VI. LABORATORY MEASURES

A. SUMMARY OF SAMPLE COLLECTION

Clinical samples to be collected by participant type are outlined in Table 1.

Table 1. Clinical samples collected for T1DGC by type of participant.

	Affected Sibling(s) and Cases	Biological Parents	Unaffected Sibling(s)	Controls
Cells for DNA, LCL (lymphoblastoid cell lines)	**X**	**X**	**X**	**X**
Serum for $IA2_{ic}$, GAD, TPO, TG, 21-OH, H/KATPase and ZnT8 analysis[1]	**X**			
Serum for storage and future assays	**X**	**X**	**X**	**X**
Plasma for storage and future assays	**X**	**X**	**X**	**X**
Cell pack sample from plasma tube for DNA extraction	**X**	**X**	**X**	**X**

[1]Autoantibody measures originally assayed were $IA2_{ic}$ and GAD; other measures were performed during the last two years of the study.

B. RATIONALE FOR COLLECTION OF SAMPLE FOR DNA ISOLATION AND LYMPHOBLASTOID CELL LINES

To avoid the potential need for repeated blood sampling of participants, the Type 1 Diabetes Genetics Consortium will establish Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCL) on all participants. The cell lines will be established in certified facilities within each network (Asia-Pacific, European, North American, and United Kingdom).

There are several justifications for establishing lymphoblastoid cell lines. First, they will provide an inexhaustible source of DNA for this and other NIDDK-supported studies of type 1 diabetes. It has been the experience of several members of the Consortium that gene identification studies often require large amounts of DNA. The inability to re-sample a participant due to informed consent restrictions requiring removing participant names from study identifiers may result in excluding entire family units from further study. In addition, certain molecular methodologies require material in addition to conventionally stored DNA. Live cells allow pursuit of a variety of biochemical studies and may therefore permit more detailed testing of specific etiologic hypotheses generated by the linkage studies. Permanent lymphoblastoid cell lines will be established by EBV transformation of lymphocytes isolated from peripheral whole blood using standard methods (1-3). Blood will be drawn by venipuncture and collected into sodium heparin or ACD tubes during their visits to the clinical center. These tubes will be

labeled with bar codes (provided by the Coordinating Center) and shipped to the Regional Network DNA Repository for transformation.

The rationale for establishing regional repositories includes: (1) samples will be sent within a smaller geographic area; (2) the network repository would be responsible for immortalization of a smaller number of participants than if there was one central DNA Repository; and (3) should a cell line fail to be established, a repeated blood sample could be obtained more easily. After establishing the cell lines in each network repository, cells will be sent to a central repository that will maintain the entire collection of cell lines for the Consortium. This central repository will be the contact point from which DNA will be obtained by investigators.

C. AUTOANTIBODY MEASURES

We will collect serum for autoantibody characterization in affected siblings only. Autoantibodies for $IA2_{ic}$ and GAD were measured to confirm a type 1 diabetes diagnosis. Additional autoantibody measures (TPO, TG, 21-OH, H/K ATPase and ZnT8) were recommended by the Autoantibody Working Group and approved by the Steering Committee during the last two years of the study.

D. CELL PACK AND CELL LINE DNA

The cell pack remaining after the plasma is aliquoted will be collected and shipped to the Regional Network DNA Repositories for DNA extraction. In addition, cell line DNA will be extracted from viable cell line samples for use in genotyping projects. Cell pack and cell line DNA will be shipped to and stored in a central repository for future use.

E. SAMPLE STORAGE

Serum and plasma samples are stored on all members of the ASP and trio families and all cases and controls for future studies and subsequent analysis.

LITERATURE CITED

1. Anderson MA, Gusella JF. Use of cyclosporin A in establishing Epstein-Barr virus transformation in human lymphoblastoid cell lines. *In Vitro* 1984; 20: 856-858.
2. Miller G, Lipman M. Release of infectious Epstein-Barr virus by transformed marmoset leukocytes. *Proc Natl Acad Sci USA* 1973; 70: 190-194.
3. Freshney RI. *Culture of Animal Cells – A Manual of Basic Techniques*. 2nd Ed. New York. Alan R Liss, 1987.