acute stress response to the psychosocial challenge of completing a timed cognitive evaluation. We provide the first survey study of oxytocin and vasopressin, social peptides involved not only in close social relationships, but also stress and blood pressure (Reyes et al., forthcoming). Finally, we continued our measures of reproductive steroids and their vaginal bioassay, key for sexuality, which is a unique part of the NSHAP survey (Galinsky et al., forthcoming) as well as frailty, a concept for aging on which Wave 2 has focused (Huisingh-Scheetz et al., forthcoming). Lastly, we collected samples to analyze for genotype and asked a subset of participants to wear an actigraph watch for 3 days after the interview, enabling measurement of daytime activity (Huisingh-Scheetz et al., forthcoming) and sleep (Lauderdale et al., forthcoming).

Recognizing the multiple systems in which each biomeasure is imbedded facilitates the multilevel integrated analyses to explicate the reciprocal interactions among social life, psychological states, and biological mechanisms. This is an important point, even when a single disease or system is the primary research focus, this paper identifies the other systems in which its biomeasures are imbedded, and may need to be included as potential sources of error or countervening forces.

Pragmatic Criteria for Expanded Wave 2 In-home Biomeasures

There are a variety of models for integrating biomeasures into population-based studies (Jaszczak, Lundeen, & Smith, 2009) ranging from centralized collection of biomeasures by health care professionals at a medical facility at one end of the spectrum to self-administered biomeasure collection using equipment that is left with or mailed to the respondent at the other. One variation of the medical facility model is the use of mobile clinics with high-quality facilities, equipment, and trained medical staff that can be moved from location to location, a model utilized by the National Health and Nutrition Examination Survey (Centers for Disease Control and Prevention, National Center for Health Statistics, 2013). In the middle of this spectrum is the use of nonmedically trained interviewers to collect biomeasures in the respondents' homes. Recent developments in technology have made a wider range of in-home biomeasure collection feasible using nonmedically trained field interviewers.

At the onset of the study, NSHAP decided to use field interviewers to collect biomeasures in the home as part of the in-person interview. Field interviewers gave us the opportunity to collect biomeasures efficiently and cost effectively. In addition, interviewers can overcome difficulties in gaining the participation of population subgroups (i.e., poor health, lack of transport, remoteness) that might otherwise be missing or underrepresented in the study population. This, combined with the knowledge that Health and Retirement Survey (Institute for Social Research, 2013) successfully administered a limited set of biomeasures using this model, lead to our decision to move forward with in-home collection.

The key operational criteria for selecting biomeasures were: (a) adaptability to in-home biomeasure collection; (b) adaptability to nonmedically trained administration; (c) adaptability to the target population of older adults; (d) choosing portable, reliable, valid, and economical equipment; (e) evaluating the administration and response burden on field interviewers, respondents, and the MSBL (for each measure and collectively in the NSHAP interview); (f) biospecimen temperature control during in-home interview, transportation, storage, and shipping; and (g) validating all protocols and assays in field pretests.

To determine whether a Wave 1 biomeasure was suitable for Wave 2, the transdisciplinary group first analyzed the Wave 1 biomeasures to determine if they were similar to the current literature, had been operationally feasible and should be retained, refined, or dropped for Wave 2. Overall, a number of high-quality biomeasure data were obtained by Wave 1 nonmedically trained interviewers; results, however, also illuminated protocols needing improvement for in-home collection, shipping, biospecimen tracking, or laboratory assays. For example, we developed novel field collection

and temperature control techniques detailed below, essential for the success of several novel biomeasures.

Next, we conducted a small pretest of instrumentation and field protocols prior to Wave 2 (n = 120). The pretest allowed the evaluation of novel field collection techniques for the new biomeasures, confirmed comparability of refined biomeasures repeated in both waves, and field testing of the temperature control techniques—guided by the same scientific and operational criteria described above. The pretest provided invaluable data crucial for finalizing the Wave 2 biomeasures (particularly for the novel field techniques), shipping the biospecimens from the field to the laboratories, choosing laboratories for assays, and refining assay methods for biospecimens collected in the home by nonmedically trained interviewers and not in a clinic setting. This process highlighted both the technical challenges and success of obtaining high-quality biomeasure data in a survey data. This article includes the details that were particularly challenging as well as the solutions.

In this overview article, we first describe the refined and novel biospecimens collected in the home for assay of an array of different biomeasures (see Tables 1 and 2), including their common protocols for collection, transport, storage and shipping, and preparation for laboratory assays to generate biomeasures. The utility of each biomeasure for aging research are covered in separate articles in this Special Issue, along with their distributions and gender differences. These articles in this Special Issue also recommend analysis methods, particularly for longitudinal analysis (Gregg, O'Doherty, Schumm, McClintock, & Huang, forthcoming; Hoffmann, You, Hedberg, Jordan, & McClintock, forthcoming; Kozloski, Schumm, & McClintock, forthcoming; Lauderdale et al., forthcoming).

Then we describe the biomeasures collected during the home interview with the result directly entered in CAPI (See Table 2). Details of these biomeasures assessing chronic diseases, frailty, neuropsychology, olfaction, and sensory function are available in separate articles in this Special Issue (Huisingh-Scheetz et al., forthcoming; Kern et al., forthcoming; Pinto et al., forthcoming; Shega et al., forthcoming; Vasilopoulos et al., forthcoming).

Refined and Expanded Biospecimen Collection

Our iterative process, balancing intellectual value with feasibility in the field and laboratory, identified four types of body fluids (blood, saliva, urine, and vaginal secretions) yielding 40 biomeasures, 29 more than in Wave 1 (Tables 1 and 2). For each biospecimen (novel, refined, and repeated), adherence to details during collection, transfer, storage, and shipping enabled substantially more types of reliable and valid biomeasure data. The continuity and changes made to biospecimens between Waves 1 and 2 are summarized in Tables 1 and 2. We had many failed and ultimately successful pilots, and anticipated and forestalled many things that could have gone wrong in the field. This article provides those details that we found essential for NSHAP's resounding success.

Controlled Biospecimen Temperature During the Home Interview, Storage, and Transportation

In a national population-based study, temperature control is a major challenge for biospecimen integrity and data reliability. Because some biomeasures are not stable at ambient temperatures, we developed new field protocols for keeping specimens cold or frozen throughout the home interview, transportation, storage, and shipping. The new field methods developed for Wave 2 also allowed us to introduce new biomeasures for Wave 2. For example, biomeasures for immune and kidney function, stress, sexuality, psychological states, and social interactions need to be assayed in unclotted whole blood and urine were enabled by new Wave 2 collection protocols.

We verified that the new Wave 2 protocols kept biospecimens cold (7 ± 3°C) in a pilot test was conducted prior to field data collection. Thermal recorders (SmartButton, Catalog No. 01-0184; ACR Systems, Surrey, BC) were placed next to mock specimens while mimicking each phase of the protocol: field interview, storage, and laboratory shipping. Specimens stayed between 4.0°C and 10.0°C for 24 hr, not reaching 22°C until 56 hr. In this way, temperature control was substantially improved over ambient temperatures in Wave 1 (temperature range = 20.5°C–24.0°C; transported in a conventional lunch bag with an Instant Cold Compress (Catalog No. M565; First Aid Only, Vancouver, WA) and shipped in a Styrofoam shipping container with an Instant Cold Compress).

The field interviewers brought coolers with frozen nontoxic refrigerant packs (Polar Pack Foam Brick, Catalog No. FPP15; ThermoSafe Brands, Arlington Heights, IL) to the interview for biospecimen transport. Designed to sustain cold temperatures for small volume biospecimens, the semirigid foam brick is impregnated with refrigerant. Solid when frozen, it requires considerably more energy than does water to transition states at 0°C from a solid to a gel/liquid; the foam retains its shape throughout. The nylon cooler (8.5"L × 7"H × 5"W; Gemline, Inc., Lawrence, MA) had thick foam walls designed to keep a six-pack cold for an extended period. Immediately after collection, the field interviewer placed each biospecimen underneath the refrigerant pack (except for DBS), immediately resealing the cooler and transporting it to his or her field base.

At the field base, the field interviewer stored the biospecimens needing refrigeration (urine, unclotted whole blood, vaginal swabs, and saliva sponge) in a Styrofoam shipping container (Mini-Mailer, Catalog No: 440; SCA Thermosafe, Arlington Heights, IL), rotating a fresh-frozen refrigerant pack every 24 hr if shipping was delayed. Passive drool saliva specimens requiring freezing were collected in a 24-place CRYO Storage Box (Catalog No: 24270-200; VWR International, Radnor, PA) and placed in a residential grade freezer (−23°C to −18°C) inside a Styrofoam box

to protect against warming during the automatic defrost cycles. Specimens stable at room temperature were stored according to biospecimen-specific protocols, which are described in the biospecimen sections.

DBS and Unclotted Whole Blood: Immunity, Metabolism, Cardiovascular Disease, and Kidney Function

Collection.—Refined blood collection protocols produced higher blood flow than in Wave 1, significantly decreasing collection time and increasing the percent of respondents, who with a single stick, could provide the requisite five drops of blood (66%–75%; the remainder required multiple sticks). It also enabled us to add a new blood specimen in Wave 2, unclotted whole blood collected and stored in a Microtainer (a small, unbreakable plastic tube with a FloTop collector), which yielded plasma for assay of 22 additional biomeasures not feasible using DBS (see Table 2). For the respondent, the two whole-blood collection protocols were seamlessly combined into a single collection of 10 drops. The co-operation rate for blood collection was 92.1% (using COOP3; The American Association for Public Opinion Research, 2011).

The Wave 2 DBS collection was based on Wave 1 (Williams & McDade, 2009 for Wave 1; see Electronic Supplement # 1 for Wave 2). Three key modifications increased blood flow and collection volume for Wave 2. First, a hand warmer improved circulation. The field interviewer began the DBS collection by activating a hand warmer and asking the respondent to hold the hand warmer in their nondominant hand for a couple of minutes before blood collection. Second, a new lancet (BD Blue Lancet, Catalog No 366594; Becton Dickinson and Company, Franklin Lakes, NJ) Third, a smaller filter paper card ([3 ½" × 2 ½"] vs. [4" × 8 ¼]; Whatman 903 Protein Saver Card, Catalog No 10534612; GE Healthcare Life Sciences, Piscataway, NJ) allowed the field interviewer to maneuver the card more easily during DBS collection, and was viewed as user friendly. The card's collection area was the same; only the administrative data area was smaller (e.g., identification numbers and labels).

After the five DBS were collected, the field interviewer immediately placed the respondent's finger over the Microtainer (BD Microtainer Tube with Dipotassium EDTA, Beadless additive BD Microgard closure, Catalog No. 365974; Becton Dickinson and Company) and collected the additional five drops of whole blood (250 µl).

Transportation and storage.—Immediately after collection, the field interviewer placed the Microtainer under the refrigerant pack in the biospecimen cooler and then set aside the DBS filter paper to dry until the end of the interview, at which point it was sealed in a Ziploc bag with a silica-gel desiccant pack (Catalog No: 10548234; Whatman, Piscataway, NJ). At the interviewer's field base, the Microtainer was shipped overnight to the University of Chicago, Flow Cytometry Facility in a Styrofoam container containing a refrigerant pack. The field interviewers removed the DBS filter paper from the Ziploc bag and placed it in a breathable plastic container with a desiccant pack to dry overnight. In the morning, the filter paper was stored in a sealed Ziploc bag along with the desiccant pack in a residential grade refrigerator (4°C) until weekly shipping to the University of Washington, Department of Medicine Biomarker Analysis Laboratory.

Assays.—The University of Washington, Department of Medicine Biomarker Analysis Laboratory assayed four analytes from the DBS. The technical details associated with selecting a new laboratory for DBS analysis are described in Gregg et al. (forthcoming). Each assay had a high yield, and improved over Wave 1 (success summarized by % valid assay values given samples collected): C-reactive protein (97.9%, 3.2% improvement over Wave 1), Epstein–Barr virus antibody (98.1%, 1.4% improvement), total hemoglobin (97.4%, 6.7% improvement), and glycosylated hemoglobin (97.6%, 12.7% improvement) (see Table 2 for multiple applications). A flow diagram of each step from the number of participants to the number of valid assay values is provided in Supplementary Figure 2 of Electronic Supplement #2 and the valid assay values are summarized in Table 3.

The NSHAP data set reports both DBS and fresh plasma values. The laboratory used an independent set of blood samples to assay both as plasma and as a DBS. Their regression equation provided the algorithm for generating plasma values from DBS values. As of the submission date of this manuscript, NSHAP also plans to analyze the DBS for cholesterol and HDL.

Unclotted whole blood in the Microtainer provided the plasma to measure key analytes characterizing immune function, metabolism, biological stress, kidney function, and psychological states (see Table 1). The unclotted blood collected in K_2EDTA Microtainers was centrifuged and the plasma was extracted and frozen (−80°C) at the University of Chicago, Flow Cytometry Facility. As of the submission date of this manuscript, a multiplex panel of 18 cytokines-chemokines are being assayed in duplicate with Luminex technology (Luminex 100 device; BioRad, München, Germany) using the BioPlex Manager Software (Version 5, BioRad; see Tables 1 and 2 for analytes and their multiple applications to health and psychosocial systems). Multiplex magnetic-bead antibody kits were used for cytokines-chemokines (GM-CSF, IFN-γ, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, MCP-1, sIL-2ra, TGF-α, TNF-α, TNF-β, and VEGF), Milliplex MAP Human Cytokine/Chemokine Panel (HCYTOMAG-60K-18; Millipore, Schwalbach, Germany) as well as for adiponectin, Lipocalin-2/neutrophil gelatinase-associated lipocalin, fibrinogen, and apolipoprotein B. See Table 2 for multiple applications.

Table 3. Percent of Valid Assay Values for Biomeasures Assayed From Biospecimens and Change From Wave 1

Biomeasures assayed from biospecimens	Biospecimen	Percent of valid assay values	Change from Wave 1
C-reactive protein	DBS	97.9%	3.2% improvement
Epstein–Barr virus antibody	DBS	98.1%	1.4% improvement
Total hemoglobin	DBS	97.4%	6.7% improvement
Glycosylated hemoglobin	DBS	97.6%	12.7% improvement
Testosterone	Passive drool	94.7%	6.6% improvement
17-beta-estradiol	Passive drool	88.8%	6.7% decrease
Progesterone	Passive drool	95.3%	1.7% improvement
DHEA	Passive drool	95.6%	4.7% improvement
Cortisol (beginning)	Saliva sponge	98.9%	n/a (new Wave 2)
Cortisol (middle)	Saliva sponge	99.1%	n/a (new Wave 2)
Cortisol (end)	Saliva sponge	99.2%	n/a (new Wave 2)
BV	Vaginal swab	98.5%	4.4% improvement
Yeast	Vaginal swab	97.3%	11.8% improvement
Oxytocin	Urine	99.6%	n/a (new Wave 2)
Vasopressin	Urine	99.6%	n/a (new Wave 2)
Creatinine	Urine	99.6%	n/a (new Wave 2)

Note. BV = bacterial vaginosis; DBS = dried blood spots; DHEA = dehydroepiandrosterone; n/a = not applicable.

In the cytokine panel, there are 18 separate bead sets, each of which is internally dyed with a specific fluorescence, and is then conjugated with antibodies that bind the analyte of interest. Each bead has antibodies for one particular molecule and a corresponding uniquely colored fluorescence. The plasma sample is then combined with the beads, dilutions are added if necessary, the mixture is stabilized, and the beads adhere to the proteins in the blood sample. The mixture is then run through the Luminex reader, where beads are detected by flow cytometry and move single file through a flow cell where red and green lasers function to detect bead color and signal strength.

Saliva Collection: Saliva Passive Drool, Saliva Sponges (Salivettes), and Oragene

Saliva production decreases during aging (Hochberg et al., 1998; Jones, Watkins, Hand, Warren, & Cowen, 2000) and is further reduced by common medications (Thomson, Chalmers, Spencer, & Slade, 2000). Nonetheless, we wanted to remeasure all four reproductive steroids (estradiol, progesterone, testosterone, dehydroepiandrosterone [DHEA]) from a saliva specimen passively drooled into the collection tube (Kozloski et al., forthcoming) and add salivary free cortisol as a biomeasure of adrenal function and stress (see Table 2), requiring three additional saliva samples. We also added genotyping, which required yet another saliva sample (Oragene).

We achieved these increased collection goals with five modifications. We worked with a laboratory needing only half the Wave 1 sample volume to measure all four reproductive steroids in duplicate (Kirschbaum, Strasburger, Jammers, & Hellhammer, 1989; Walpurger, Pietrowsky, Kirschbaum, & Wolf, 2004). We employed Salivette-cortisol sponges designed for duplicate assays of small volumes, which are chewed, stimulating saliva production. Finally, we collected saliva for genetics with Oragene, at the very end of the interview when respondents had had time to replenish their saliva. We also dropped the Wave 1 assay of salivary cotinine because it correlated highly with self-reported smoking in Wave 1, a question retained in Wave 2. We also dropped the Wave 1 saliva test for HIV (Orasure) as we found its prevalence was exceptionally low among 57- to 85-year-olds in the United States (0.236% ± 0.002 standard error of the population estimate).

Saliva Passive Drool: Reproductive Steroids

Collection.—The Wave 2 passive drool saliva collection protocol was nearly identical to Wave 1 (Gavrilova & Lindau, 2009), except for collecting less volume in a smaller vial (see Electronic Supplement #1). The co-operation rate was 94.9% (using COOP3; The American Association for Public Opinion Research, 2011).

Transportation and storage.—The field interviewer placed the passive drool saliva container in the cooler under the refrigerant pack and transported it to the interviewer's field base for storage in a residential grade freezer (−20°C). The specimens were batch-shipped monthly in a Styrofoam container on dry ice to the MSBL. Upon receipt at the MSBL, each sample was stored at −80°C until shipment to the Dresden LabService GmbH, Germany. No samples were lost in transit (see Supplementary Figure 3).

Assays.—We collaborated with Clemens Kirschbaum, PhD (Technisch Universität Dresden LabService GmbH, Dresden; Kirschbaum et al., 1989) to optimize assay kits and procedures for duplicate assays in small volumes and to help ensure comparability between Waves 1 and 2

Table 4. Overview of Wave 2 Biospecimen Shipping and Assay Laboratories

Biospecimen	Shipping schedule	Temperature requirements	Shipping destination	Biomeasure assay laboratory
Cytology vaginal swab (red)	Daily	Cold	Martha K. McClintock, PhD, Director, University of Chicago, McClintock Survey Biomeasure Laboratory (MSBL)	Martha K. McClintock, PhD, Director, University of Chicago, MSBL
Urine (three tubes)	Daily	Cold	MSBL	Toni Ziegler, PhD, Director, Assay Services Laboratories at the Wisconsin National Primate Research Center, NIH RR00016
Saliva sponge: Salivette (three swabs)	Daily	Cold	MSBL	Clemens Kirschbaum, PhD, Director, Technisch Universität, Dresden LabService GmbH, Germany
Saliva spit: Oragene	Daily	Room temperature	MSBL	To be selected
Microbiology vaginal swab (blue)	Daily	Cold	Jeanne A. Jordan, PhD, Laboratory Director-GWU Clinical Research Site[a]	Jeanne A. Jordan, PhD, Laboratory Director-GWU Clinical Research Site
Whole blood in Microtainer	Daily	Cold	Ryan Duggan, BA, Technical Director, University of Chicago, Flow Cytometry Facility	Ryan Duggan, BA, Technical Director, University of Chicago, Flow Cytometry Facility
Dried blood spots	Weekly	Cold/room temperature	Alan Potter, PhD, Technical Director, University of Washington, Department of Medicine Biomarker Analysis Laboratory	Alan Potter, PhD, Technical Director, University of Washington, Department of Medicine Biomarker Analysis Laboratory
Saliva passive drool	Monthly	Frozen	MSBL	Clemens Kirschbaum, PhD, Director, Technisch Universität, Dresden LabService GmbH, Germany

Note. [a]Full title is Jeanne A. Jordan, PhD, Laboratory Director-GWU Clinical Research Site; Director-Laboratory Science Program, Department of Epidemiology and Biostatistics, School of Public Health and Health Services, The George Washington University.

(see details in Kozloski et al., forthcoming). We chose the same commercial competitive-enzyme immunoassay kits (Salimetrics, LLC, State College, PA) used in Wave 1 to assay free (biologically available) levels of four reproductive steroids: testosterone, 17-beta-estradiol (E2), progesterone, and DHEA (see Table 2 for multiple applications).

The procedures of the Dresden LabService GmbH yielded high assay success rates, most improved over Wave 1 (success summarized by % valid assay values given samples collected): testosterone (94.7%, 6.6% improvement over Wave 1), 17-beta-estradiol (E2, 88.8%, 6.7% decrease), progesterone (95.3%, 1.7% improvement), and DHEA, 95.6%, 4.7% improvement); see Tables 1 and 2. The Electronic Supplement #2 Supplementary Figure 3 is a flow diagram of each step from the number of participants to the number of valid assay values and the valid assay values are summarized in Table 3.

Saliva Sponges (Salivette): Free Cortisol

Collection.—Saliva containing "free" (i.e., biologically available) cortisol was collected by asking respondents to gently chew a small cylindrical sponge made from synthetic fiber called a Salivette, (Salivette-Cortisol; Catalog No. 51.1534.500; SARSTEDT Group, Nümbrecht, Germany) for 1 min. Samples were collected at the beginning, middle, and end of the interview with co-operation rates of 95.0%, 94.8%, and 91.9% (using COOP3; The American Association for Public Opinion Research, 2011). The Salivette collection technique is specialized for measuring cortisol accurately in the smaller saliva volumes expected from this older adult population.

Transportation and storage.—The Salivette specimens were immediately placed in the cooler under the refrigerant pack along with other refrigerated specimens, and shipped overnight to the MSBL in a Styrofoam container with a replacement frozen refrigerant pack. Upon receipt at the MSBL, specimens were stored at −80°C until shipment to Dresden LabService GmbH. No samples were lost in transit (see Supplementary Figure 4).

Assays.—Saliva was centrifuged and extracted from the Salivette and analyzed for free cortisol at Dresden LabService GmbH (Cortisol Luminescence Immunoassay, RE62011; IBL International, Hamburg, Germany). The assay success rates for each Salivette were (see Supplementary Figure 4 in Electronic Supplement #2 and Table 3): first sample at beginning of interview (98.9%), second sample in middle of interview (99.1%), and third sample at end of interview (99.2%).

Oragene®: Genotype

Collection.—At the conclusion of the Wave 2 interview, respondents were asked to give a final 2-ml saliva specimen for genetic analyses collected with an Oragene kit (Oragene DNA, Catalog No. OG-250; DNA Genotek Inc., Ontario, Canada; see Electronic Supplement #1). The respondent co-operation rate was 83.0% (using COOP3;

The American Association for Public Opinion Research, 2011).

Transportation and storage.—Oragene specimens were shipped daily at room temperatures via overnight shipment to the MSBL where they are being stored at 22°C with controlled humidity. Once saliva is mixed with the Oragene preservative, specimens remain stable for DNA extraction for at least 5 years at room temperature (Iwasiow, Desbois, & Birnboim, 2011).

Assay.—At the time of this article's submission, DNA specimens are being stored at the MSBL until DNA extraction.

Vaginal Swabs

Collection.—Compliance collecting specimens for vaginal microbiology and cytology was slightly higher in Wave 2, and most importantly, yielded assay results detecting the expected effect of age (see Hoffman et al., forthcoming, for detailed specimen collection refinements and analysis). The swabs were self-collected by female respondents using field methods similar to Wave 1 (see Lindau et al., 2009 for Wave 1, see Electronic Supplement #1 for Wave 2) with a co-operation rate of 74.3% (using COOP3; The American Association for Public Opinion Research, 2011). The most important refinements were placing the swabs directly into the transport tube, rather than a paper bag, using assay-specific swabs, and maintaining a cool temperature throughout storage and shipping. Indeed the microbiology laboratory commented that the sample quality was as if they had been sent from within the hospital. We did not include a swab for the human papillomavirus (HPV) swab as Wave 1 established a low prevalence of high-risk HPV (6%, Lindau, Drum, Gaumer, Surawska, & Jordan, 2008).

Transportation and storage.—The field interviewer placed the returned swab transport tubes to, under the refrigerant pack in the cooler for transport to the field base. The swabs were shipped overnight in Styrofoam containers with replacement frozen refrigerant packs to two separate laboratories: Jeanne A. Jordan, PhD, Laboratory Director-GWU Clinical Research Site Director and MSBL. Upon receipt at GWU and MSBL, specimens were processed on to microscope slides the same day.

Assays.—The vaginal microbiology swab was analyzed for the presence of bacterial vaginosis (BV) and yeast by Jeanne A. Jordan, PhD, Laboratory Director-GWU Clinical Research Site Director (see Hoffmann et al., forthcoming). The assay success rates and improvements of Wave 1 adequacy rates were (see Supplementary Figure 5 in Electronic Supplement #2 and Table 3): BV (98.5%, increase of 4.4%) and yeast (97.3%, increase of 11.8%). At the time of the submission of this manuscript, analysis of the vaginal cytology swab was in process at the MSBL, M. K. McClintock, Director. The same laboratories were used in Wave 1.

Urine

Collection.—Urine was a new biospecimen for Wave 2. We were interested in using urine specimens for various biomeasures, including social proteins and kidney function (Table 1; Reyes et al., forthcoming) but we were concerned about multiple freeze-thaw cycles, which could potentially interfere with some of the protein assays, if we collected one large specimen. This meant asking the field interviewers to create aliquots in the home, which was not appealing for those concerned about handling urine. Therefore, we utilized a collection device that could first be completely sealed and then three aliquots made from that sealed container (see Electronic Supplement #1). The respondent co-operation rate was 87.5% (using COOP3; The American Association for Public Opinion Research, 2011).

Transportation and storage.—The three urine tube specimens were immediately placed in the cooler under the refrigerant pack for the duration of the interview in preparation for transportation to the field base. The urine tubes were shipped overnight in Styrofoam containers with replacement frozen refrigerant packs to MSBL. Upon receipt at the MSBL, specimens were stored at −80°C.

Assays.—The urine specimens were analyzed for oxytocin, vasopressin, and creatinine by Toni Ziegler, PhD. Director of the Assay Services Laboratories at the Wisconsin National Primate Research Center, (NIH RR000167; see Reyes et al., forthcoming). The assay success rates were (see Supplementary Figure 5 in Electronic Supplement #2 and Table 3): oxytocin (99.6%), vasopressin (99.6%), and creatinine (99.6%).

Biospecimen Shipping and Tracking

The collection and assay of the biospecimens required a detailed and comprehensive shipping plan. The specimens had specific temperature requirements (i.e., room temperature, cold, or frozen) and shipping schedules (i.e., daily, weekly, or monthly) in order to maintain specimen integrity. Table 4 summarizes the specimen shipping schedule, temperature requirements, destination, and assay laboratories for each biospecimen. As in Wave 1, the biomeasure working group created step-by-step shipping protocols that adhered to International Air Transport Association and FedEx shipping guidelines. See Electronic Supplement #1 for information on temperature control, shipping co-ordination and schedule, centralized biomeasure laboratory, direct shipments, and tracking.

Refined and Expanded Biomeasures Collected During Home Interview

The biomeasures were collected during the in-person interview with the result directly entered in CAPI, with the exception of the Actiwatch that was collected postinterview. These are also summarized in Table 2 categorized by systems: (a) anthropometrics; (b) cardiovascular; (c) frailty, activity, and sleep, (d) neuropsychology; and (e) sensory. Anthropometrics included height, weight, waist circumference, and hip circumference. Height, weight, and waist circumference were collected using the Wave 1 protocols for consistency across waves and hip circumference was a new measure for Wave 2 (see Electronic Supplement #1). Blood pressure, heart rate, and preventricular contraction were measured with a digital blood pressure monitor (Lifesource, UA 767 Plus: A&D Medical, Milpitas, CA). The same blood pressure monitor model and collection protocol was used across both waves (see Electronic Supplement #1).

The timed walk and chair stands were refined for Wave 2 and is described in Huisingh-Scheetz et al. (forthcoming). In addition, a measure of sleep and activity using an Actiwatch was added to Wave 2 (Lauderdale et al., forthcoming). The neuropsychology measures include orientation, memory, attention, visuoconstruction, naming, language, executive function, and abstraction. The collection procedures are described in Shega et al. (forthcoming). The smell measure was refined for Wave 2 and is described in Kern et al. (forthcoming). Objective measures of taste, touch, and vision were collected in Wave 1, but dropped in Wave 2. A description of the sensory measures across waves, including self-report and objective measures is described in Pinto et al. (forthcoming). Co-operation rates for the in-home biomeasures were high with all eight measures having co-operation rates over 93% and actigraphy, which was collected postinterview over 3 days, achieving a co-operation rate of 77.7% (see Jaszczak et al., forthcoming, for measure specific rates).

Discussion

This article summarizes the conceptual rational and detailed protocols for the novel biospecimens and biomeasures successfully collected in Wave 2, as well as those repeated from Wave 1. The effects of age and gender on these measures, and hence their applicability to aging research, are presented in the specific biomeasure articles of this Special Issue (Gregg et al., forthcoming; Hoffmann et al., forthcoming, Kozloski et al., forthcoming; Lauderdale et al., forthcoming). Investigators using the biomeasure variables generated in Waves 1 and 2 should refer to these details as biospecimen handling can affect the assay results and their interpretation (see specific biomeasure articles for further details). This overview article summarizing the biomeasure methods can be cited in methods sections of data analysis articles to be published in medical, biopsychological, or clinical journals that typically require such methodological detail. Although modifications were made, our working group focused on making those changes that would still permit and facilitate consistent longitudinal analyses of the biomeasures across waves.

Respondents were willing to provide the biospecimens as evidenced by the high co-operation rates (89.2% average biospecimen co-operation rate). In addition, the success rates for biospecimen collection, storage, and shipping protocols (see Electronic Supplement #2) yielded a high percent of usable data, typically an improvement over the Wave 1 rates (97.3% average assay success rate). Finally, we encourage other surveys and field research to utilize and improve upon these methods, providing a basis for comparing biomeasure data from large scale surveys.

Key Points

- The National Social Life, Health, and Aging Project Wave 2 established continuity across waves for the biomeasures, while also making refinements to the protocols for in-home collection, shipping, tracking, and laboratory assays.
- This paper provides the detailed field methods and biospecimen collection protocols needed for publications, either by referencing this article or summarizing its information.
- Instituted changes permit and facilitate longitudinal analyses of the data set.
- New and innovative biomeasures were added to test new hypotheses about the prevalent diseases, aging conditions, health and psychosocial factors observed at Wave 1.
- Temperature sensors verified that the improved refrigerant packs and coolers kept biospecimens appropriately cool during the interview and shipping to laboratories.
- Protocols for four new biospecimens were added in Wave 2: urine, unclotted whole blood to assay biomeasure levels in plasma, saliva sponges for cortisol, and saliva preserved for genetic analysis.

Supplementary Material

Supplementary material can be found at: http://psychsocgerontology.oxfordjournals.org/

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