PhenX Toolkit

Data Collection Worksheet

Please Note: The Data Collection Worksheet (DCW) is a tool to aid integration of a PhenX protocol into a study. The PhenX DCW is not designed to be a data collection instrument. Investigators will need to decide the best way to collect data for the PhenX protocol in their study. Variables captured in the DCW, along with variable names and unique PhenX variable identifiers, are included in the PhenX Data Dictionary (DD) files.

The protocol for blood collection and processing is found in the Type 1 Diabetes Genetics Consortium Blood Collection and Processing Manual of Operations ([alink[T1DGC-MOOP.pdf|T1DGC Manual of Operations]]). The protocol for the extraction of DNA from whole blood is found in Rosinger et al. 2010. The protocol for human leukocyte antigen genotyping is found in Mychaleckyj et al 2010. Please note that the immobilized linear arrays used to genotype the samples were provided by Roche Molecular Systems and are not commercially available.

The following is a summary version of the full Type 1 Diabetes Genetics Consortium protocols. **It is not intended to replace the actual protocols listed above.**

Venipuncture / Blood Collection Procedures

Editors Note: Please review chapter VI of the Type 1 Diabetes Genetics Consortium Blood Collection and Processing Manual of Operations ([alink[Chapter6-Blood_Collection_and_Processing.pdf|T1DGC Manual of Operations - Chapter VI]]) for a full description of Phlebotomy procedures.

General steps:

- Blood is collected from the best available vein.
- Blood for DNA analysis should be collected in a 4.9-mL purple top tube (EDTA)
- The tubes are handled in a way that prevents hemolysis
- The tube contents are mixed by inverting eight times
- Record date and time of blood collection
- Record the reason why a blood sample was not collected.

Blood Processing

Editors Note: Please review chapter VI of the Type 1 Diabetes Genetics Consortium Blood Collection and Processing Manual of Operations ([alink[Chapter6-Blood_Collection_and_Processing.pdf|T1DGC Manual of Operations - Chapter VI]]) for a full description of Blood Processing procedures.

- The sample is placed in an ice and water bath. Incubate the sample between 30 and 60 minutes.
- Do not let **any** of the samples stand in direct sunlight or at extreme temperatures.
- The sample is centrifuged, to separate the plasma from the cells.
- The plasma is pipetted away from the sample without disturbing the cell pack.
- The cell pack is shipped at ambient temperature to the DNA repository for DNA extraction.

DNA Extraction from Whole Blood

Please review Rosinger et al., 2010 for the full description of the DNA extraction protocol.

- DNA is isolated by a modified salting out procedure or by cholorform extraction
- DNA concentration is determined by fluorescence using a double-stranded DNA quantification reagent.
- DNA quality is confirmed by comparison to an appropriate standard (ladder) on an agarose gel.

Laboratory Assay for Human Leukocyte Antigen

Please review Mychaleckyj et al., 2010 for a full description of the HLA genotyping methods. Please note that the immobilized linear arrays used to genotype the samples were provided by Roche Molecular Systems and are not commercially available.

- DNA (5 ug total: 250 ul at 20ng/uL) is shipped to the HLA genotyping laboratory in screw top tubes.
- Each human leukocyte antigen region is amplified from 60 ng of genomic DNA by polymerase chain reaction (PCR) on a separate 96 well plate according to standardized protocol (see Mychaleckyj et al., 2010 for details of the polymerase chain reaction reaction)
- Polymerase chain reaction products are hybridized to oligonucleotide probes attached to nylon-backed membranes. The probes correspond to specific human leukocyte antigen DNA sequences. (Please note that the immobilized linear arrays used to genotype the samples were provided by Roche Molecular Systems and are not commercially available.)
- Hybridized probes are visualized and assigned a genotype by software.