NCS Formative Research Template for OIRA Clearance

TO BE COMPLETED BY STUDY CENTER:

LOI #: LOI2-BIO-19
Title of Formative Research: Integration of salivary analytes into the NCS: Evaluation of Feasibility, Efficiency, and Benefits (from LOI Round 2 Request Item category 4, "Methods for biospecimen collection and analysis and environmental exposure sensors)
Participating Institutions: **Johns Hopkins University (JHU); Emory University**
Recruitment Study Arms:
SME: **Jack Moye**
COTR: **John Lumpkin**

Purpose of the Study: The NCS seeks to establish optimal protocols for the collection and assay of saliva samples for use in the NCS Vanguard and Main Studies. This multi-site (JHU and Emory) project has multiple component parts that are complementary.

The first goal of this substudy is to use standard immunoassay techniques to develop, evaluate, and validate saliva assays in order to make recommendations for the NCS Vanguard and Main Studies 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 (for example, hair, urine, and blood). We will determine a) whether hormonal status, environmental contaminants, markers of inflammation and oral health, oxidative stress, and pathogen-specific antibodies 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 hair, serum, and urine.

The second goal is to evaluate the feasibility and acceptability of self-collection methods, procedures, and instruction processes from mothers and small children (ages 3 months through 3 years), and to understand the implications of different procedures on assay integrity and performance. The approach is to obtain key information about the feasibility and acceptability of collecting saliva from a sample of families (mother-child dyads) that are demographically similar to NCS participants. Specifically, we will determine the advantages and limitations to saliva sampling from the perspective of study participants. Discovering obstacles that occur during interactions with participants will enable investigators to address and overcome any such issues during the Vanguard and Main Studies and increase perceived acceptability, reduce missing samples as a result of noncompliance, educate participants on correct techniques and procedures to reduce error, and reduce attrition. This data will enable the NCS team to make evidence-based decisions about how we approach the design of saliva collections and sampling scheme, with a focus on instructions and packaging to maximize success.

Benefit to NCS Vanguard or Main Study: The NCS Vanguard Study has revealed concern that participant burden related to biospecimen collection might impact recruitment and retention in the NCS Vanguard and Main Studies. Currently, the saliva collection is employed to measure only a limited number of analytes; however, if the number of analytes that can be measured by saliva is increased, thus eliminating the need for more invasive and burdensome biospecimen collection such as venipuncture and urine collection, the NCS may increase retention and compliance with biospecimen collection overall. The NCS Vanguard and Main Studies will benefit if we are able to show that the saliva samples collected can be assayed for a broad range of potentially important analytes and associated with other measures. Specific efforts to streamline and improve participant collection instructions and procedures will also serve to increase saliva collection compliance and sample integrity and viablility.

Study Design: This is a multi-site, multi-stage project coordinated through the National Children's Study sites at JHU and Emory, with JHU acting as the lead site. JHU will be responsible for consenting, collecting, and distributing its de-identified samples to Emory. Emory will be responsible for collecting and de-identifying specimens from its sample of participants. A group of designated JHU faculty and staff will have responsibility for monitoring and oversight of adverse events and other protocol events for this research.

Goal I: Assay Development and Validation

150 adults age 18-35 (50% male, 50% non-pregnant female), will be recruited for this study using flyers posted on the campuses of the JHU Schools of Nursing, Medicine, and Public Health. Please note: concerns about blinding, routine care, therapies, etc. are not applicable to this study. All recruitment and data collection activities for this portion of the substudy will occur at the JHU study location. Study staff will conduct screening interviews over the phone with people who respond to the recruitment flyers ("Attach 1b Goal 1_TelephoneScreeningScript).

Eligible participants will meet a study team member for an in-person visit at the Johns Hopkins General Research Clinical Center. The study team member will complete the consent process ("Attach 1c Goal 1_Consent Form") with the participant, including review of consent form and answering any questions, then conduct a demographic/health/behavior interview ("Attach1d Goal 1_Demographic Health Behavior Questionnaire") with the participant. 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.

Next, saliva, urine and blood collection will occur during the in-person visit as described below:

> **Saliva Sample Donation:** Participants will gently force approximately 15 mL (1 tablespoon) of pooled saliva through a straw into a container.
>
> **Urine sample Donation:** Participants will void urine in a private bathroom. Participants will be instructed to use the clean catch method to collect urine into a BD clean catch collection container.
>
> **Venipuncture**: Collection of 29 mL (1.93 tablespoons) of blood will occur via venipuncture.

Following sample collection, participants will be provided with a monetary incentive thanking them for their time and a copy of their consent form, which includes contact names and phone numbers for concerns about the study or medical questions concerning sample collection. 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 and consent.

Sample aliquots will be distributed between study sites (JHU and Emory). Once specimens are distributed, individual site laboratories will process the specimens according to their laboratory SOPs.

We will use the resulting data to 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) Oxidative stress (Emory), and (5) Environmental chemicals (Emory).

Additionally, both sites will identify an absorbent material that is acceptable to individuals who are demographically similar to NCS participants that can be used in all study phases and across age groups, absorbs sufficient saliva sample, enables high volume recovery, and creates minimal interference with the key salivary analytes of interest to this substudy and to the NCS Vanguard and Main Studies.

The resulting data will be returned to JHU for integration into a master data set. The master data set, stripped of any PII, will be distributed to each site for statistical analysis. Residual sample materials will then be destroyed at The School of Nursing Center for Interdisciplinary Salivary Bioscience Research and at Emory. Project staff at the respective substudy sites will perform this task. Results of assays on the samples provided by participants will not be made available to the participants.

Goal II: Evaluation of Sample Collection Protocols

Separately, we will develop a prototype saliva sampling protocol based on information already present in the literature. The resulting protocol will be pilot tested, and feedback regarding feasibility and acceptability of the protocol will be collected from a total of 66 mother-child dyads. That feedback will be incorporated into a revised protocol, and the revised protocol will be pilot tested in a second, different group of 66 mother-child dyads.

JHU is the lead center for compiling materials and conducting analyses. Emory will contribute to instrument development. Both JHU and Emory will administer surveys and prototype collection materials to invited participants. Both sites will then work together to refine the sampling approach.

The JHU team will create a prototype saliva sample collection kit and will train the JHU and Emory data collection teams on how to use the kit. Prototype collection kit will contain commercially-available foam swabs (1 x 4 cm for adults; 1 x 12 cm for children), short drinking straws, and 15 mL and 2 mL collection vials. Regional NCS subcontractors will administer these sample collection kits to 66 mother-child dyads. Each site will be responsible for administering sample collection kits and protocols to 33 dyads.

Day 1: A home visitor will guide the participant through consent process (“Attach 2b Goal 2_Consent Form”), conduct a brief demographics questionnaire (“Attach 2c Goal 2_Home Visit Demographics Questionnaire”), demonstrate the saliva collection, storage, and shipping procedures in person (“Attach S1 Goal II Home Visit Saliva Collection Guide,” “Attach S3 Goal II Saliva Collection Diagrams,” “Attach S4 Goal II Saliva Collection Do's and Don'ts,” and “Attach S5 Goal II Sample Shipping Instructions”) and then give the participants illustrated instructions and materials concerning the procedures (“Attach S2 Goal II DemoMotherInfantChild Saliva Collection Sheets”). Subjects will then self-collect saliva samples on the next day. We will monitor compliance with and quality of performance of the collection steps.

Day 2: Each participating mother will collect saliva 4 times at specified times of day from herself and from her child. Participants will store samples in their freezers in a small insulated box overnight.

Day 3: Participants will complete the mailing process as instructed by NCS staff. The samples will be mailed to JHU via FedEx or UPS.

Day 4: NCS staff will call participants to complete a follow-up interview (“Attach 2d Goal 2_ Follow Up Telephone Interview”) concerning the sample collection process, instructions, and packaging. The interview

will include performance indicators such as whether sufficient sample volumes are actually collected, whether the specimens as collected on schedule, and whether the samples are packaged and returned in the manner necessary to maintain sample integrity. All samples will be de-identified using bar codes, and returned to JHU, where volume and sample integrity will be assessed. Samples will subsequently be destroyed. Samples will not be stored or assayed for any biological markers.

At JHU, the material received will be evaluated for the number of complete specimens, the sample volume generated, whether instructions were followed, how samples were packaged and shipped, and the maximum temperature the specimens reached during transport. The saliva collection kit will be revised based on the feedback provided in the follow-up interview from the first cohort of 66 mothers. This may involve, for example, making changes to the instructions and/or to the size or shape of the swab material.

The revised collection kit will then be deployed using the same process (outlined above) as the prototype, to another 66 mother-child dyads (33 dyads each at JHU and Emory). The mothers of the second cohort will be also interviewed as above. As in the initial collection, no assays will be performed on the samples collected. All samples will be destroyed.

Target Respondents:

Goal I: We will invite a convenience sample of participants via flyers ("Attach 1a Goal I Exemplar Recruitment Flyer") and a study coordinator on the JHU campus. Participants may be male or non-pregnant female, English-speaking adults between the ages of 18 and 35. Individuals who are currently taking prescription medications other than birth control will be excluded from this substudy. Participants in this portion of the substudy will not be current Vanguard Study participants. Recruitment will continue until targets are met; to meet this recruitment target, we anticipate that we will need to conduct a screening interview on a total of 165 adults.

Goal II: We will invite mothers with small children (ages 3 months through 3 years) who are demographically similar to participants in the NCS Vanguard Study, but who may be geographically ineligible for participation in the Vanguard Study, to participate in this similar to the population to be targeted for the larger NCS study. A total of roughly 132 mother-child dyads will be recruited, with 66 recruited at each site. The first 66 dyads (33 at each site) will receive the initial prototype system of saliva collection demonstration/instructions, and then be interviewed for feedback about the process. Modifications will be made to the saliva collection system, then deployed to the next 66 dyads (again, 33 at each site). Recruitment will continue until targets are met; to meet this recruitment target, we anticipate that we will need to conduct a screening interview on a total of 145 mothers of young children (about 72 parents, or 36 per participating study site, for each testing group).

Sample Size Calculation:

Goal I: We will collect blood, urine, and saliva from a total of 75 adult males and 75 adult females. These samples will be used to evaluate saliva-to-urine and saliva-to-blood associations for various salivary analytes. Effect sizes for serum-saliva correlations are expected to fall between.25 and .95 across the various analytes (N > 25) to be studied, with the majority of coefficients in the .40 range. We estimate the within gender cell size needs to be a minimum of 50 to detect significance at $p < .05$ level after including covariates. 25 samples from males and 25 samples from females will be allocated into a control (untreated) group, or filtered through 3 different swab types based on density. Correlations will be computed among the 4 different conditions to

evaluate the impact of the treatments on individual differences. We expect these associations to be in the .80 to .95 range, and after including covariates anticipate 25 within each gender cell is sufficient to find these associations significant at the $p < .05$ level.

Goal II: In the first wave of assessments (engaging cohort 1), we will pilot test multiple sample collection techniques (e.g., swabs, collection aids) and methods to encourage participants to collect samples on a set schedule. In the second wave of assessments (engaging cohort 2) in Part II, we will recruit a second cohort of 66 dyads and present a prototype version of the sample collection approach based on refinements learned from data derived from the analysis of cohort 1. Recall, that we are not assaying these samples for salivary biomarkers of any type. Our main outcome measures are self-reported acceptance of the procedures derived from a post-collection interview, as well as objective measures derived from examining the samples (e.g., max temperature reached during shipping, sample volumes) when they are returned by mail to JHU. It is critical that the sampling involves both mothers and their children.

For this study, we had initially proposed to involve 2 cohorts of 33 dyads at each of three original study sites (JHU, Emory, and UCLA). The overall target sample size was therefore 2 cohorts of 99-100 dyads. Given the diverse nature of the dependent variables to be measured we expected considerable variation in the assessments. Initially, we expected that we would employ the major independent variables between dyads. With cohort size N = 100, we would have had approximately 25-30 dyads in each design cell. When UCLA discontinued their involvement in this substidy, this reduced the number to 2 cohorts of 66 dyads. With this reduction in sample size, JHU and Emory investigators have refined the design such that the main independent variables will be run within subjects. This enables us to accomplish our feasibility assessments with the reduced cohort size of 66.

Method of Recruiting:

Goal I: Flyers announcing this portion of the substudy will be posted in common areas in the JHU Schools of Nursing, Medicine, and Public Health ("Attach 1a Goal 1_Exemplar Recruitment Flyer"). Those announcements will briefly describe the substudy and provide a contact number for the project. Potential participants who call the listed phone number will be screened by phone ("Attach 1b Goal 1_TelephoneScreeningScript") to determine if they qualify for the study. If the participant accepts, we will be explain to him/her that he/she will be required to make one visit to the Johns Hopkins East Baltimore campus for roughly 45 minutes. During this visit, we will 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. For this study, the nursing staff in the JHU Institute of Clinical and Translational Research (ICTR) will be supporting the NCS with biospecimen collection. The ICTR clinics at Johns Hopkins is designed to support a wide range of research studies. The study visit will take place in the ICTR clinic with nursing staff assigned to provide services for our research study. There is no additional burden carried by the nursing staff in helping us with biospecimen collection. After the screening call, an appointment will be made for a study team member to meet participants at the ITCR site. Concerns about blinding, routine care, therapies, etc are not applicable to this study.

Goal II: At both participating NCS study locations, located in the Atlanta and Baltimore/Washington Metropolitan areas, there are extensive recruitment procedures already in place for the larger NCS study. These include announcements in a variety of public media (radio ads, print ads on in newspaper, posters). These announcements direct interested participants to local subcontractors (primarily Battelle in the Atlanta and Baltimore/Washington Metropolitan areas) responsible for consenting, and implementing data collection

procedures. These subcontractors are provided with inclusion and exclusion criteria for participation in the NCS Vanguard Study. Potential participants who respond to these advertisements but who do not qualify for the larger NCS study (e.g., they live outside of the geographic sampling area) will then be screened by the sub-contractors for inclusion and exclusion criteria in this substudy. Participants for this portion of the LOI2-BIO-19 substudy will be English-speaking between the ages of 18 and 35 and have children between 3 months and 3 years of age. Following the screening process, participants meeting the above criteria will be invited to participate. Subcontractors will provide each subject with Hopkins study coordinator contact information in case she has further questions, but the subcontractors themselves will arrange home visits with each participant directly.

Confidentiality:

Goal I: 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. Once signed consent forms will be stored separately from all other project data in a locked filing cabinet in the PI's office. Only the PI will have access to these documents. Questionnaires and biospecimens will be labeled with sequential bar-code numbers. Only the PI will have access to the codebook that links the information on the consent form containing PII to the bar-code ID. Laboratories at Emory and JHU will only received bar-code specimens. They will have no access to the questionnaire information or any other information about the study. Data transferred from the laboratories back to the JHU data core will be de-identified. 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. All staff member have been required to complete data security training.

Goal II: Information will only be used and disclosed by Johns Hopkins as described in the consent form and in Notice of Privacy Practices. The participant may 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. Participants are informed during recruitment and consent that if researchers witness any abuse, neglect, or criminal activity, it must be reported to local authorities.

IRB Approval: Local IRB clearance for this activity has been obtained by the participating Study Centers. Please see the attached IRB approval letters.

Incentives: Upon completion of substudy activities, participants will receive cash or gift cards as a token of appreciation. Incentive amounts described below are in accordance with the currently approved incentive structure for the NCS Vanguard Study: $25 in total for a questionnaire, and an additional $25, in total, for a set of biospecimens collected during a single visit.

Goal I. Participants may receive at total of $50.00 upon completing this study (including participation in a demographic and health questionnaire and providing a set of biospecimens). Partial incentives may be given based on the number of samples provided if the participant decides to stop participation in the project midway through sample collection. Participant removal criteria include: failure to follow instructions, cancellation of studies, or other reasons unforeseeable at the time of recruitment. This is explained during screening and on the consent form.

Goal II. Participants will receive at total of $25.00 for completing this study. Payment will be mailed to them when the samples are received at Johns Hopkins University. If subjects only complete the follow-up phone interview, they may receive partial payment of $15.00.

Sensitive Questions: We will not ask sensitive questions as a component of this substudy.

Proposed Project Schedule: We will begin this project upon receipt of all regulatory approvals.

Data Collection Burden:

Estimates of Annual Hour Burden – Goal I

Data Collection Activity	Type of Respondent	Estimated Number of Respondents	Estimated Number of Responses per Respondent	Average Burden Hours Per Response (in hours)	Estimated Total Annual Burden Hours
Screening	Adult	165*	1	15/60	41
Questionnaire	Adult	150	1	15/60	38
Saliva Collection	Adult	150	1	15/60	38
Urine Collection	Adult	150	1	15/60	38
Blood Collection	Adult	150	1	15/60	38
Total		**165**			**193**

*Screening total is the number of estimated consented participants plus 10%.

Goal II

Data Collection Activity	Type of Respondent	Estimated Number of Respondents	Estimated Number of Responses per Respondent	Average Burden Hours Per Response (in hours)	Estimated Total Annual Burden Hours
Screening	Parent/ Guardian	145*	1	15/60	36
Home Visit Demographics Questionnaire	Parent/ Guardian	132	1	2/60	4
Saliva Collection Instructions	Parent/ Guardian	132	1	24/60	53
Saliva Collection	Parent/ Guardian	132	4	10/60	88
Saliva Collection	Child	132	4	10/60	88

Follow-up Telephone Interview	Parent/ Guardian	132	1	15/60	33
Total		**277**			**302**

*Screening total is the number of estimated consented parents / guardians plus 10%.

Annualized Cost to Respondents –

Project I

Data Collection Activity	**Type of Respondent**	**Estimated Total Annual Burden Hours**	**Hourly Wage Rate**	**Respondent Cost**
Screening	Adult	41	$10.00	$410.00
Questionnaire	Adult	38	$10.00	$380.00
Saliva Collection	Adult	38	$10.00	$380.00
Urine Collection	Adult	38	$10.00	$380.00
Blood Collection	Adult	38	$10.00	$380.00
Total		**193**	$10.00	**$1,930.00**

Project II

Data Collection Activity	**Type of Respondent**	**Estimated Total Annual Burden Hours**	**Hourly Wage Rate**	**Respondent Cost**
Screening	Adult	36	$10.00	$360.00
Home Visit Demographics Questionnaire	Adult	4	$10.00	$40.00
Saliva Collection Instructions	Adult	53	$10.00	$530.00
Saliva Collection	Adult	88	$10.00	$880.00
Saliva Collection	Child	88	--	--
Follow-up Telephone Interview	Adult	33	$10.00	$330.00
Total		**302**	$10.00	**$2,140.00**

☒ **Please check here after ensuring that all calculations have been verified**

Estimated Costs:
Project I
Staff Hours: 386 hours
Supervisor Hours: 97 hours

Project II
Staff Hours: 604 hours
Supervisor Hours: 151 hours

Attachments: Attach 1a Goal 1_Exemplar Recruitment Flyer, Attach 1b Goal 1_TelephoneScreeningScript, Attach 1c Goal 1_Consent Form, Attach 1d Goal 1_Demographic Health Behavior Questionnaire, Attach 1e Goal 1_IRB Protocol, Attach 1f Goal 1_IRB Approval, Attach 2a Goal 2_TelephoneScreeningScript, Attach 2b Goal 2_Consent Form, Attach 2c Goal 2_Home Visit Demographics Questionnaire, Attach 2d Goal 2_ Follow Up Telephone Interview, Attach 2e Goal 2_IRB Protocol, Attach 2f Goal II_IRB Approval Letter, Attach S1 Goal II Home Visit Saliva Collection Guide, Attach S2 Goal II DemoMotherInfantChild Saliva Collection Sheets, Attach S3 Goal II Saliva Collection Diagrams, Attach S4 Goal II Saliva Collection Do's and Don'ts, Attach S5 Goal II Sample Shipping Instructions, Attach S6 Goal II Saliva Kit Choking Hazard Warning

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Public reporting burden for this collection of information is estimated to average X minutes per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB control number. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to: NIH, Project Clearance Branch, 6705 Rockledge Drive, MSC 7974, Bethesda, MD 20892-7974, ATTN: PRA (0925-0647). Do not return the completed form to this address.

Appendix 1. Maximum NCS Incentives, by Study Activity and Impact on Participants (Approved by OMB 1/5/12)

Data Collection Activity Characteristics	**Initial NCS Vanguard Study**	**NCS Recruitment Substudy and Formative Research**		
		Phase 1	Phase 2	Formative Research
Time for encounter	3 hours	0.5 to 1 hour	0.5 to 1 hour	0.5 to 1 hour
Sensitivity of questions	Sensitive, including sexual activity	Few sensitive questions	Few sensitive questions	Few sensitive questions
Physical measures	Yes	No	No	Yes*
Environmental specimens	Yes	No	Yes	Yes*
Biospecimens	Yes	No	Yes	Yes*
Participant observation	Yes	No	No	No
Monetary incentive, per visit	$100	$25	$25 for the group of study questionnaires, plus $25, in total, for any bio-specimens collected during a contact and, where appropriate for environmental specimens	$25, in total, for any bio-specimens collected during a contact. For questionnaires, or any environmental specimens – up to $25 when deemed necessary
Non-monetary incentives (tote bags, post its, key chains, etc.)	In addition to the monetary incentive, non-monetary incentives valued at $25 or less may be offered to participants	As an alternative to the monetary incentive, NCS logo gifts valued at $25 or less may be offered to the participants in lieu of cash or local incentives not exceeding $25 in value and deemed non-coercive by local IRBs	In addition to the monetary incentive, NCS logo gifts valued at $25 or less may be offered to the participants if these are deemed acceptable by local IRBs	Instead of monetary incentives, NCS logo gifts valued at $25 or less may be offered to the participants if these are deemed acceptable by local IRBs