Design and Rationale of the Biomarker Center of the Household Air Pollution Intervention Network (HAPIN) Trial

Dana Boyd Barr,1 Naveen Puttaswamy,2 Lindsay M. Jaacks,3 Kyle Steenland,1 Sarah Rajkumar,2 Savannah Gupton,1 P. Barry Ryan,1 Kalpana Balakrishnan,4 Jennifer L. Peel,4 William Checkley,5,6 Thomas Clasen,1 and Maggie L. Clark4 (HAPIN Investigative Team)

1Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, Atlanta, Georgia, USA
2Department of Environmental Health Engineering, ICMR Center for Advanced Research on Air Quality, Climate and Health, New Delhi, India
3Department of global Health and Population, Harvard T.H. Chan School of Public Health, Boston, Massachusetts, USA
4Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, Colorado, USA
5Division of Pulmonary and Critical Care, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA
6Center for Global Non-communicable Disease Research and Training, School of Medicine, Johns Hopkins University, Baltimore, Maryland, USA

BACKGROUND: Biomarkers of exposure, susceptibility, and effect are fundamental for understanding environmental exposures, mechanistic pathways of effect, and monitoring early adverse outcomes. To date, no study has comprehensively evaluated a large suite and variety of biomarkers in household air pollution (HAP) studies in concert with exposure and outcome data. The Household Air Pollution Intervention Network (HAPIN) trial is a liquidified petroleum gas (LPG) fuel/stove randomized intervention trial enrolling 800 pregnant women in each of four countries (i.e., Peru, Guatemala, Rwanda, and India). Their offspring will be followed from birth through 12 months of age to evaluate the role of pre- and postnatal exposure to HAP from biomass burning stoves in the control arm and LPG stoves in the intervention arm on growth and respiratory outcomes. In addition, up to 200 older adult women per site are being recruited in the same households to evaluate indicators of cardiopulmonary, metabolic, and cancer outcomes.

OBJECTIVES: Here we describe the rationale and ultimate design of a comprehensive biomarker plan to enable us to explore more fully how exposure is related to disease outcome.

METHODS: HAPIN enrollment and data collection began in May 2018 and will continue through August 2021. As a part of data collection, dried blood spot (DBS) and urine samples are being collected three times during pregnancy in pregnant women and older adult women. DBS are collected at birth for the child. DBS and urine samples are being collected from the older adult women and children three times throughout the child’s first year of life. Exposure biomarkers that will be longitudinally measured in all participants include urinary hydroxy-polycyclic aromatic hydrocarbons, volatile organic chemical metabolites, metals/metalloids, levoglucosan, and cotinine. Biomarkers of effect, including inflammation, endothelial and oxidative stress biomarkers, lung cancer markers, and other clinically relevant measures will be analyzed in urine, DBS, or blood products from the older adult women. Similarly, genomic/epigenetic markers, microbiome, and metabolomics will be measured in older adult women samples.

DISCUSSION: Our study design will yield a wealth of biomarker data to evaluate, in great detail, the link between exposures and health outcomes. In addition, our design is comprehensive and innovative by including cutting-edge measures such as metabolomics and epigenetics.

Introduction

Understanding individual exposures and effects is critical in successful epidemiologic investigations to avoid misclassification of exposures or outcomes (Antó et al. 2000; Kogevinas 2011); however, intra- and inter-person variation in predictors of exposures (e.g., behaviors, microactivity patterns, work- and home-related tasks), genetic susceptibility, and toxicokinetics make quantitative assessment difficult without individual-level data (Cohen Hubal et al. 2019; Lioy 1999; Lioy and Smith 2013; Vincent 2012). Biomarkers are useful tools for understanding environmental exposures, susceptibility, and biological responses leading to disease outcomes (Barr et al. 2005; Needham et al. 2005b). Further, the collection of temporally resolved biological samples enables the individual analysis of markers of exposure and disease accounting for this intra- and inter-person variation (Barr et al. 2005).

This manuscript describes a comprehensive biomarker approach to enable us to evaluate household air pollution (HAP) exposures, susceptibility, and early effects for a variety of health outcomes as a part of a large randomized controlled trial called the Household Air Pollution Intervention Network (HAPIN) trial. HAPIN was designed to evaluate the effect of a randomized liquidified petroleum gas (LPG) stoves and fuel intervention on health among family members in 800 households in each of four diverse biomass-using low- and middle-income countries (LMICs; Guatemala, India, Peru and Rwanda) populations using exposure–response (i.e., evaluation of how exposure relates to biomarker or disease outcome) analyses and comparisons between the study arms to which participants were assigned, regardless of their actual adherence to the intended condition. These LMIC locations were purposefully selected to be diverse in characteristics such as altitude, population density, cooking practices, fuel types, and baseline levels of pollution to improve the study’s generalizability. Briefly, HAPIN is enrolling 800 eligible pregnant women (at 9 to <20 weeks gestation) at each of the LMIC countries referred to as International Research Collaboration (IRC) sites and following these women through pregnancy and their child to 1 year of age. In approximately one-fourth of these households, up to 200 older adult women (OAW) are also being enrolled. Households are randomized into control and intervention arms (1:1) with the intervention arm receiving an LPG stove and gas supply for the duration of the study. The primary health outcomes of HAPIN are birth weight, severe pneumonia in the first 12 months of life, stunting at 12 months of age, and blood pressure in the OAW. The study protocol has been reviewed and approved by institutional review boards (IRBs) or ethics committees of all participating institutions. The study has been registered with ClinicalTrials.gov and is

Address correspondence to Dana Boyd Barr, Emory University, RSPH, 1518 Clifton Rd., CNR2007, Atlanta, GA 30322 USA. Telephone: (404) 727-9605. Email: dbbarr@emory.edu

The authors declare they have no actual or potential competing financial interests.

Received 15 June 2019; Revised 5 February 2020; Accepted 11 February 2020; Published 29 April 2020.

Note to readers with disabilities: EHP strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in EHP articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehponline@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.
overseen by an independent Data Safety Monitoring Board (DSMB). Recruitment and data collection began in May 2018 and expected completion is August 2021. The HAPIN trial is described in more detail elsewhere (Clasen et al. 2020).

The HAPIN trial is composed of many core components, including exposure assessment [e.g., particulate matter ≤2.5 μm in aerodynamic diameter (PM$_{2.5}$), black carbon measures], biomarker measurements, stov e use monitoring, surveillance, and data management. In particular, one of the aims of HAPIN is to evaluate associations between targeted and untargeted/exploratory biomarkers of exposure and effect with intervention status or exposure defined from personal or household air pollution measurements (detailed in Johnson et al. 2020). To successfully achieve this aim, we developed a comprehensive biomarker center (BMC) comprising scientists from each IRC and collaborative institutions involved with HAPIN, including experts in exposure science, HAP, analytical chemistry, epidemiology, and toxicology to ensure the most appropriate biomarkers are measured in the most viable and logistically feasible matrix to provide maximum exposure and health information in the HAPIN cohort. In addition, the BMC is supported by two analytical biomarker laboratories: (a) the Laboratory for Exposure Analysis and Development in Environmental Research (LEADER) at Rollins School of Public Health, Emory University, Atlanta, Georgia; and (b) the laboratory at Sri Ramachandra Institute of Higher Education and Research (SRHIER), Chennai, India. The overall goal of our BMC is to provide high-capacity, high-quality, and high-throughput analysis of a wide range of biomarkers in samples collected from participants. This entails four primary objectives: (a) to provide training and monitoring compliance of sample collection, handling, and storage including developing collection and aliquoting protocols, ensuring sample integrity throughout the process and developing a short- and long-term archival system; (b) to identify, prioritize, and measure specific biomarkers of HAP exposure and effect in urine and dried blood spots (DBS); (c) to develop local laboratory capacity and harmonize biomarker measures across IRCs; and (d) to develop and validate novel biomarkers that will provide insight into the broader mechanistic questions linking HAP exposure to disease development.

Rationale for the HAPIN Biomarker Design

HAPIN is a complex and costly study funded by multiple agencies with different missions. Because of this, we sought to collect as many biological matrices as were feasible from both a cost and participant and field staff burden perspective. Thus, we wanted to evaluate biomarkers related to outcomes including cardiovascular, metabolic, cancer, and respiratory disease, as well as birth outcome and child development. This included a detailed prioritization scheme that included strength of evidence linking exposure or outcome and biomarker, our ability to measure the biomarker, and the stability/validation of the biomarker in the specified matrix. With this in mind, we sought out the most viable biomarkers that could comprehensively evaluate the components of HAP exposure and these health outcomes. Furthermore, we wanted to allow for innovation in our design by incorporating cutting-edge biomarkers, including epigenetic alterations and metabolic alterations. Recognizing the field is ever changing and new biomarkers are discovered each year, we also implemented a Biomarker Nomination process whereby newly emerging biomarkers such as fibrinogen and telomeres could be continually included throughout the duration of the study, given financial and laboratory limitations. Although all of the newly nominated biomarkers may not be discussed in this paper, we are working toward formalizing plans for their eventual measurement. Finally, we also had to consider the reality that a minimum of two laboratories would be involved in these measurements because biological samples from India cannot be shipped to another country or analyzed by a non-Indian laboratory (with the exception of small subsets of samples used for cross-validation purposes). This required that we evaluate both the capabilities and capacities of the laboratories, including instrumentation that differed widely. In an ideal scenario, one laboratory would perform all analyses to allow for easy comparison of data between countries. To ensure we would be able to compare data, we developed a thorough quality assurance/quality control scheme that is described in detail below. This scheme extended beyond the laboratories, however, and included metrics of quality and success in field activities that involved collecting the biological specimens. This multifaceted biomarker design required careful planning and coordination to enable successful execution that was continually evaluated for quality and completeness.

### Biological Matrix Selection

Because of the large number of participants and the already burdensome exposure, behavioral, and intervention measures in place in the HAPIN trial, we opted for a biospecimen approach that would enable us to maximize the number of appropriate samples collected while minimizing participant burden and risk (Table 1). For participating pregnant women, children, and OAW, we chose to collect routine blood samples as DBS on a five-spot Guthrie filter paper card, a method that overcomes collection, transport, and storage limitations of venipuncture sampling (McDade et al. 2007), and to collect a convenience or spot urine sample. The use of DBS is a novel aspect of our study because it also allows us to collect blood from the children, which is often not feasible when venipuncture is required. As such, the measurements of biomarkers that are traditionally measured in whole blood or serum will be validated against DBS measurements. We will use venous blood/DBS pairs collected in the formative pilot phases of our research to ensure these measurements are stable and interpretable. All biomarkers will be similarly validated in one or both of the BMC laboratories. Ideally, serum and

| Table 1. Biosample collection timeline |
|---------------------------------------|
| Child age (study time point)          | 24–28 weeks gestation (1–3 months post-randomization) | 32–36 weeks gestation/birth (3–5 months post-randomization) | ~3–7 months of age (~9 months post-randomization) | ~6 months of age (~12 months post-randomization) | ~12 months of age (~18 months post-randomization) |
| Control | OAW | Control | OAW | Control | OAW | Control | OAW | Control | OAW | Control | OAW |
| Urine | X | − | X | − | X | − | X | − | X | − | X | − |
| Dried blood spots | X | − | X | − | X | − | X | − | X | − | X | − |
| Oral rinse* | − | − | X | − | − | − | X | − | − | − | X | − |
| Nasal turbinate sample* | − | − | X | − | − | − | X | − | − | − | X | − |
| Buccal scrape* | − | − | X | − | − | − | X | − | − | − | X | − |
| Venous blood (RBC, BC and plasma)* | − | − | X | − | − | − | X | − | − | − | X | − |

Note: −, not applicable; BC, buffy coat; C, child; NM, new mother; OAW, older adult woman; PW, pregnant woman; RBC, red blood cells.

*Peru and Guatemala international research collaborating sites only.
Table 2. Target biomarkers for the HAPIN trial.

| Biomarker                                                                 | Biomarker no. for Table 2 | Reason for selection | Matrix | Method | Method reference |
|---------------------------------------------------------------------------|----------------------------|----------------------|--------|--------|------------------|
| Intercellular adhesion molecule 1 (ICAM-1)                               | 1                          | Endothelial marker of cardiovascular function | DBS    | Immunoassay | Barnett and Ware 2011; Hecht et al. 2011; McElrath et al. 2011, 2013 |
| Vascular cellular adhesion molecule 1 (VCAM-1)                           | 2                          | Endothelial marker of cardiovascular function | DBS    | Immunoassay | McElrath et al. 2011 |
| Endothelin-1                                                             | 3                          | Endothelial marker of cardiovascular function | DBS    | Immunoassay | Goddard and Webb 2000 |
| E-selectin                                                               | 4                          | Endothelial marker of cardiovascular function | DBS    | Immunoassay | Barnett and Ware 2011; McElrath et al. 2011, 2013 |
| C-reactive protein (CRP)                                                  | 5                          | Inflammation marker | DBS    | Immunoassay | Barnett and Ware 2011; McElrath et al. 2011, 2013 |
| Interleukin 6 (IL-6)                                                     | 6                          | Inflammation marker | DBS    | Immunoassay | Barnett and Ware 2011; McElrath et al. 2011, 2013 |
| von Willebrand factor antigen (vWF)                                      | 7                          | Blood coagulation protein | DBS    | Immunoassay | Barnett and Ware 2011; Mannucci 1998 |
| Hemoglobin A1c (HbA1c)                                                   | 8                          | Marker of glycemic control | DBS, capillary blood | POC, LC-MS/MS | Dubach et al. 2019; Jeppsson et al. 2002 |
| Hemoglobin (Hb)                                                         | 9                          | Clinical biomarker | DBS, capillary blood | POC, LC-MS/MS | Jeppsson et al. 2002; Osborn et al. 2019 |
| Lipids                                                                   | 10                         | Clinical biomarkers | DBS    | Immunoassay | Xu et al. 2019 |
| P53 Tumor-associated antigen antibodies (p53 TAA antibodies)            | 11                         | Lung and other cancer biomarker | DBS    | Immunoassay | Barnett and Ware 2011; McElrath et al. 2011, 2013 |
| F2-Isoprostanes                                                          | 12                         | Inflammation markers | DBS    | Immunoassay | Soffler et al. 2010 |
| Clara cell protein (CC16)                                                 | 13                         | Lung insult marker | DBS    | Immunoassay | Broeckaert and Bernard 2000 |
| 8-OH-deoxyguanosine (8OHdG)                                               | 14                         | Oxidative stress marker | Urine  | LC-MS/MS | Marocco et al. 2017 |
| Myeloperoxidase (MPO)                                                    | 15                         | Oxidative stress marker | DBS    | Immunoassay | Marocco et al. 2017 |
| Malondialdehyde (MDA)                                                   | 16                         | Oxidative stress marker | Urine  | LC-MS/MS | Kartvenka et al. 2019a |
| Cytochrome P450 (Cyp450)                                                 | 17                         | Enzyme induction | DBS    | Immunoassay | Lake et al. 2009 |
| 3-OH cotinine; cotinine, and 4-(methylisoxamino)-1-(3-pyridyl)-1-butanol (NNAL) | 18                         | Short-term and longer-term (¿6 weeks) tobacco smoke biomarkers | Urine  | LC-MS/MS | Avila-Tang et al. 2013; Braun et al. 2010; Carmella et al. 2003; Needham et al. 2005a; Sexton et al. 2011; Yuan et al. 2014 |
| Polycyclic aromatic hydrocarbons (1-OH pyrene, 1-/2-naphthol, 2-/3-hydroxyfluorene, 2-/3-/4-hydroxyphenanthrene, phenanthrene tetrol) | 19                         | Carcinogen exposure biomarker | Urine  | GC-MS/MS | Aquilina et al. 2010; Perera et al. 2005 |
| Volatile organic chemicals [mercapturate metabolites including S-phenylmercapturate (benzene metabolite), S-benzylmercapturate (toluene metabolite), S-1-phenyl-2-hydroxyethylmercapturate (stilbene metabolite), and S-2-hydroxyethylmercapturate (acrylonitrile, vinyl chloride, ethylene oxide metabolite)] | 20                         | Carcinogen exposure biomarker | Urine  | LC-MS/MS | Alsos et al. 2012; Barr and Ashley 1998; Calafat et al. 1999 |
| Heavy metals/metalloids (lead, mercury, cadmium, arsenic)                 | 21                         | Air pollution exposure, neurotoxicants | DBS, urine | ICP-MS | Back Louis et al. 2012; Jones et al. 2010; Needham et al. 2005a; Rubin et al. 2007; Sexton et al. 2011 |
| Levoglucosan                                                             | 22                         | Wood exposure biomarker | Urine  | LC-MS/MS | Naheer et al. 2013 |
| Metabolome                                                               | 23                         | Biomarker discovery | DBS, serum | HRMS | Burgess et al. 2015; Frediani et al. 2014; Go et al. 2015; Roede et al. 2013 |
| microRNA                                                                 | 24                         | Biomarker discovery | NT, plasma | RT-PCR | Harrison et al. 2000; Ponnusamy et al. 2015 |
| mRNA                                                                     | 25                         | Biomarker discovery | NT | RT-PCR | Harrison et al. 2000 |
| DNA methylation                                                         | 26                         | Biomarker discovery | NT, BC, Bead chip | RT-PCR | Paquette et al. 2016 |
| Oral microbiome                                                          | 27                         | Biomarker discovery | Oral rinse | 16S rDNA | Jovel et al. 2016 |
| Novel inflammation cancer markers [serum amyloid A, soluble tumor necrosis factor receptor 2, chemokine (C-X-C motif ligand 9 or monokine induced by γ-interferon)] | 28                         | Cancer risk evaluation | Plasma | Immunoassay | Shieh et al. 2017 |

Note: BC, buffy coat; Bead, buccal, buccal cells; DBS, dried blood spots; GC-MS/MS, gas chromatography-tandem mass spectrometry; HAPIN, Household Air Pollution Intervention Network; HRMS, high resolution mass spectrometry; ICP-MS, inductively coupled plasma-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NT, nasal turbinate swab; POC, point-of-care; RT-PCR, reverse transcription-polymerase chain reaction.

*Measured in all other adult women.
*Measured in all participants including pregnant women, children, and other adult women.
*Measured in a subset of samples.
DBS measurements are highly correlated. In instances where they are not, we may still have internal validity to use the markers as pre-intervention and post-intervention measures, although the concentrations may not be comparable to clinically interpretable values.

Materials for urine collection are dropped off at the home 1 d prior to sample collection so the participants can collect a first morning void sample, record the collection time on the label, and store it in the biospecimen-certified cooler boxes provided. The field staff collect the cooler boxes that morning and transport them back to satellite or central laboratories. These cooler boxes either have refrigerant gel inside the walls such that the entire cooler box is pre-frozen or use hard-walled ice packs that are pre-frozen. Our experiments indicated that both types of boxes retained sufficient cooling temperature for the 48-h period in which the cooler box was deployed in the field (unpublished data). When field staff arrive, they collect a DBS sample from the pregnant woman and OAW via finger prick after warming the hands and massaging the arms to encourage adequate blood flow for collection. For infants, DBS samples are collected by heel stick until the 6-month visit, after which they are collected via finger prick, consistent with the World Health Organization and American Association for Clinical Chemistry guidelines (WHO 2010). For infant urine samples, a pediatric urine collection bag is affixed to the infant’s genital area and a urine sample is collected over 2–3 h. We explored the use of disposable diapers for urine collection during the formative research phases of HAPIN where methods, procedures and equipment were pilot tested in the field, and both field staff and parents preferred the more traditional urine collection bag that is used in clinical settings. Likely, this is because diapers are not commonly used in our target populations, so we opted for this more straightforward collection approach. To date, our success rates collecting child urine this way has been high [i.e., Guatemala 94% (609/646), Rwanda 97% (692/717), Peru 72% (243/336), India 94% (580/614)]. A field blank (described below) is collected after every 100 urine samples are collected. Additional biosamples are collected from the OAW in Peru and Guatemala to enable the exploration of novel cancer biomarkers (Bassig et al. 2017; Shiels et al. 2017) and omics analyses (see details below). For these women, nasal turbinate swabs, an oral rinse, buccal scrapes, and a venous blood draw are obtained. The venous blood samples are collected in serum separator tubes for serum and in ethylenediaminetraacetic acid-preserved tubes to enable the separation of plasma,uffy coat, and red blood cells for storage and analysis. The venous blood and urine samples are processed and aliquoted in the field laboratories of each IRC within 4 h of collection (or within 8 h if refrigerated). All samples are stored at −20°C at the IRC laboratories for the short term (~3–7 months) until shipped to Emory University (Peru, Guatemala, and Rwanda samples) or SRHHER (India samples), where they are stored at −80°C until analysis or archival in the biorepository.

Biomarker Selection
The biomarkers selected for analysis (Table 2) were chosen because of previous links to air pollution/HAP exposure. Moreover, because the four major chronic diseases (i.e., cardiovascular disease, cancer, chronic respiratory disease, and diabetes), which together account for >75% of all chronic disease deaths globally, share common pathophysiological mechanisms (e.g., inflammation and oxidative stress) (Jha et al. 2012), these markers were considered important to measure.

Biomarkers of exposure [polycyclic aromatic hydrocarbons (PAHs), volatile organic chemicals (VOCs), and levoglucosan] will be measured in all or a subset of all longitudinally collected urine samples from all participants (Table 1). In the OAW, we will measure a suite of biomarkers of endothelial function [intercellular adhesion molecule 1 (ICAM-1), vascular cellular adhesion molecule 1 (VCAM-1), endothelin-1, E-selectin, von Willebrand factor antigen (vWF)] (Poggesi et al. 2016), inflammation (C-reactive protein (CRP), interleukin 6 (IL-6), F2-Isoprostanes) (Ghezzi et al. 2018), blood coagulation (vWF) (Wiseman et al. 2014), oxidative stress [8-hydroxy-deoxyguanosine (8OHdG), peroxidation [i.e., myeloperoxidase (MPO)]] (Marrocco et al. 2017), glycemic control/diabetes [hemoglobin A1C (HbA1c)] (Jia 2016), a marker with specific relevance to lung cancer [P53 tumor-associated antigen (TAA) antibodies] (Fortner et al. 2017; Shi et al. 2015), enzyme induction [cytchrome P450 (Cyp450)], and a marker of lung insult/inflammation [Clara cell protein (CC16)] (Broeckaert and Bernard 2000; Wong et al. 2009).

Because this is a large randomized controlled trial, it provides an ideal mechanism for discovery of novel biomarkers of exposure and effects associated with HAP. In collaboration with the National Cancer Institute of the National Institutes of Health, an ancillary study was incorporated that involves additional sample collections in the OAW participants of Peru and Guatemala. These additional samples will be collected among all OAW at baseline and at the visit occurring approximately 12 months after the intervention (n = 400 samples; Table 1). Novel inflammatory cancer markers will be measured and epigenetic and omics techniques will be used for biomarker discovery. In venous blood, the inflammatory markers serum amyloid A, soluble tumor necrosis factor receptor 2, chemokine (C-X-C motif) ligand 9 or monokine induced by γ-interferon will be evaluated, along with CRP to evaluate lung cancer risk (Shiels et al. 2015, 2017). In addition, measurement of miRNA, microRNA (miRNA), DNA methylation, the metabolome, and the microbiome in complementary samples will enable us to gain a better understanding of the response of these measures to exposure and intervention (Robles and Harris 2017; Vargas and Harris 2016).

Biomarker Measurements
Across the course of the study, over 55,000 samples will be collected from participants, so it is not logistically feasible to analyze every biomarker in every sample. Thus, we have developed a biomarker analysis scheme that will enable us to maximize the data collected while still keeping the costs and human resource needs within budgetary constraints (Table 3). Our rationale for this measurement scheme relates to the health outcomes evaluated in each participant subset. For example, because cardiovascular outcomes will be assessed in the OAW, most clinical markers of cardiovascular disease will only be measured in those samples. Exposure markers will be measured in all participants, including children for whom direct exposure measurements will not be available. Further, we will measure all analytes in all longitudinal samples of a 5% subsample of the population to finalize our biomarker prioritization scheme. These data will provide information on within- and between-person variability in biomarker concentrations and on estimates for exposure–response that will inform the most efficient analysis scheme (i.e., to maximize the information gained for each sample type and aliquot by determining the number of longitudinal measures needed to efficiently answer our research questions). At minimum, this will include longitudinal measurements collected at baseline and after randomization. This process is currently underway as sample collection continues.

Clinical Biomarkers in DBS
To more efficiently use DBS samples for clinical markers, a full spot will be sampled and eluted in 1.5 mL phosphate buffered saline (PBS). Portions of this stock extract will be used in each of
the biomarker measurements, which will require two aliquots from this same stock to allow for a replicate analysis.

**Endothelial, cardiovascular, inflammation, and oxidative stress (i.e., MPO) markers.** These biomarkers are measured using commercially available multiplexed immunoassays (Meso Scale Discovery Multiplex Immunoassay Reader) with customized V-Plex kits. The PBS extracts are placed in 96-well plates, in duplicate, and prepared according to the standard assay protocol. The resulting reaction products are analyzed on a multiplex plate reader with a full set of calibrants and quality control samples. Values are averaged before reporting. Calculated values of replicates that have differences greater than 20% are repeated or flagged as suspect. Similarly, samples are repeated if the quality control samples indicate a failure of proper measurement. Our current limits of detection (LODs) vary by biomarker but are generally in the low picograms-per-milliliter range with relative standard deviations (RSDs) of <15%. Our approach is further supported by our preliminary results from a cross-sectional study among Nicaraguan women (n = 54) using wood-burning cookstoves. For example, a 25% increase in kitchen PM2.5 was associated with a 7.4% increase [95% confidence interval (CI): 0.7, 14.5] in CRP (Young et al. 2019).

**Hemoglobin and HbA1c.** Hemoglobin (Hb) and HbA1c are measured using a latex-enhanced immunoassay that changes the solution turbidity proportional to the amount of Hb and HbA1c in the sample. Percentage HbA1c is the ratio of HbA1c to Hb. This method is superior to traditional immunoassays because of limited interference, especially by fetal Hb (Rohlfling et al. 2008). Although less precise than the immunoassay and other standard reference methods (Rohlfling et al. 2008; Wittenmeier et al. 2019), we will also measure Hb (HemoCue® Hb 201 system, HemoCue® AB) and HbA1c (A1CNow+ system, Chek Diagnostics) using point-of-care devices during visits in order to provide immediate results back to the pregnant women (i.e., Hb), children (i.e., Hb at the 6- and 12-month visits), and OAW (i.e., HbA1c) on data forms that they can share with their physicians. We believe that immediately sharing some results is of value to the participants and will help keep them engaged in the study. Our previous results among Honduran primary cooks (n = 142) utilized the same point-of-care device to measure HbA1c; when HbA1c cut points were used to define diabetes risk, we observed evidence supporting an association between HAP and prevalent prediabetes/diabetes, for example, the prevalence ratio per interquartile range increase (84 μg/mL) for 24-h personal PM2.5 was 1.49 (95% CI: 1.11, 2.01) (Rajkumar et al. 2018). Suggestive evidence of a positive association between PM2.5 and continuously assessed HbA1c was also observed, particularly among women >49 years of age (Rajkumar et al. 2018).

**Lipids.** HDL, LDL, cholesterol, and triglycerides will be measured using standard clinical methods (e.g., ultracentrifugation and fractionation of cholesterol) (Linné and Ringsrud 1999;WHO 2003). Evidence linking air pollution and metabolic syndrome or its components such as blood lipid profiles comes mainly from studies of ambient air pollution in high-income countries (Chuang et al. 2010; Clementi et al. 2019; Lee et al. 2019; Matthiessen et al. 2018; Yang et al. 2018; Yitshak Sade et al. 2016). However, we estimated positive associations between measures of personal HAP and metabolic syndrome among Honduran women (Rajkumar et al. 2019). We did not observe cross-sectional associations between HAP exposure and individual blood lipids, although blood pressure seemed to modify an association between PM2.5 and total cholesterol (Rajkumar et al. 2019).

**Tumor-associated antigen antibodies.** Antibodies associated with the p53 signaling pathway have been associated with respiratory cancers as well as other cancers. A commercially available p53 antibody array will be used that evaluates 196 highly specific antibodies related to the p53 signaling pathway (RayBio® Human p53 enzyme-linked immunosorbent assay kit, RayBiotech). This array contains site-specific and phospho-specific antibodies, enabling evaluation of tyrosine and serine/threonine phosphorylation at specific sites.

**Cytochrome P450 induction.** Cyp450 will be measured using standard spectrophotometry and the ferrous carbon monoxide (CO) difference spectrum, the difference between absorbance at 450 nm wavelength of totally reduced Cyp450 (artificially bound to CO using sodium dithionate) and the sample. The concentration of Cyp450 in the blood is calculated using the Beer-Lambert law and an average extinction coefficient of 91,000 M−1/cm.

**Other Biomarkers in DBS**

**Heavy metals/metalloids analysis.** Heavy metals/metalloids such as lead, cadmium, and mercury are common air pollutants and constituents of smoke from biomass burning and can be measured in DBS and urine. For DBS, a spot is digested using nitric acid on a heater block to break down the molecular components of the blood and the filter paper. Pedersen et al. (2017) reported essentially quantitative recovery of a variety of metals from DBS. The digested matrix is diluted and injected into the inductively coupled plasma–mass spectrometry (ICP-MS) (Jones et al. 2010; Sexton et al. 2011). Multiple isotopes of each metal are monitored to ensure reliable results. Indium, iridium, lutetium, and rhodium are used as internal standards. The method LODs range from 0.1–0.5 ng/mL with RSDs of <10%.

**Metabolomics/miRNA in DBS.** In a subset of intervention and control participants, a comprehensive metabolomics analysis will be performed. One blood spot will be dissolved in PBS and subsequently extracted with acetonitrile containing internal standards. Samples are analyzed using liquid chromatography (LC)–high-resolution MS, enabling accurate mass calculation. Data are

### Table 3. Biomarker class to be measured in each participant.

| Biomarker class                              | Biomarker no. from Table 2 | Mother | OAW | Child |
|---------------------------------------------|----------------------------|-------|-----|-------|
| Cardiovascular/endothelial markers          | 1–4                        |       | X   |       |
| Oxidative stress markers                    | 14–16                      |       | X   |       |
| Lipids                                      | 10                         |       | X   |       |
| HbA1c                                      | 8                          |       | X   | X    |
| Hb                                          | X                          |       |     | X    |
| Other clinical biomarkers                   | 7, 11, 13, 17              |       | X   |       |
| Exposure biomarkers                         | 18–22                      |       | X   | X    |
| Inflammation markers                        | 5, 6, 12                   |       |     |       |
| Metabolome (subset)                         | 23                         |       | X   | X    |
| Microbiome                                  | 27                         |       |     | X    |
| mRNA/miRNA                                  | 24–25                      |       | X   | X    |
| DNA methylation                             | 26                         |       |     |       |
| Novel inflammation markers (subset)         | 28                         |       |     |       |

Note: —, not applicable; Hb, hemoglobin; HbA1c, hemoglobin A1c; OAW, older adult woman.
subjected to linear regression and principle component analyses to identify features that are significantly related to exposure status with an initial false discovery rate of 20%. Data are matched to standard metabolomics databases to tentatively identify over 10,000 features (unique mass to charge ratios coupled with retention times). Once these biomarkers are discovered, the specificity and sensitivity will be determined in a validation study. Similarly, miRNA discovery and profiling arrays will be generated in the same subset of DBS using TaqMan technology that is commercially available, and then searches will be performed to identify the miRNA present. miRNAs have recently been shown to be measurable in DBS (Ponnusamy et al. 2015).

**Biomarkers in Urine**

**Markers of oxidative stress.** Most oxidative stress biomarkers, with the exception of MPO, are measured in both urine and DBS. 8-OHdG and F2-isoprostanates are measured in urine using LC–tandem MS (LC-MS/MS) methods.

**PAH metabolites.** The targeted PAH metabolites are readily excreted in urine and have been shown to be representative of the carcinogenic fraction of air pollution primarily from combustion processes (Lee et al. 1999). Because exposure biomarkers integrate exposure routes, the specific routes of exposure that have differential toxicities (i.e., inhalation or ingestion) cannot be determined by the biomarker measurement (Barr et al. 1999); however, we anticipate that the inhalational pathway will dominate ingestion and, when coupled with exposure data collected in the field, we should be able to apportion exposure sources. The target PAH metabolites are urinary 1-OH pyrene, 1- and 2-naphthols, and phenanthrene tetroil. The urinary biomarkers 1-OH pyrene and 1- and 2-naphthol are predictive of exposures to the carcinogenic components of PAHs that are mostly fecally excreted (e.g., benzo[a]pyrene) rather than excreted in urine (Uziel and Haglund 1988). Phenanthrene tetroil is a marker of the diol epoxide metabolic activation pathway and a potential biomarker of lung cancer susceptibility (Hecht 2002; Wang et al. 2012). We will measure PAHs using a method already established in our laboratory (Kartavenka et al. 2019b). One-half milliliter urine will be aliquoted into a test tube and spiked with isotopically labeled internal standards for automatic recovery correction and normalization of mass spectral data. After homogenization of the matrix, the PAH metabolites will be extracted using a mixed-mode phase solid phase extraction. The extract will be subjected to a derivatization process with diazomethane to protect the polar hydroxyl groups, then the concentrated extract will be analyzed using gas chromatography–tandem MS (GC-MS/MS) (Smith et al. 2002). PAH metabolites do not respond well to LC-MS/MS analysis because of the nonspecific loss of water and lack of further fragmentation thus require a derivatization and GC-MS/MS analysis. Each analyte has either separation by time or separation by mass (or both). Two precursor → product ions (quantification and confirmation ion transitions for MS/MS) are monitored for each analyte and its isotope analog. The LODs for all analytes are 0.1 ng/mL with RSDs of <12%.

**3-OH cotinine, 4-(methyl nitrosamo)I-(3-pyridyl)I-1-butanol, levoglucosan, and VOC metabolites.** We will use methods established or implemented in our lab to measure 3-OH cotinine (Braun et al. 2010), 4-(methyl nitrosamo)I-1-(3-pyridyl)I-1-butanol (NNAL) (Carmella et al. 1997), levoglucosan (Naehr et al. 2013), and VOC metabolites (Alwis et al. 2012; Barr and Ashley 1998). 3-OH cotinine and NNAL are short-term (i.e., 2-d) and long-term (i.e., 6-week) markers, respectively, of exposure to tobacco smoke. Levoglucosan is a specific marker of wood burning. VOC metabolites are detoxification products of glutathione binding of the electrophile VOCs where the terminal amino acids are cleaved and the cysteine is N-acetylated and excreted in urine. Other urinary metabolites are mostly bound to glucuronide or sulfates and require a deconjugation step that involves incubation at physiologic temperature with enzymes that contain β-glucuronidase and/or sulfatase activities. Although this process has been optimized for each method, this is typically performed overnight to ensure quantitative deconjugation of the bound analyte. The urine aliquots (typically from 0.1–1 mL) are spiked with isotopically labeled internal standards and mixed. They are treated with an acetate buffer at pH 4.5–5 and a glucuronidase/sulfatase enzyme solution and then deconjugated overnight at 37°C. The hydrolysate is extracted using solid phase extraction (utilizing mixed-mode cartridges), and the extracts are concentrated for analysis by high performance LC-MS/MS (HPLC-MS/MS) with electrospray ionization (Alwis et al. 2012; Barr and Ashley 1998; Carmella et al. 1997; Naehr et al. 2013; Stepanov et al. 2006). Similar to our PAH analysis, two precursor → product ions (quantification and confirmation ion transitions) are monitored for each analyte and its isotope analog. The LODs are in the low picograms-per-milliliter range with RSDs of <10%.

**Biomarkers in Other Biosamples (Peru and Guatemala Only)**

**Microbiome/metabolome/mRNA/miRNA in additional tissues.** The oral microbiome, metabolome, mRNA, and miRNA will be measured in a subset of OAW in Peru and Guatemala. S16 rDNA sequencing will be performed on oral rinse samples to obtain microbiome data using established techniques (Jovel et al. 2016). The samples will have been appropriately preserved with RNAProtect (Qiagen) to stabilize the RNA to enable this analysis. miRNA and mRNA will be analyzed using reverse transcription–polymerase chain reaction (Harrison et al. 2000) and DNA methylation will be measured using BeadChip technology (Paquette et al. 2016). Metabolomics will be performed in serum samples, as described above.

**Statistical Analysis**

Descriptive statistics will be conducted and correlations (Pearson or Spearman, as appropriate) will be obtained for all measured pollutant concentrations with the urinary biomarkers of exposure. We will compare biomarker results between the two arms using a mixed model where the biomarker (or its transformation) is the outcome (either continuous or dichotomous), where each subject who is tested repeatedly has a random intercept, and where the independent variable is the treatment arm. We will also conduct exposure–response analyses where the biomarker is again the outcome of interest and the exposure of interest is PM$_{2.5}$. Covariates will include age, sex, body mass index, season, and other potential confounders. In both of these types of analyses we will check model assumptions. PM$_{2.5}$ will be modeled as continuous or transformed, as well as categorically and via splines. We will use a false discovery rate correction to account for multiple comparisons (Benjamini and Hochberg 1995).

We will then consider our primary and secondary outcomes as the outcomes in biomarker–response regressions in which the biomarkers of exposure are predictors. These mixed model regressions will again include a random intercept for the subject with repeated biomarker measurements and will include both binary (pneumonia/stunting) and continuous outcomes (birthweight, blood pressure). These models will not be restricted to post-intervention data but will include baseline data. We will also consider using the average post-intervention biomarker level as a predictor of outcome in these models. In the case of blood pressure, the outcomes will also be repeated measures in the OAW, although again we may model the average blood pressure after the intervention and also consider an interaction between time and the biomarker of interest. Various function of biomarkers will be used, including as continuous or transformed (e.g., log, square root), as well as categorically, and via restricted
cubic splines. Again, we will use a false discovery rate correction to account for multiple comparisons (Benjamini and Hochberg 1995).

Finally, based on results observed in the prior analyses for biomarkers that appear to mediate the effect of PM$_{2.5}$ on an outcome, we will consider a mediation analysis in which we attempt to separate out the direct and indirect effects of PM$_{2.5}$ exposure (Richiardi et al. 2013; Vanderweele 2016). We will use directed acyclic graphs to illustrate the mediations under consideration. The basic idea here is to partition the effect of the exposure on outcome between that which passes through an intermediate variable (the biomarker) and that which has a direct effect on the outcome regardless of the level of the biomarker. Such analyses are a natural complement for biomarkers that are thought to be predicted by the exposure and have an effect on the outcome. For example, if PM$_{2.5}$ has an effect on blood pressure, one would want to assess whether that effect is mediated by biomarkers of inflammation.

For the omics analyses, we will utilize the R packages xSMannotator (Uppal et al. 2017) and xMSpanda (https://rdrr.io/github/kuppal2/xmsPANDA/), which were both developed at Emory. xMSpanda is based on linear models for microarray data, partial least squares discriminant analysis, and random forests. We will also conduct multivariable analyses within a linear regression framework, using a false discovery rate correction (Benjamini and Hochberg 1995).

Quality Assurance/Quality Control (Intra- and Inter-Laboratory Validation)

Method Validation

All methods used in the BMC are fully validated before use. Validation parameters include determination of LODs and limits of quantification (LOQs), accuracy, precision, robustness, and analytical specificity. The method LOD is defined as $3s_0$ (method LOQ $= 10s_0$), where $s_0$ is the standard deviation at zero concentration. Most often this is established as the lowest analytical standard with an average signal-to-noise ratio of 3. In instances where the blank samples contain the target analyte from contamination, the LOD is the concentration + $3s_0$. The LOD will be verified visually by analysis of samples spiked at the LOD concentration. Because the LOD is not a static number but, rather, is dynamic, changing over the course of analysis on the basis of the constituents of the sample, the analyst conducting the measurements, the status of the instrument, and so on, and average LODs will be reported for each study. Accuracy will be established using National Institute of Standards Standard Reference Materials (NIST SRMs). In addition, spiked recovery experiments where known concentrations are spiked into matrices and quantified as unknowns will be performed to ensure that accuracy is within 20% of the known value as per U.S. Food and Drug Administration regulations. Precision will be established using quality control pools. A continuing Shewart plot will be used to evaluate precision over time. Both intraday and interday precision will be established. Robustness testing of each method will be performed by intentionally altering at least three parameters of the method to determine the effect on the resulting data. The results of these tests will be used to ensure reliability of the methods. In instances where the quality control fails, analyses will cease until the problem is identified and corrective action is taken. The method performance and quality control of the system will be reestablished before analysis recommences.

Because, by law, Indian biological samples cannot be analyzed outside of India, we are prevented from having all analyses across IRCs performed in a single laboratory. To ensure that we have the most valid results across IRCs, we have embarked on an extensive cross-laboratory validation process for which we have much experience (Barr et al. 2007; Prapamontol et al. 2014). Both the LEADER laboratory at Emory and the SRIHER laboratory in India are actively validating methods in-house but also exchanging samples to ensure we have suitable method agreement. India does allow a quality control set of samples to be exported to allow for cross-validation (Jaacks et al. 2019). To date, we have cross-validated PAH biomarkers and several of the cardiovascular and endothelial markers with good agreement. This validation process also involves on-site training and evaluation in the analysis of biomarkers. So far, our cross-validation has proven fruitful, enabling us to combine results rather seamlessly. These validation studies are being published elsewhere.

Biorepositories in the United States and India

We have begun the development and maintenance of a long-term biorepository to store biological specimens in a manner that retains sample integrity and is only linked to identifiable participant data through sample coding. Urine samples are aliquoted into cryovials to enable access to the samples without repeated thaw-refreeze cycles to maintain sample integrity. DBS cards are stored in zip top bags with desiccant pouches to prevent deterioration of samples. The biorepository consists of four secure −80°C freezers linked to a monitoring and alarm system that generates automatically transmitted alarms (via email and phone) when the temperature deviates within 10°C of the target temperature. In the case of freezer failure, a fifth overflow freezer will be used to temporarily house the samples until the freezer is repaired. Regular freezer maintenance (i.e., defrosting and calibrating temperature gauges and alarms) will be performed every 6 months and documented.

The freezers will be fully mapped with each sample location (shelf, box, position) and volume. The map will be retained and updated, as needed, using the OpenSpecimen tracking system. Each freezer will remain locked, with access granted only by the BMC co-directors, in accordance with the study protocol or at the request of the study primary investigators or steering committee. Access to the study samples outside of the current protocol will require a vetting process similar to that of the National Heart, Lung, and Blood Institute (NHLBI) Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC) repository. This entails the development of a study protocol and budget that must be reviewed and approved by the HAPIN steering committee as well as all participating IRBs and our DSMB. All procedures for collection, labeling, and storage are compatible with the NHLBI BioLINCC repository. A limited data set will be provided to the NHLBI BioLINCC repository as well.

Metrics of Success

In addition to the logistics of sample collection and analysis, we have worked into our BMC scheme several ways to evaluate our mechanisms and measure success. These measures include both field- and laboratory-based evaluations to ensure data collection and sample integrity as well as quality biomarker measurements.

Sample Collection/Biosample Data Collection

We evaluate the sample collection processes at each IRC one time a year to ensure that field teams are collecting and processing samples according to our standard operating procedures. These evaluations are conducted by two BMC members who are not affiliated with any IRC or field team and who have deep understanding of the protocols and the impact deviations can have on data collection. To do this, the evaluators accompany the field staff on typical participant visits and observe firsthand the specimen collection techniques. If they notice deviations or improper practices, these are corrected quickly and the potentially affected sample identities are recorded. At the end of a visit, an evaluation report is presented to...
the team, including items to improve adherence to the protocols. On a monthly basis, we monitor various sample collection metrics, including time between urine collection and laboratory processing, the amount of time a sample remains at room temperature prior to freezing, and the number of DBS collected per participant. We discuss these parameters monthly with BMC staff and offer remedies when necessary. We also monitor for missing or nonrecorded information and will have those variables promptly corrected.

Field Blanks
Field blanks are collected for every 100 participant samples collected to ensure we do not have contamination in the exposure biomarkers. The field blank consists of a purified water sample collected at the location that a urine sample was collected. We use blank spots on the Guthrie filter cards for blanks for DBS samples. These are analyzed alongside participant samples to ensure no field contamination has occurred.

Laboratory Analysis
We perform cross-validation to ensure comparable results across the two laboratories. In addition, we routinely participate in proficiency testing programs (e.g., German External Quality Assessment Scheme, New York State Department of Health Trace Elements Testing Program, and the Quebec Multielement External Quality Assessment Scheme), where available, to ensure our data are comparable to other participating laboratories. We also include NIST SRMs in every run for target chemicals that have SRMs. Unfortunately, NIST materials are not available for blood spots, but they can be applied to Guthrie cards and analyzed similarly to unknown samples.

Conclusions
We have developed a comprehensive biomarker measurement scheme for the HAPIN study that includes targeted exposure, susceptibility, and effect biomarkers, and we have incorporated an untargeted omics analysis for biomarker discovery. Our biomarker plan includes the largest set of biomarkers measured in a HAP study and includes early markers of disease risk. We have incorporated quality measures to ensure accurate data that are comparable among the two laboratories measuring biomarkers. By combining all of these aspects, we have created a strong biomarker component for the HAPIN study.

Acknowledgments
We thank N. Rothman and Q. Lan from the National Institutes of Health (NIH) National Cancer Institute (NCI) for their advice and discussions regarding the novel biomarkers to be measured in Peru and Guatemala.

The HAPIN trial is funded by the U.S. National Institutes of Health (cooperative agreement 1U1HL134590) in collaboration with the Bill & Melinda Gates Foundation (OPP1131279). Additional support was provided by Emory’s NIH-funded Human Exposure Research Center (P30ES019776). A multidisciplinary, independent Data and Safety Monitoring Board (DSMB) appointed by the National Heart, Lung, and Blood Institute (NHLBI) monitors the quality of the data and protects the safety of patients enrolled in the HAPIN trial.

Members of the NHLBI DSMB: N.R. Cook, S. Hecht, C. Karr, J. Millum, N. Sathiakumar (Chair), P.K. Whelton, and G.G. Weinmann (Executive Secretary).

Program Coordination: G. Rodgers, Bill & Melinda Gates Foundation; C.L. Thompson, National Institute of Environmental Health Science; M.J. Parascandola, National Cancer Institute; D.M. Krotoski and M. Koso-Thomas, Eunice Kennedy Shriver National Institute of Child Health and Human Development; J.P. Rosenthal, Fogarty International Center; C.R. Nierras, NIH Office of Strategic Coordination Common Fund; K. Kavounis, D. Kim, A. Punturieri, and B.S. Schmetter, NHLBI. The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the US National Institutes of Health or Department of Health and Human Services.

Members of the HAPIN Investigative Team: V. Aravinda-alochan, K. Balakrishnan, D. Boyd Barr, V. Burrowes, D. Campbell, J. McPeek Campbell, E. Canuz, A. Castañaza, H. Chang, W. Checkley, Y. Chen, M. Chiang, M.L. Clark, T. Clasen, R. Craik, M. Crocker, V. Davila-Roman, L. de las Fuentes, O. De Léon, A. Diaz-Artiga, E. Dusabimana, L. Elon, J.G. Espinoza, I.S. Pineda Fuentes, S. Garg, D. Goodman, S. Gupton, M. Hardison, S. Hartinger, S.A. Harvey, M. Hengstermann, P. Herrera, S. Hossen, P. Howards, L. Jaacks, S. Jabbarzadeh, M.A. Johnson, A. Jones, K. Kearns, M. Kirby, J. Kremer, M. Lawes, J. Liao, A. Lovvorn, F. Majorin, E. McCollum, J. McCracken, R. Meyers, J.J. Miranda, E. Mollinedo, L. Moultou, K. Mukhopadhyay, L. Naether, A. Nambajimana, F. Ndagijimana, A. Nizam, J. de Dieu Nitvuguruzwa, A. Papageorghiou, J. Peel, R. Piedrahita, A. Pillarsetti, N. Puttaswamy, E. Puzzolo, A. Quinn, S. Rajkumar, U. Ramakrishnan, D. Reardon, G. Rosa, J. Rosenthal, P.B. Ryan, Z. Sakas, S. Sambandam, J. Sarnat, S. Simkovich, S.S. Sinharoy, K.R. Smith, K. Steenland, D. Swearing, G. Thangavel, L.M. Thompson, A. Toenjes, L. Underhill, J.D. Uwizeyimana, V. Valdes, A. Verma, L. Waller, M. Warnock, K. Williams, W. Ye, and B. Young.

References
Akins JR, Waldrep K, Bernett JT Jr. 1989. The estimation of total serum lipids by a completely enzymatic ‘summation’ method. Clin Chim Acta 184(3):219–226, PMID: 2611996, https://doi.org/10.1016/0009-8981(89)90054-5.
Alvis KU, Blount BC, Britt AS, Patel D, Ashley DL. 2012. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). Anal Chim Acta 750:152–160, PMID: 23062436, https://doi.org/10.1016/j.aca.2012.04.009.
Antó JM, Sunyer J, Kogevinas M. 2000. Environment and health: the long journey of environmental epidemiology at the turn of the millennium. J Epidemiol Biostat 5(1):49–60, PMID: 10885871.
Aquilina NJ, Delgado-Saborit JM, Meddings C, Baker S, Harrison RM, Jacob P III, et al. 2010. Environmental and biological monitoring of exposures to PAHs and ETS in the general population. Environ Int 36(7):763–771, PMID: 20591483, https://doi.org/10.1016/j.envint.2010.05.015.
Avila-Tang E, Al-Delaimy WK, Ashley DL, Benowitz N, Bernett JT, Kim S, et al. 2013. Assessing secondhand smoke using biological markers. Tob Control 22(3):164–171, PMID: 22940677, https://doi.org/10.1136/tobaccocontrol-2011-050296.
Barnett N, Ware LB. 2011. Biomarkers in acute lung injury—marking forward progress. Crit Care Clin 27(3):681–683, PMID: 21742222, https://doi.org/10.1016/j.ccc.2011.04.001.
Barr DB, Ashley DL. 1998. A rapid, sensitive method for the quantitation of N-acetyl-S-(2-hydroxyethyl)-cytochrome in human urine using isotope-dilution HPLC-MS-MS. J Anal Toxicol 22(2):96–104, PMID: 9547405, https://doi.org/10.1033/jat.22.9.96.
Barr DB, Barr JR, Drikski JW, Hii RH Jr, Ashley DL, Needham LL, et al. 1999. Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicol Ind Health 15(1–2):168–179, PMID: 10188199, https://doi.org/10.1179/0748233997846556.
Barr DB, Barr JR, Drikski JW, Hii RH Jr, Ashley DL, Needham LL, et al. 1999. Strategies for biological monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children’s Study. Environ Health Perspect 111(8):1083–1091, PMID: 16079083, https://doi.org/10.1289/ehp.7617.
Bassing BA, Dai Y, Vermeulen R, Ren D, Hu W, Duan H, et al. 2017. Occupational exposure to diesel engine exhaust and alterations in immune/ inflammatory markers: a cross-sectional molecular epidemiology study in China. Carcinogenesis 38(11):1104–1111, PMID: 28968774, https://doi.org/10.1093/carcin/bqx081.
complication. Am J Obstet Gynecol 204(5):418.e1–418.e12, PMID: 21349490, https://doi.org/10.1016/j.aojog.2010.12.010.

Naeher LP, Barr DB, Needham LL, Simpson CD. 2013. Urinary levogucosan as a biomarker for woodsmoke exposure in wildland firefighters. Int J Occup Environ Health 19(4):304–310, PMID: 24588036, https://doi.org/10.1179/1078952913y.0000000037.

Needham LL, Barr DB, Caudill SP, Pirkle JL, Turner WE, Osterling J, et al. 2005a. Concentrations of environmental chemicals associated with neurodevelopmental effects in U.S. population. Neurotoxicology 26(4):531–545, PMID: 16123195, https://doi.org/10.1016/j.neuro.2004.09.005.

Needham LL, Özkaynak H, Whyatt RM, Barr DB, Wang RY, Naeher L, et al. 2005b. Exposure assessment in the National Children’s Study: introduction. Environ Health Perspect 113(8):1076–1082, PMID: 16097802, https://doi.org/10.1289/ehp.7613.

Osborn ZT, Villanilla N, Derickson PR, Sewatsky T, Wager AP, Freeman K. 2019. Accuracy of point-of-care testing for anemia in the emergency department. Respir Care 64(11):1343–1350, PMID: 30914402, https://doi.org/10.1097/rc.0000000000000364.

Paquette AG, Houseman EA, Green BB, Lester B, et al. 2016. Regions of variable DNA methylation in human placenta associated with newborn neurobehavior. Epigenetics 11(8):603–613, PMID: 27366929, https://doi.org/10.1080/15592294.2016.1195534.

Pedersen L, Andersen-Ranberg K, Hölgaard M, Nybo M. 2017. Quantification of multiple elements in dried blood spot samples. Clin Biochem 50(12):703–709, PMID: 28121917, https://doi.org/10.1016/j.clinbiochem.2017.01.010.

Perera FP, Rauh V, Whyatt RM, Barr DB, Wang RY, Naeher L, et al. 2005b. Exposure assessment in the National Children’s Study: introduction. Environ Health Perspect 113(8):1076–1082, PMID: 24588036, https://doi.org/10.1179/1078952913y.0000000037.

Peduzzi P, Aschoff P, Pescini F, Pantoni L, Inzitari D. 2016. Circulating biologic markers of endothelial dysfunction in cerebral small vessel disease: a review. J Cereb Blood Flow Metab 36(1):72–94, PMID: 26558955, https://doi.org/10.1038/jcbfm.2015.116.

Ponnsamy V, Kapellou O, Yip E, Evason J, Wong LF, Michael-Titus A, et al. 2015. A study of microRNAs from dried blood spots in newborns after perinatal asphyxia: a simple and feasible biosampling method. Pediatr Res 79(5):799–805, PMID: 26720806, https://doi.org/10.1007/pr.2015.276.

Prapamontol T, Sutan K, Laoyong S, Hongbsong S, Lee G, Yano Y, et al. 2014. Cross validation of gas chromatography-flame photometric detection and gas chromatography-mass spectrometry methods for measuring dialkylphosphate metabolites of organophosphate pesticides in human urine. Int J Hyg Environ Health 217(4):555–566, PMID: 24280299, https://doi.org/10.1016/j.ijheh.2012.10.005.

Rajkumar S, Clark ML, Young BN, Benka-Coker ML, Bachand AM, Brook RD, et al. 2018. Exposure to household air pollution from biomass-burning cookstoves and HbA1c and diabetic status among Honduran women. Indoor Air 28(5):768–776, PMID: 29886912, https://doi.org/10.1111/ina.12484.

Rajkumar S, Young BN, Clark ML, Benka-Coker ML, Bachand AM, Brook RD, et al. 2019. Household air pollution from biomass-burning cookstoves and household metabolic syndrome, blood lipids, and waist circumference in Honduran women: a cross-sectional study. Environ Res 170:46–55, PMID: 30557691, https://doi.org/10.1016/j.envres.2018.12.010.

Richardi L, Bellocco R, Zugna D. 2013. Mediation analysis in epidemiology: methods, interpretation and bias. Int J Epidemiol 42(5):1511–1519, PMID: 24019424, https://doi.org/10.1093/ije/djt127.

Roth AE, Harris CC. 2013. Integration of multiple “OMIC” biomarkers: a precision medicine strategy for lung cancer. Lung Cancer 107:50–58, PMID: 23744275, https://doi.org/10.1016/j.lungcan.2016.06.003.

Roede JR, Uppal K, Park Y, Lee K, Tran V, Walker D, et al. 2013. Serum metabolomics of slow vs. rapid motor progression Parkinson’s disease: a pilot study. PLoS One 8(10):e77629, PMID: 24167579, https://doi.org/10.1371/journal.pone.0077629.

Rohlfing CL, Connolly SM, England JD, Hanson SE, Moellering CM, Bachelder JR, et al. 2005. The effect of elevated fetal hemoglobin on hemoglobin Aβ results: five common hemoglobin Aβ methods compared with the FFC reference method. Am J Clin Pathol 129(5):811–814, PMID: 18426743, https://doi.org/10.1309/FVTUDUGHJFJD16H.

Rubin CS, Holmes AK, Benson MG, Jones RL, Flanders WD, Kieszak SM, et al. 2007. Investigating childhood leukemia in Churchill County, Nevada. Environ Health Perspect 115(1):151–157, PMID: 17266926, https://doi.org/10.1289/ehp.9022.

Sexton K, Ryan AD, Adgate JL, Barr DB, Needham LL. 2011. Biomarker measurements of concurrent exposure to multiple environmental chemicals and dietary classes in children. J Toxicol Environ Health A 74(14):927–942, PMID: 21623537, https://doi.org/10.1080/1556976X.2011.573745.

Shi JX, Qin JJ, Ye H, Wang P, Wang KJ, Zhang JY. 2015. Tumor associated antigens as anti-TAA biomarkers in the diagnosis of ovarian cancer: a systematic review with meta-analysis. Expert Rev Mol Diagn 15(6):822–852, PMID: 25959246, https://doi.org/10.1586/14737519.2015.1035713.