

Johns Hopkins Medicine - eForm A

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1. Abstract

This is a component project of NICHD contract No: HHSN275200800033c, contract work assignment for National Children's Study titled "Integration of Salivary Analytes into the NCS: Evaluation of Feasibility, Efficiency, and Benefits" (LOI Round #2, Project 19). The NCS Vanguard studies have revealed concern that participant burden regarding biospecimens in the larger NCS project (100,000 participants to be followed from birth to age 20) might impact recruitment and retention. Poor recruitment and retention in the massive project will result in significant cost overruns and potential compromise the scientific value of the information obtained. Total project cost is estimated at approximately \$3 billion dollars (without overruns). The use of saliva in the NCS has the potential to increase participation and retention in the larger study. This contract was assigned to explore whether oral fluid samples have the potential to reduce participant burden and to use standard immunoassay techniques to develop, evaluate, and validate saliva assays so as to make recommendations to the NCS regarding the breadth of analytes that can be measured in saliva and the relation of the levels of analytes in oral fluid to those in traditional biospecimens (urine and blood).

This specific component of this work assignment involves demonstrating the feasibility of laboratory assays for assessing hormonal status, environmental contaminants, markers of inflammation and oral health, oxidative stress, and pathogen-specific antibodies and DNA sequences in saliva samples. This subproject will collect matched serum, saliva, and urine samples from 150 adults (age 18-35; 50% male, 50% non-pregnant female, not currently taking prescription medications other than birth control) for use in laboratory method development projects in order to determine whether hormonal status, environmental contaminants, markers of inflammation and oral health, oxidative stress, and pathogen-specific antibodies and DNA sequences can be accurately measured in oral fluid specimens, as well as to determine the relationships between the levels of these analytes and biomarkers in saliva to their respective levels in urine and/or blood. Samples will be collected at JHU, de-identified, aliquotted, and distributed to the two partner institutions (JHU and Emory). JHU will work on assay protocols related to hormones, oral inflammation, and disease specific antibodies, as well as optimizing DNA extraction from saliva specimens and microarray analyses. Emory will work on assays for oxidative stress and environmental chemical exposure. Of the total 150 subjects to be recruited, samples from 100 of the individuals will be used to refine and optimize assays (see Objective A below), while samples from 50 of the individuals will be used to optimize saliva collection device.

2. Objectives

The overarching goal is to use standard immunoassay techniques to develop, evaluate, and validate saliva assays in order to make recommendations to the NCS regarding the breadth of analytes that can be measured in saliva and the relation of the levels of analytes in oral fluid to those in traditional biospecimens (urine and blood). This study aims to determine a) whether hormonal status, environmental contaminants,

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markers of inflammation and oral health, oxidative stress, and pathogen-specific antibodies and DNA sequences can be accurately measured in oral fluid specimens, and b) what relationships exist between the levels of these analytes and biomarkers in saliva and their respective levels in urine and/or blood.

Objective A : Refine and optimize assays for each of the following categories of analytes: (1) hormones (JHU), (2) oral inflammation (JHU), (3) Disease specific Antibodies (JHU), (4) DNA extraction and assay (UCLA), (5) Oxidative Stress (EMORY), and (6) Environmental Chemicals (EMORY).

Approach: Modify assay protocols for use with saliva analytes, or refine existing research protocols. Document range of sensitivity, spike recovery, linearity on dilution, intra-and inter-assay coefficient of variation using standard criteria. Document range of variation in a normative sample, as well as associations or lack thereof between saliva and traditional biospecimens.

Action items:

JHU COMPONENT:

1. Collect relevant demographic, health, and behavior information along with matched serum/plasma/urine and whole saliva from 100 healthy young adult participants (ages 18-35, not currently taking prescription medications other than birth control) from the JHU community, 50% male, 50% (non-pregnant) female for assay validation work. Distribute aliquots (volume to be determined) of each specimen between JHU and Emory.
2. Compare and contrast different commercial sources of salivary hormone/peptides (e.g., cortisol, testosterone, estradiol, progesterone, DHEA(s), NPY) assays and present data to team to select recommended protocols.
3. Review literature on markers of oral inflammation (e.g., IL-1b, MMP-8, CRP, IL-6, TNF-a) -- select representative analytes, evaluate and validate assays reported in the literature for these assays.
4. Review literature on salivary measures of disease specific antibodies (e.g., EBV, CMV, HSV)-- outline basic assay platforms and refine techniques to develop experimental assay protocols.
5. Extract DNA from salivary and blood samples and hybridize to Affymetrix genome wide SNP 6.0 microarrays.
6. Evaluate performance of salivary vs. blood derived DNA by agreement between sample sources, technical reproducibility, and frequency of Mendelian errors, in addition to standard microarray quality control procedures. Define and/or modify any algorithmic approaches to genotyping as necessary to improve performance.
7. Deliverables: Assay protocols, standard validation and performance report, genotyping accuracy as a function of sample source, algorithmic approaches to improving genotype accuracy.

EMORY Component:

1. Exchange samples with JHU core facility

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2. Identify candidate analytes for markers of oxidative stress, and select representative toxicants from most recognized classes of environmental chemicals. Develop and refine assays protocols. Address quality assurance issues and establish limits of detection and sample size necessary for quantifiable results.
3. Deliverables: Assay protocols, standard validation and performance report, data analyses of ranges and relationships of measures between biospecimen types.

Objective B: Identify an absorbent material that is acceptable to NCS participants, can be used in all study phases (with infants, children, and adults), absorbs sufficient sample, enables high volume recovery, and creates minimal interference with the key salivary analytes of interest to project.

Actions items:

1. Obtain 3,000 1 cm x 10 cm (cylinder) samples of prototype swab material
2. Collect relevant demographic, health, and behavior information along with matched serum/plasma/urine and whole saliva from 50 young adult (ages 18-35, not currently taking prescription medications other than birth control) participants from the JHU community, 50% male, 50%(non-pregnant) female. Filter whole saliva samples through device on bench. Aliquot and distribute (filtered and unfiltered saliva; urine; and plasma/serum—volume to be determined) samples between Emory and JHU colleagues to determine impact on salivary analytes of interest. Run direct comparisons between filtered and unfiltered saliva, and correlate levels with those in traditional biospecimens (plasma/serum and urine).
 - A. JHU --Evaluate effect of swab on representative candidate analytes (n in parentheses) from each of the following categories : steroids (1), polypeptides (1), markers of oral inflammation (2), disease specific antibodies (1)
Deliverable: Report confirming that saliva collection material can be use (with extraction or without) to assay hormones, markers of oral inflammation, disease specific antibodies.

Evaluate DNA extraction from swab-collected samples and determine DNA stability and reproducibility under standard and typical collection conditions. Compare data from such samples to current salivary collection protocols. Determine if any separate DNA collection strategy is required compared to other analytes.
Deliverable: Report confirming that saliva collection material does or does not interfere with the analysis of DNA from saliva samples.
 - B. Emory –
Evaluate material for contamination during the manufacturing process.
Deliverable: Feedback to manufacturer on production contamination possibilities.
Evaluate Environmental Chemicals and Markers of Oxidative Stress in the experimental samples. Deliverable: Report confirming that saliva whether collection material can be used (with extraction or without) to assay markers of oxidative-stress and environmental chemicals.

3. Based on results, modify treatment of material, and re-rerun the saliva filtering subcomponent of the experiment again.
4. Based on results, determine whether extraction is needed from the material, determine equations needed to normalize saliva values if there is a consistent high/low bias.

3. Background

Advances in biotechnology, coupled with the recent characterization of a vast array of analytes and biomarkers in saliva, have created the opportunity to measure components of biological systems in oral fluids and apply knowledge gained from those measurements to a diverse spectrum of research. More than 25 years of basic research documents that many analytes can be measured in oral fluids. Much of this empirical work has focused on establishing the reliability, precision, and validity of the measurement of salivary analytes and biomarkers. In the past decade, the focus has shifted away from measurement issues to describing phenomenon.

This study is being done to further develop understanding of saliva as a research specimen with the specific goal of increasing recruitment/retention levels for the NCS study by decreasing burden on the subjects involved. The measurements of interest include testing analytes that are naturally present (i.e., hormones, DNA) as well as analytes present due to environmental exposures (i.e., tobacco smoke, pesticides) in order to determine whether the levels measured in saliva compare to measurements of those chemicals in urine and blood.

Saliva Collection Procedure:

Collection of saliva is generally regarded as a somewhat vexatious issue from a research perspective. It is clear when reviewing the scientific literature, that many methods have been implemented for collecting saliva (9,10).

Debate has focused on issues such as:

- whether to collect stimulated or unstimulated saliva, and if collecting stimulated saliva then how best to do it
- whether to collect saliva directly from the salivary glands or from 'pooled' saliva
- whether to rinse mouth or not before collecting saliva
- whether samples should be collected at the same time of day for all volunteers.

These issues will be briefly discussed in turn.

Stimulated vs. unstimulated, and how to stimulate saliva:

There is an argument that it is actually impossible to collect unstimulated saliva, because the very act of collecting saliva will stimulate its flow. Thinking about saliva can stimulate saliva flow, touching the lips with a collecting vessel stimulates saliva flow, and of course, thoughts about food can stimulate saliva flow. It is therefore generally accepted that unstimulated saliva is probably impossible to collect. Nonetheless, the commonly accepted method of collecting 'unstimulated' saliva is via 'passive drooling' – that is, the volunteer sits in a chair with their head tipped forward, mouth slightly open, with a collection vessel against the lips, and the saliva that pools in the mouth flows into the vessel, with help from the volunteer to expectorate it.

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Saliva flow varies considerably between individuals, and is generally estimated to be somewhere around 1 ml/min. For this project, a moderate volume of saliva (12-15 ml) will be required to allow for the analysis of all the different biomarkers. Collecting 15 ml of saliva would therefore typically take approximately 5 to 10 mins on average.

Many studies have collected 'stimulated' saliva, with volunteers chewing on blocks of paraffin wax, or chewing rubber bands, or having the tongue stimulated with a drop of lemon juice. None of these methods are appropriate for the present study, however, as these may all affect the assays that will subsequently be conducted. For example, biomarkers in saliva may adhere to wax or rubber, which would reduce their relative levels in the saliva samples. We did consider using a glass marble in the mouth for stimulation, but we were concerned about the potential choking hazard. Furthermore, since we are only collecting a moderate amount of saliva per participant, we do not believe stimulation is necessary.

Whether samples should be collected at the same time of day:

It is evident that there are diurnal variations in saliva flow. Some researchers have argued therefore that all samples should be obtained at the same time of day to attempt to standardize for this. This is typically done in the mid-morning, around 10-11am, at least 1 hour after food, and hopefully before the volunteer starts to get hungry for their lunch. Researchers have even gone to great lengths to supposedly standardize saliva collection, e.g. by adjusting the lighting, temperature, and noise levels in the collection room.

However, in the real world, when a saliva diagnostic test is developed, it will be used at all times of day and in all sorts of conditions. Therefore, to improve the transferability of the data, it is our contention that saliva samples in this research can be collected at various times during the day, though at least 1 hour after eating.

Despite these technical challenges, collection of saliva as a diagnostic fluid is seen to have huge potential for the future. The advantages of saliva in this regard are that its collection is seen as 'safe', acceptable and non-invasive by patients, it could eliminate the need for blood samples, self-collection is possible, it would allow for community- or home-based collection in special populations, it is economical, and it is relatively simple to collect (11).

Taking all of the above into consideration, the ideal method of collecting saliva in this study will incorporate the following:

- be safe, quick, simple
- not involve stimulation with any substances that may ultimately interfere with the assay, or to which mediators might bind, thereby reducing their concentration in saliva (therefore, rubber bands and paraffin wax should not be used)
- not utilize any sort of absorbent collection methods (e.g. chewing on gauze swabs or cotton wool rolls), as mediators may adhere to these, and therefore not enter the assay
- not involve any sort of suction approach, as this will not allow adequate time for mixing of GCF and saliva.

Taking these factors into consideration, the method that will be employed in this study is as follows:

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This will be a passive drool sample, with subjects allowing saliva to flow through a straw into a sterile 50 ml polypropylene universal container. Collection will continue until approximately 15 ml saliva has been collected.

This method has been established as a safe and practical method of collecting saliva for studies such as this.

To minimize protein breakdown, samples will be kept on ice during the collection procedure, and flash frozen immediately after collection is completed (14).

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4. Study Procedures

This is a multi-site project coordinated through the National Children's Study sites at JHU and Emory, with JHU acting as the lead site. The project is coordinated via biweekly conference calls, and over the course of the 14 month project period there will be 3 in person meetings. The first such meeting was in January 2011 to launch the project planning phase. Communication between partner sites will occur through a combination of emails and conference calls throughout this project to discuss progress as well as any challenges or deviations that may arise.

Emory and UCLA have received a copy of the project protocol (NA_00046518_eIRB eFormA), screening tool (NA_00046518_Recruitment-TelephoneScreeningScript), questionnaire (NA_00046518_Demographic

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Health Behavior Questionnaire), and the consent form (NA_00046518_Consent) submitted as part of this application to the JHU SOM IRB. They will be applying for "Non-human subjects" research approval for the project at their study sites.

JHU will be responsible for consenting, collecting, and distributing de-identified samples to Emory and UCLA. A group of designated JH faculty/staff will have responsibility for monitoring, oversight of adverse events and other protocol events for this research.

Sequence of Steps:

- 1. Recruitment:** Recruitment of research participants will be conducted on the JHU campus by a study coordinator. Announcements for the project will be posted in common areas in the JHU Schools of Nursing, Medicine, and Public Health. Those announcements will describe the opportunity briefly and provide a contact number for the project. Potential participants who contact the study office will be screened by phone (using submitted telephone screening questionnaire) to determine if they qualify for the study: male or non-pregnant female, English-speaking adult between the ages of 18 and 35, not currently taking prescription medications other than birth control). (Please note: all exclusion/inclusion criteria are included in this initial screening questionnaire ("NA_00046518_Recruitment-TelephoneScreeningScript") and should not be confused with the information being collected in the demographic/health/behavior questionnaire ("NA_00046518_Demographic Health Behavior Questionnaire") to be administered on site.) If participant accepts, it will be explained to him/her that he/she will be required to make one visit to the Johns Hopkins East Baltimore campus for roughly 45 minutes in order to complete the consent process and a demographic/health/behavior questionnaire with a study coordinator, then provide blood, saliva, and urine samples with the assistance of a nurse. Participant removal criteria include failure to follow instructions, cancellation of studies, or other reasons unforeseeable at the time of recruitment. If participants stop participating, they may receive partial compensation depending upon the samples donated, as explained during screening ("NA_00046518_Recruitment-TelephoneScreeningScript") and on the consent form ("NA_00046518_Consent").
- 2. Appointment at CTSA Clinic:** After the screening call, an appointment will be made for a study team member to meet qualified participants at the ITCR site. Please note: concerns about blinding, routine care, therapies, etc are not applicable to this study.
- 3. Consent and Questionnaire:** A study team member will complete the consent process ("NA_00046518_Consent") with the subject (including review of consent form and answering of any questions), then conduct a demographic/health/behavior interview ("NA_00046518_Demographic Health Behavior Questionnaire") with the subject. The demographic/health/behavior questionnaire does not include questions that will determine inclusion/exclusion criteria; rather the information collected by this questionnaire is relevant to analyzing assay results as the results may be influenced by one or more of the factors examined.
- 4. Sample collection:** Saliva, urine and blood collection will occur as described below:

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Saliva Sample Donation: Saliva collection will require that the subject gently force approximately 15 mL (1 tablespoon) of pooled saliva through a straw into a container.

- a) 15mL of saliva will be collected
- b) Saliva will be aliquoted into 15 x 1mL aliquots and flash frozen at -80C

Urine sample Donation: Urine will be voided by the subject in a private bathroom. Subject will be instructed to use the clean catch method to collect urine into a BD clean catch collection container.

- a) Approximately 25mL (1.69 tablespoons) of urine will be collected in a BD Urine Collection Device
- b) 5mL urine will be transferred into 4x 15mL blue top conical tubes (direct transfer)

Venipuncture (CTSA Staff): Collection of 29 mL (1.93 tablespoons) of blood will occur via venipuncture.

- a) 1x 3mL Lavender metal top/EDTA (**METAL TOP MUST BE FIRST BLOOD DRAW**)
 1. Immediately after collection mix well by inversion 8-10 times
 2. DO NOT SPIN!
 3. CISBR staff will place on ice and bring back to lab and store at 4C
- b) 2x 3mL Lavender top/ EDTA (**for DNA**)
 1. Immediately after collection mix well by inversion 8-10 times
 2. DO NOT SPIN!
 3. Using transfer pipette, spot 50uL onto each of 4 circles on a Whatman blood card. Once dried, store cards at room temperature with humidity indicators and dessicant packs.
 4. Whole blood will be aliquoted into 5x 1mL Sarstedt pre-labeled tubes (CISBR staff will provide transfer pipettes and labeled tubes).
- c) 3x 3mL Lavender top/ EDTA (**for plasma**)
 1. Mix well by inversion 8-10 times
 2. Spin for 15 minutes at Room Temperature
 3. Transfer 1mL of plasma 4x 2mL Sarstedt pre-labeled tubes
- d) 1x 10mL SST tube (**for serum**)
 1. Mix well by inversion 5 times
 - a. Allow to sit at room temperature for 30 minutes to allow clot activation (but no longer than 2 hours before spinning)
 - b. Spin 1000-1300 RCF for 10 min
 - c. Transfer 1 mL of serum from 10 mL SST into 4x 2 mL Sarstedt pre-labeled tubes.

5. Conclusion of appointment with subject: Subjects are given financial compensation for donation of samples and a copy of the consent form, which includes contact names and phone numbers for concerns about the study (PI) or medical questions concerning sample collection (MD). Results of assays on the samples provided by the subject will not be made available to the subject afterwards. This is made clear to participants during both screening (“NA_00046518_Recruitment-TelephoneScreeningScript”) and consent (“NA_00046518_Consent”).

6. Transfer of samples from CTSA to CISBR (project staff).
7. Aliquots will be distributed between sites (JHU and Emory). Once specimens are distributed, individual site laboratories will process the specimens according to their laboratory SOPs.
 - a) Objective A is the refinement and optimization of assays for each of the following categories of analytes: (1) hormones (JHU), (2) oral inflammation (JHU), (3) Disease specific Antibodies (JHU), (4) DNA extraction and assay (JHU), (5) Oxidative Stress (EMORY), and (6) Environmental Chemicals (EMORY).
 - b) Objective B (shared by all sites) is the identification of an absorbent material that is acceptable to NCS participants, can be used in all study phases (with infants, children, and adults), absorbs sufficient sample, enables high volume recovery, and creates minimal interference with the key salivary analytes of interest to project.
Please see “Objectives” section for further details.
8. The resulting data will be returned to JHU for integration into a master data set.
9. The master data set, stripped of any potentially identifying data records, will be distributed to each site for statistical analysis.
10. Samples destroyed at CISBR and Emory (project staff at respective sites). Results of assays on the samples provided by the subject will not be made available to the subject.

Inclusion/Exclusion Criteria

Not currently taking prescription medications other than birth control, English-speaking men and non-pregnant women between the ages of 18 and 35 will be recruited.

Drugs/ Substances/ Devices:

- a. According to the JHMI IRB Guidelines, a serious risk device poses a “potential for serious risk to the health, safety, or welfare of a subject.” A device is a serious risk if it: is intended as an implant; is purported or represented to be for a use in supporting or sustaining human life; is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health; and/or otherwise presents a potential for serious risk to the health, safety, or welfare of a subject. Non-significant risk devices are devices which do not meet these criteria. None of the devices used in this study meet the above criteria for serious risk, therefore all devices used in this study are “non-serious risk devices”.
- b. Blood and urine sample collection devices are standard devices routinely used by the Johns Hopkins Clinical Research Unit for these purposes.
- c. Saliva sample collection kits: Please note that we use the term "collection kit" within our study in a manner that is not the same as how the FDA is using this term. Our "collection kits" include research tools that are used to facilitate saliva collection. It is not required for these tools to be reviewed by the FDA. We are neither using them in clinical trials, nor using them for diagnostics, nor linking them to an FDA approved/cleared IVD. They are for research use only, not diagnostic use. They are for 2 methods of saliva collection: (1) drooling saliva through a straw, or (2) putting a foam swab under the tongue.

The foam swabs have been qualified by the FDA as “Food Grade Substance”, but the other collection kit materials have not been reviewed by the FDA. These tools are not sold commercially. We are in the process of creating the parameters to optimize their acceptability and utility in the field. Medical, physical, psychological, emotional, and/or social risks to which participants in our study will be exposed due to “devices” used in this study do not have a greater probability and magnitude of harm or discomfort than those ordinarily encountered in daily life or during the performance of routine physical examinations or tests.

- d. Assays: This project will use commercially available immunoassay kits, as well as some assays that are being developed in our lab, for the assay of saliva, serum and urine. Some of the commercially available assays may be marketed by the manufacturer for diagnostic use, while others may be for research use only. Our intention is not to use any of these assays for diagnostic purposes. We are simply using them to benchmark the concentration of the analyte in traditional biospecimens so that we can correlate those concentrations with the concentration of that analyte in oral fluid. The assays we will use for serum and urine will be produced by Diagnostic Systems Laboratories, American Laboratory Products, Enzo Life Sciences, Diagnostic Products Corporation, R & D Systems. These assays, for use with serum and urine will be performed without modification to the manufacturer's recommended protocols. Over the course of the project we may find that particular assays perform below our expectations, and thus we may change to a different assay (made by a different maker) over the project period. For saliva assays, we will use assays from Salimetrics, IBL, DRG, American Laboratory Products, that are specifically designed for use with saliva. Some of the salivary assays may be 510k cleared or 510k exempt (DHEA, Progesterone, Estradiol), however, 510k status is not essential for our research questions. We will use some commercially available assays designed for use with saliva that are marketed for research use only. We will also obtain assays commercially marketed for us with multiple biological specimens (R and D systems) and modify them for use with oral fluids. Dr. Granger has been modifying commercially available serum assays for use with saliva since the early 1990s. Our project is not focused on creating "diagnostic devices" for oral fluids. It is simply establishing the measurement feasibility of a range of different analytes in oral fluids. Thus, the FDA "status" of the device is not important to our research question, since we are not studying patients, nor are not conducting a clinical trial. Therefore, it is not relevant for this particular project whether these assays have been cleared by the FDA as IVDs. Medical, physical, psychological, emotional, and/or social risks to which participants in our study will be exposed due to “devices” used in this study do not have a greater probability and magnitude of harm or discomfort than those ordinarily encountered in daily life or during the performance of routine physical examinations or tests.

Study Statistics

We will use standard laboratory criteria to determine the lower and upper limits of sensitivity of the assays, document spike recovery and linearity on dilution, document inter and intra-assay coefficients of variation. For salivary analytes that pass criteria for measurement reliability and validity we will compare levels obtained to those obtained in either urine, blood, or both. Comparisons will be made between saliva and traditional specimen levels using t-tests, after appropriate transformation and screening for outliers. We will also compute correlations between levels obtain in saliva and or urine or blood.

Risks

Medical

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Venipuncture can be associated with slight discomfort as a thin needle is inserted into a vein in your arm.. There is a risk that the venipuncture used to collect the blood sample may cause redness or mild bruising. Venipuncture is conducted by a trained nurse to minimize risks. There is also a minimal risk of choking hazard during saliva collection. There may be other discomforts that are not yet known. Any unanticipated medical problems will be immediately reported to the medical doctor of our study team. A group of designated JH faculty/staff will have responsibility for monitoring, oversight of adverse events and other protocol events for this research and reporting them to the IRB.

According to the JHMI IRB Guidelines, a serious risk device poses a “potential for serious risk to the health, safety, or welfare of a subject.” A device is a serious risk if it: is intended as an implant; is purported or represented to be for a use in supporting or sustaining human life; is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health; and/or otherwise presents a potential for serious risk to the health, safety, or welfare of a subject. Non-significant risk devices are devices which do not meet these criteria. None of the devices used in this study meet the above criteria for serious risk, therefore all devices used in this study are “non-serious risk devices”.

Legal/Breach of Privacy/Confidentiality

There is a small risk that others could see sensitive personal information about the subject, which could cause embarrassment or have legal penalties. The staff working on the study and at times others working at Hopkins will see information collected from the subject. This includes people who review the research studies, their staff, lawyers, or other Johns Hopkins staff. The information may also be viewed by people outside of Johns Hopkins, such as government groups (such as the Food and Drug Administration), safety monitors, other hospitals in the study and companies that sponsor the study. Information will only be used and disclosed by Johns Hopkins as described in the consent form and in Notice of Privacy Practices; however, it cannot be guaranteed that people outside Johns Hopkins who receive the information will keep it confidential. The subject can cancel permission to use and disclose his/her information by contacting the Johns Hopkins Privacy Officer (instructions are included on the consent form). If permission is cancelled, no further information will be collected from that point, but information already collected in this study would not be affected. Results of assays on the samples provided by the subject will not be made available to the subject. Subjects are informed during recruitment (“NA_00046518_Recruitment-TelephoneScreeningScript”) and consent (“NA_00046518_Consent”) that if researchers witness any abuse, neglect, or criminal activity, it must be reported to local authorities.

Data Protection Plan (to minimize risks)

The four types of data include (1) participants signatures on consent forms, (2) participants responses to a brief questionnaire, (3) biospecimens, and (4) laboratory results from the assay of the biospecimens.

1. Once signed consent forms will be stored separately from all other project data in a locked filing cabinet in Dr. Granger's office. Only Dr. Granger will have access to these documents.
2. Questionnaires and biospecimens will be labeled with sequential bar-code numbers. Only Dr.

Granger will have access to the codebook that links the information on the consent form to the bar-code ID.

3. Laboratories at Emory and JHU will only receive bar-code specimens. They will have no access to the questionnaire information or any other information about the study.
4. Data transferred from the laboratories back to the JHU data core will be de-identified.
5. At JHU data will be stored in password protected computers, according to the NCS FISMA plan coordinated and implemented through the NCS JHU study site in the JHU SPH.
6. All staff have been required to complete data security training.

Financial

Though there is no direct cost to participate in the study, Johns Hopkins and the federal government do not have a program to pay the subject if he/she suffers adverse events from being in the study.

- If subject has health insurance: The costs for any treatment or hospital care received as the result of a study-related injury will be billed to subject's health insurer. Any costs that are not paid for by the health insurer will be billed to the subject.
- If subject does not have health insurance: Subject will be billed for the costs of any treatment or hospital care received as the result of a study-related injury.
- Study-related injury (provided the costs are not the result of care required to treat an underlying disease or condition). Any costs that are not paid for by the study sponsor will be billed to the subject.

5. Benefits

There is no direct benefit to the subject from being in this study; however, if a person takes part in this study, he/she may help others in the future. The samples donated will be used to explore new methods and develop new tests for use with saliva samples. Eventually, this study will help show which analytes have the potential to be included in the larger project. If we are able to demonstrate that saliva can be used to measure chemicals of interest, this observation has potential to minimize discomfort of the participants in the larger study on maternal and child health, increasing the chances that subjects will participate.

6. Payment and Remuneration

Participants will receive a total of \$50.00 for completing this study. Partial payment will be given based on the number of samples provided if the subject decides to stop participation in the project (\$15 for saliva, \$15 for urine, and/or \$20 for blood).

7. Costs

There is no cost associated with participating in this study, but participation will require transportation to and from the Johns Hopkins Medical Institutes Campus.