

SHOW protocol for testing, shipping, and storing plasma, serum, whole blood, and urine

### **Initial blood sample testing**

Marshfield Labs, a private company, is contracted to provide lab values as described below according to the internal Laboratory Manual of Operations. Coded bio-specimens are shipped in a timely manner for immediate testing and reporting. The following tests (at a minimum) are run on these samples:

- complete blood cell count with differential
- serum glucose
- serum cholesterol
- serum high density lipoprotein (HDL) cholesterol
- serum triglycerides
- serum creatinine
- serum glycosylated hemoglobin (hemoglobin A1c)
- C-reactive protein (beginning in 2021)

### **Biological Sample Transportation**

With consent of the subject, coded aliquots of the biological samples, are frozen, stored, and batch-shipped to a private company, Prevention Genetics, in Marshfield, WI (<http://preventiongenetics.com>), for DNA extraction and storage in their biorepository. The remaining aliquots of plasma (including the paxgene tube), serum aliquots, and urine aliquots are frozen in the SHOW biorepository (-80C) and stored for future unspecified research.

All shipments of samples follow IATA regulations on transport and shipping of biosamples and the use of dry ice. The phlebotomist is certified in specimen shipping. Samples are tracked via the bar-coding labeling system. Staff will wear personal protective equipment and devices during all stages of sample processing and shipping to ensure safety of staff.

Confidentiality of samples is protected by the barcode label system, which provides a link to the subject, but linked data is not directly available. No personal identifiers or health information is applied to the sample tube or provided to Marshfield Labs or Prevention Genetics. Linked health information is available to qualified researchers with the appropriate SHOW Data and Biosample request approvals and IRB approval.

### **Sample Storage and Distribution**

Most of the blood sample aliquots are stored in the SHOW Biorepository in -80C freezers for future unspecified testing. All plasma, serum, urine, and whole blood aliquots are logged and tracked in the biorepository management software, Freezerworks ®. This includes DNA and mRNA that will be saved for future genetic testing. Participants are asked to provide consent regarding sample storage and additional consent specifically for storage of genetic material. The samples will be available for sharing with researchers outside the original SHOW protocol upon request. Outside requests for use of existing SHOW biorepository data will have to complete a formal data request from SHOW and obtain IRB approval for use of samples. A systematic tracking system of sample sharing requests and use has been developed. Samples are provided to outside researchers only if SHOW study participants have consented to this. There are no plans to commercialize the genetic material and it will only be used for research.

The following procedures have been established for distribution and use of SHOW biologic samples collected for future unspecified testing:

1. If researchers request samples, they will be required to submit a formal request and application to the SHOW Scientific Core Review Committee. The Scientific Committee is chaired by Kristen Malecki, SHOW director and PI and includes several Population Health

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Sciences Faculty affiliated with the SHOW study. The scientific merit for the request and consistency with SHOW's mission and policies are evaluated by the science team.

2. Once the hypothesis-driven or evaluation research has been approved by the scientific review committee, researchers are asked to complete a formal sample request form.
3. The form is used for identification and tracking of samples within the biorepository.
4. Before samples are provided to researchers, SHOW will obtain documentation of IRB approval or exemption for the specific proposed use of samples.
5. Any use of biologic samples from the SHOW biorepository requires review by the SHOW committee and IRB approval for sample analysis.
6. SHOW samples will remain coded and will not be linked to identifiable data such as names, addresses, and birthdates. All researchers outside of SHOW (not IRB key personnel) that have access to banked samples will not have access to information that would make it possible to link names to coded materials. Any linkage of analytic results with a more detailed analytic dataset will occur in the main SHOW office, unless otherwise authorized by the IRB.

### **Genetic Analysis Completed on SHOW samples**

Dr. Malecki (SHOW director) received a X01 award for genetic analysis of up to 650 SHOW DNA samples. NIH provides funding for DNA analysis in CIDR (Center for Inherited Disease Research) at John Hopkins University. The criteria for selection of DNA samples will be a) participant's consent for DNA analysis in future research, b) participation in SHOW Microbiome project in 2016-2017, c) providing blood sample for DNA extraction. Based on these criteria we have between 610-650 DNA samples available to be sent to CIDR. DNA samples sent to CIDR will be re-coded with an 8-10 digit ID number with no additional information. CIDR will not have access to the key linking re-coded ID with SHOW ID. CIDR will perform and analyze data for two arrays: Multi-Ethnic Global array and Methylation EPIC array. All data generated from these analyses will be sent back to SHOW.

Illumina Multi-Ethnic Global array: it is a multi-purpose, multi-ethnic genotyping array. The array tests for > 1.7 million selected markers (SNP) enabling identification of genetic associations with common and rare traits. We will get back data on SNPs genotypes and sequencing variant (e.g. allele variant for specific gene). Although we do not test for specific diseases or carrier status, array data will provide investigators invaluable tool for identification of new genetic traits associated with specific diseases.

Illumina methylation EPIC array: test 850,000 CpG sites on the genome for methylation status. We will get back data on methylation status (0-100%) for each CpG. DNA methylation is an important biological pathway that regulates gene expression. Although the genome (genotype) is determined at the conception and does not change over the life course, the phenotype is largely determined by methylation status of specific genes. Methylation status can be changed throughout the life by many factors including environmental exposures (examples - smoking, diet, exposure to lead, etc.).

Thus, the results from these two arrays together with existing SHOW data on a number of health determinants will provide invaluable tools for researchers to better understand determinants of health and disease in diverse populations (by race, gender, age, urban/rural, etc.).

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SHOW, as required by receiving NIH-funding for the CIDR genome wide association studies, must submit genotyping information to a federal database named dbGaP so that it can be shared with other researchers. Genetic data entered in dbGaP are assigned a new unique id before sharing so that data users will not have access to identifiable information. The key for linking to other data and identifiers collected by SHOW will be kept secure on SHOW servers. In order to submit data to dbGaP, we used the command-line program ascp from Aspera.