HBCD Biospecimen Working Group

Recommendations for HBCD Phase II (April, 2020)

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Reviewed by the Biospecimen working group membership

1. **Membership:**

Name (*Site PI)	**Institution**
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2. General principles and considerations for recommendations:

The Biospecimen Working Group (WG) initiated the work by identifying key biospecimen domains based on their documented influence on fetal, infant, and child brain and behavioral development. We identified timing of collection, windows of detection, and biological matrices. The group also mapped proposed biospecimen collection by a study participant (mother, child, father).

As a disclaimer, we emphasize that these recommendations are preliminary and are based on the best-case scenario of recruitment during second/third trimester in pregnancy. Based on the final recommendations from the Study Design Working group, an 'adaptive design' with alternate biospecimen collection will need to be planned if a subset of maternal-infant pairs will be enrolled at or shortly after birth. We also acknowledge that the feasibility of specimen collection and processing might vary across sites. We have tried to differentiate essential and recommended data elements based on feasibility of collection and expertise at sites participating in Phase I, as well as other principles described in this summary. Moreover, differences across regions, cultures, and specific vulnerabilities in the local study populations may necessitate alternate sample collection.

We have not yet focused on cost estimates associated with analyses and identification of specific panels at this time since those will be driven largely by the centralized laboratory and agreements reached during the start-up phase of HBCD Phase II. Instead, our WG has primarily focused on guiding principles of biospecimen collection, feasibility and frequency of collection, and scientific value and rigor as per NIH recommendations.

The following **guiding principles** were developed to identify and prioritize potential biospecimens:

- Utility as a biological matrix/biomarker to assess the impact on brain development
- Collection during the critical periods of exposure
- Ease of collection and transportation
- Cultural acceptability
- Minimal invasiveness
- Flexibility (i.e., matrix could be used to evaluate a range of potential targets or processes of interest)
- High sensitivity and specificity of the biomarkers in the evaluated matrix
- Detection window (broader window was preferred, particularly for the prenatal period to cover as much fetal exposure as possible)
- Anticipated expertise and infrastructure at participating sites
- Availability and accuracy of alternative non-biological measures

The following **key domains** which are related to child brain development were identified:

1. **Licit and illicit substance exposures (targeted exposures)**. This includes opioids, alcohol, tobacco, and other substance, as well as medications for treatment of opioid use disorder such as buprenorphine and methadone (MOUD). Some substances may be prescribed or legal in some states (e.g. opioids, cannabis), therefore use may not necessarily be 'illicit.'

2. **Pre- and postnatal co-exposures of interest**: This includes environmental contaminants, nutriture, and the microbiome.

3. **Genetic, epigenetic, stress, and inflammatory markers.** Factors that may mediate, moderate, or confound substance exposure associations with brain development.

Subsequently, HBCD subgroups were formed to identify potential biospecimens, articulate rationale for their collection, and identify appropriate biospecimen collection methods within each domain.

During the formative stages of our work, we reviewed materials and protocols from other national studies and repositories (e.g., ECHO Birth Cohorts, NIEHS CHEAR/HHEAR Program). Similar to the ECHO-wide cohort, we adopted the following data elements:

- **Essential** ("preferred" in ECHO): core measures that sufficiently balance innovation with feasibility, burden with efficiency, and breadth with depth across diverse the anticipated HBCD cohort. Those are suggested to be collected by all sites. If collection of an essential data element is not feasible, an alternative measure should be used (see below).

- **Recommended** ("acceptable" in ECHO): measures that might be needed to pursue specific research questions and hypotheses but might not be feasible for all sites to collect since they generally require more resources for collection and might present some challenges from the recruitment and participant burden standpoints.

  Note, that the "acceptable" data elements in the ECHO protocol are defined as "measures which reduce participant and/or researcher burden while maintaining acceptable scientific rigor", while we identified the recommended data elements as those which can help support unique hypotheses and more fully characterize the study population, but would require substantial resources to collect, process, and analyze and will increase participants' burden. Thus, they are not expected to be collected across all HBCD sites.

- **Alternative** (viable alternatives to the essential measures): measures that reduce participant and/or researcher burden while maintaining acceptable scientific rigor. Might be used by some sites as viable alternatives when collection of essential data elements is not feasible.

The biological specimens were mapped by developmental periods similar to those established in the ECHO cohort with minor modifications. We felt that it was necessary to separate the perinatal period into the delivery/hospital stay and the neonatal period. Consistent with the adaptive design principles developed by the Study Design working group, we acknowledge that it will be important to provide options to individual sites that allow for delivery-attended and non-attended specimen collection. The number of visits will depend on the recommendations from the Study Design WG. The following **developmental periods** were outlined, recognizing that the number of time points for collection will ultimately be determined by the Study Design working group.

- Prenatal (Post-LMP to pre-birth)
- Delivery/hospital stay (birth, including presentation for delivery)
- Neonatal period (first month of life)
- Infancy (2 months to 11 months 30 days)
- Early childhood (12 months to 4 years 11 months 30 days)
- Middle childhood (5 years to 10 years)

3. Special considerations for vulnerable families, including members of the tribal communities:

A Special Interest Group (SIG) was formed (Co-Chairs: Julie Croff and Ludmila Bakhireva) with members from the Biospecimen and Ethics/Legal working groups who have experience working with tribal communities. Patients with intersecting vulnerabilities, including those who are socially and economically disadvantaged deserve special attention.

To date, the SIG has conducted a literature review, exchanged our own research experience, and is currently working on a summary of the important considerations pertinent to ethnic minorities, including tribal communities. The summary report will be presented in the form of a white paper or editorial. To date, important considerations can be broadly summarized as follows:

General considerations and unique challenges:

- There are considerable health disparities in Native communities, including in substance use disorders, mental health, and maternal/child health.
- Historical trauma and history of mistreatment of tribal communities, including with misuse of specimens in biorepository and genetic analysis for purposes beyond what was stated in the consent, is an important consideration for any large-scale studies.
- Community's history, culture, values, and wishes as important contributes of successful research efforts.
- Heterogeneity of the American Indian community and tribal-specific cultural practices, preferences, as well as a broad variability in prevalence of specific conditions among tribal communities do not warrant generalization to all American Indians as a homogeneous group.
- While American Indians represent less than 5% of the US population, exclusion as a group is not appropriate.
- Confidentiality, especially in small tribal communities
- Moratorium in genetic analysis in some tribes (e.g., Navajo) deserve careful considerations.
- Challenges associated with the use of banked specimens for future exploratory analyses.

Potential facilitators:

- Collection of biospecimens honoring cultural practices, ownership of the samples and return of unused samples to the tribe are paramount.
- Prior research indicate that typically tribal communities are much more receptive to providing biospecimens for hypothesis-driven/targeted analyses rather than broad hypothesis-generating investigations (e.g., genome-wide analyses).
- Beneficence of HBCD efforts to specific communities will need to be discussed and demonstrated.
- Need to establish true mutually-beneficial partnership. Community-based participatory research might be one approach to consider.
- Research methods needs to incorporate cultural practices.
- Participants might be more receptive to self-sampling vs. clinician/clinic-based sampling.
- Culturally appropriate messaging is an important consideration of the study design.

4. Integration of recommendations with other HBCD national working groups:

The Biospecimens WG held ‘exchange sessions’ with the Study Design WG, Maternal, Neurodevelopmental & Contextual Assessments WG, and Ethics/Legal WG. A brief summary of key recommendations is summarized below:

Maternal, Neurodevelopmental & Contextual Assessments WG: Self-reported data on substance exposures is being mapped by the prenatal and postnatal/family environment subgroups of the Maternal, Neurodevelopmental & Contextual Assessments WG. The ‘gold standard’ self-reported measure, Timeline Followback Interviews, will be used. Medical record review of prenatal and delivery data will augment prenatal exposures and other biospecimen information. Our two WGs are in a process of integrating recommendations for the proposed biospecimen and self-reported data elements.

Study Design WG: The ‘ideal case’ scenario assumes prenatal recruitment and delivery-attended specimen collection. However, we know that not all participants will enroll before birth and therefore prenatal biospecimens will not be possible. Alternate methods may be employed at birth, during the hospital stay or at the neonatal visit to acquire samples as proximal to gestation as possible to estimate influential biological influences during the fetal period. Similarly, some sites will not be able to do delivery-attend specimen collection such as meconium, cord tissue/blood, blood spots or placenta. It will be important to develop

alternate plans to get the best data possible under these circumstances, and coordinate efforts for such 'adaptive design' with the Study Design WG.

We also identified the need to coordinate efforts with the "Service subgroup," which is developing overarching recommendations for referral and access to services based on the assessments employed in the HBCD study, including results of laboratory analyses.

Ethics/Legal WG: We have consulted with members of the Ethical/Legal WG to identify general best practice principles in biospecimen collection including issues of legal risk for participants, informed consent, privacy protection, responsible custodianship, access to biospecimens and data, and sharing results with subjects. The HBCD Ethical/Legal WG is investigating and producing manuscripts and resources on practices specific to the vulnerable populations that HBCDII will enroll and is also compiling resources that are already validated and tested. Members of both WGs are participating in the SIG on tribal communities.

5. Essential, Recommended, and Alternative Data Elements

Tables 1 and 2 below summarize the proposed essential and recommended data elements by participant (mother, child, father) and developmental period. They assume the prenatal recruitment and delivery-attended collection scenarios.

Table 1. Essential Biospecimens by Participant and Developmental Period

Participant	Procedure	ESSENTIAL Biospecimens	Prenatal	Delivery Hospital	Neonatal Visit	Infancy	Early Childhood	Middle Childhood
Mother	Venipuncture	Plasma/Serum	X	X				
		Whole Blood	X	X				
	Urine Collection	Urine	X	X				
	Saliva *	Saliva	X		X	X	X	X
	Nail Collection	Finger/toe nail	X					
Child	Urine Collection	Non-gel or cotton-gauze diapers		X		X	X	
	Stool	Stool				X	X	
	Saliva Collection	Saliva			X	X	X	X
	Cord Tissue	Cord Tissue		X				
	Meconium	Meconium		alt				
	Nail Collection	Finger/toe nail					X	X
	Shed Teeth *	Shed Teeth					X	X

* Biospecimen may be mailed in

Alt: Alternative Biospecimen (i.e. Meconium is an alternative specimen to Cord tissue)

Table 2. Recommended Biospecimens by Participant and Developmental Period

Participant	Procedure	RECOMMENDED Biospecimens	Prenatal	Delivery Hospital	Neonatal Visit	Infancy	Early Childhood	Middle Childhood
Mother	Venipuncture	Plasma/Serum/Whole Blood				X	X	X
	Vaginal Swab	Vaginal Swab	X					
	Expired Air/Breathalyzer	Alcohol, Cotinine	X					
	Fecal Collection	Stool	X		X			
	Nail Collection	Finger/toe nail						
	Milk Collection/Child Nutrition	Breast Milk		X	X	X		
	Hair Collection	Hair	X			X	X	X
Child	Cord/Placenta	Cord Blood		X				
		Cord Tissue						
		Placenta		X				
	Nail Collection	Finger/toe nail		X				
	Heel Stick/Finger Prick	Blood Spots		X				X
	Venipuncture	Plasma/Serum/Whole Blood						X
	Hair Collection	Hair				X	X	X
Father	Hair Collection	Hair	alt	alt	alt	X	X	X
	Saliva Collection	Saliva	alt	alt	alt	X	X	X
	Venipuncture	Plasma/Serum/Whole Blood	alt	alt	alt	X	X	X

Alt: Alternative Biospecimen (paternal specimens in the Infancy-Middle childhood are recommended, and collection in other life stages are alternatives)

6. Potential analytes of interest within each domain:

6.1. Targets of interest for the **Substances of Abuse domain**:

- Drug panels
- Ethanol direct or indirect metabolites
- Nicotine metabolites

6.2. Targets of interest for the **Co-Exposures domain**:

Environmental Contaminants

- Metals (lead, cadmium)
- Arsenic
- Other environmental chemicals (e.g., CPFCs, flame retardants, phenols, PFAS, pesticides, phthalates, polycyclic aromatic hydrocarbons, VOCs)

Nutriture

- Micronutrients including trace minerals (iron, zinc), vitamin B6, folate, vitamin B12, choline, fatty acids (Omega 3, 6 and 9)
- Potentially metabolites of prescription medications (e.g. SSRI) to augment self-report.
- Breastmilk samples

Microbiome

- Gut, oral, vaginal

Note: Some group members recommended considering molecular assays for detecting SARS-CoV-2 in selected speciments to ascertain the effect of COVID-19 on the perinatal outcomes.

6.3. Targets of interest for the **Genetic, Epigenetic, Stress, and Inflammatory markers domain:**

- Genome-wide (or sequence) data on static DNA sequence (saliva)
- Epigenome-wide data (methylation chip)
- RNA Data (array or seq)
- Cortisol
- Inflammatory proteins
- Stress exposure (teeth)

7. Areas deserving additional considerations and input from key stakeholders and investigators:

In our discussions of Biospecimen selection, consensus was not always reached and some topics were controversial. These include:

- Testing for prenatal substance use may put mothers at legal risk. The Ethical/Legal WG is investigating using a Memorandum of Understanding (MOU) with authorities in some states with highest restrictions, to protect pregnant women who reveal their substance use (or are being identified by biomarker testing) in pregnancy.

- Although it would be informative to have biological confirmation information on postnatal substance exposures in addition to the self-report, testing postnatally for substance exposures in either mother or child was largely not recommended (with exceptions to assessing exposure to second-hand smoking via nicotine metabolites) due to legal risk to families. Additionally, we assumed that the primary focus of interest of HBCD-II is the effect of prenatal exposures on infant/child development, while the effect of family and postnatal environment (as important confounders) is being captured by the measures developed by the Maternal, Neurodevelopmental & Contextual Assessments WG.
- Biomarker testing for neonatal exposure to opioids used in treatment for NOWS/NAS at sites where it is difficult to access the medical record in some hospitals, especially if multiple hospitals are involved at a single site. It will be important to abstract the data on the type of NOWS treatment, dose and duration from medical record.
- Testing maternal hair and/or fingernail for drug exposures at neonatal/postpartum visit since it might reflect both prenatal and postnatal exposures and increase legal risks to families.
- Testing for prenatal drug exposure in shed teeth
- Testing for drug-taking in middle childhood stage using urine collection.
- Potential issues with genome-wide studies in populations with overlapping vulnerabilities, particularly tribal communities and other ethnic minorities.
- The need to allow for 'opt out' options for collection of genetic material.
- Total testing burden across longitudinal studies and its effect on recruitment and retention.

The proposed essential and recommended data elements will need to be integrated with the recommendations from other working groups to ascertain overall burden (for the research subjects), feasibility of multi-modal collection at each study visit (neuroimaging and neurodevelopmental assessments, questionnaires, physiological measures, and biospecimens), cost, and implications for recruitment and retention, as well as any potential legal repercussions.