

SAMPLES PROVIDED BY NIDDK FOR PAR-04-076
(PROTEOMIC AND METABOLOMIC APPROACHES TO DIAGNOSE DIABETES AND
PRE-DIABETES)

All awardees for the R21 phase will be provided with the following samples:

- o 1ml (4X250µl) of plasma from 10 patients with normal glucose tolerance (NGT)
- o 1ml (4X250µl) of plasma from 10 patients with impaired glucose tolerance (IGT)
- o 1ml (4X250µl) of plasma from 10 patients with newly diagnosed type 2 diabetes
- o The corresponding blood cell pellet and buffy coat from each NGT, IGT and diabetic patient samples will also be provided.

The cell pellet for each patient will not be divided in four aliquots as for the plasma but will be provided in a single vial.

The exclusion criteria, the data provided with the samples, and the protocol used for preparing the samples are as follow.

Patients' exclusion criteria

- Known to have diabetes
- Known to have illness that caused them to miss work during the past week
- Known to have had acute infections or illness during the past week (in the opinion of the subject).
- Known to be pregnant.

Data provided with samples

- *Oral Glucose Tolerance Test* (After a fast of 8 to 12 hours, a person's blood glucose is measured before and 2 hours after drinking a solution containing 75gr of glucose) and *Fasting Plasma Glucose* will be used to categorize the patients. Normal Glucose Tolerance (NGT), Impaired Glucose Tolerance (IGT) and Diabetes will be identified according to WHO criteria. *NGT* - Fasting plasma glucose < 126 mg/dl and glucose rises to no higher than 140 mg/dl 2 hours after the drink.

IGT - Fasting plasma glucose < 126 mg/dl and the glucose level is between 140 and 199 mg/dl 2 hours after the drink.

Diabetes - Fasting plasma glucose > 126 mg/dl and/or the blood glucose rises to 200 mg/dl or above.

- *Anthropometric information*: height, weight, BMI, blood pressure, waist circumference, and waist/hip ratio.

- *Metabolic profile*: prior to the OGTT, venous blood will also be obtained for measurement of hemoglobin Alc (HbAlc), plasma total cholesterol, triglycerides, HDL cholesterol, LDL

cholesterol, and C-reactive protein.

Protocol used for preparing the citrated plasma samples

Collect signed IRB approved informed consent forms from each donor.

Seat the Subject for at least five minutes prior to blood collection

Under the direction of a qualified and licensed physician, trained phlebotomists will collect blood from each donor into evacuated blood collection tubes (BD Glass Sodium Citrate, 0.105M, 10 mL, catalog# 366007)

From each individual consenting donor approximately 30 mL of blood will be collected into 3 tubes by venipuncture. A discard tubes will be drawn first to prime the tubing.

Immediately after collection the tube will be inverted for 4 times

All tubes and specimen will be kept always in wet ice or refrigerated at 4 °C.

The specimens will be centrifuged in a swinging bucket centrifuge at 1,500g (see centrifuge instruction for conversion to rpm) for 15 minutes at 4°C.

The resultant plasma from the three tubes will be pooled into a secondary 50 ml conical Falcon[®] tube.

Remove buffy coat from the 3 tubes and pool them in a single 5 ml vial. Suspend buffy coat cells in freezing solution (0.9% saline containing 10% DMSO) and make 6 aliquots (approximately 0.2 mL each) in 6 labeled microcentrifuge tube. Store at -70°C.

The secondary tube (50 mL Falcon) containing the plasma will be centrifuged at 1500 rpm for 15 min at 4°C to remove all potentially remaining cellular material. Transfer top 90% into new tube to ensure that no cellular material is collected.

Make 24 aliquots (0.250 mL each) in labeled 0.5 ml cryovials.

Suspend the remaining red blood cell pellet from the 3 citrated tubes in 15 ml freezing solution (0.9% saline containing 10% DMSO) and pool cells in a single 50 mL falcon tube. Make 6 aliquots (2 mL each) in 2 mL cryovial and store at -70°C.

The above protocol will be completed within 75 minutes from time of specimen collection.

Samples provided for the R33 phase awardees

For the R33 phase we expect to be able to provide plasma, blood cell, and buffy coat samples from approximately 200 patients with IGT, 200 patients with NGT and 50 diabetic patients. These samples will be provided using the protocols described above for the R21 phase

For any inquiry regarding this protocol please contact:

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