Viability of capillary blood collection for use in population-based health research

J. Josh Snodgrass, Sharon R. Williams, and Thomas W. McDade

J. Josh Snodgrass
Department of Anthropology
University of Oregon
Eugene, OR 97403
541-346-5102 (phone)
541-346-0668 (fax)
jjosh@darkwing.uoregon.edu

Sharon R. Williams
Center on Aging
University of Chicago and
Department of Anthropology
Northwestern University
Evanston, IL 60208
847-491-7207
847-467-1778 (fax)
s-williams2@northwestern.edu

Thomas W. McDade
Department of Anthropology
Northwestern University
Evanston, IL 60208
847-467-4304 (phone)
847-467-1778 (fax)
t-mcdade@northwestern.edu

Extended Abstract:

A major impediment to the measurement of biomarkers in population-based research is the requirement for venous blood. Venipuncture is a relatively invasive procedure that is unacceptable to many research participants, must be performed by a trained phlebotomist (usually in a clinical setting), and requires immediate access to facilities where blood samples can be promptly processed and stored under controlled conditions. Recent innovations in immunoassay technology have made it possible to simultaneously quantify multiple analytes in a single sample; existing techniques (e.g., ELISA) allow only one analyte to be assayed at a time. This advance is made possible by the development of Luminex multi-analyte profiling (xMAP) technology, which is essentially a flow cytometer modified for use with polystyrene microspheres. These microspheres are available in 100 different sets, each of which has a unique fluorescent signature based on the relative proportions of red and orange fluorescent dyes. Data are acquired by running the samples through the flow analyzer, which identifies and separates each microsphere set, and quantifies the amount of bound analyte. Analyte concentrations in each sample are calculated from standard curves generated from calibrators for each analyte. Recent work has successfully used this technique to simultaneously measure up to 15 cytokines in 50 μL of human plasma.

The increased sensitivity afforded by this technology promises to expand the range of biomarkers that can be measured in small sample volumes. While plasma samples are typically collected via venipuncture, the minimal requirements for sample volume of the Luminex protocols make blood collected in capillary tubes following finger prick a viable option. A sterile, disposable lancet is used to deliver a controlled puncture that stimulates capillary blood flow, and sufficient quantities of whole blood (over 200uL) are collected and transported in capillary tubes. This minimally-invasive blood collection technique serves to reduce the discomfort and risks associated with standard venipuncture, and is particularly useful when working with children, older adults, and non-clinical populations, as well as those who are hesitant to volunteer for venous blood draws.

In the present study, we validate a protocol for measuring multiple analytes in capillary tube samples using a commercial microsphere-based immunoassay kit (Linco Research, St. Charles, MO). The study has four main objectives: 1) to examine analyte stability at different temperatures; 2) to compare analyte concentrations in plasma recovered from centrifuged versus uncentrifuged samples; 3) to quantify plasma recovery volume; and 4) to assess the time needed to collect capillary blood samples.

Methods

In the first component of the project, venous blood samples were obtained from three non-fasting adults (2 males, 1 female) by antecubital venipuncture. For each participant, 14 mL of blood were collected in 2 untreated Vacutainer tubes (BD, Franklin Lakes, NJ). 350 μL of whole blood from each participant were immediately aliquoted into 22 separate EDTA-treated Microtainer 1 mL capillary collection tubes (BD, Franklin Lakes, NJ). Three baseline samples were used for each participant; these samples were centrifuged immediately at 500 x g for five minutes and the plasma fraction was transferred into polystyrene microcentrifuge tubes using disposable pipettes. Plasma samples were stored at -30°C until analysis. All non-baseline samples were subjected to one of two environmental conditions: room temperature (23°C) or refrigeration (4°C). Each sample was maintained at the designated temperature for 1, 2, 3, 4, 6, 8, 12, or 24 hours prior to centrifugation. At the appropriate time, each capillary tube was

centrifuged at 500 x g for five minutes and the plasma fraction was aliquoted into polystyrene microcentrifuge tubes using disposable pipettes. Plasma samples were stored at -30°C until analysis. Plasma samples were analyzed with a commercially available human serum/plasma adipokine kit (Linco Research, St. Charles, MO) for leptin, interleukin-6 (IL-6) and insulin using the Luminex 100 (Luminex Corporation, Austin, TX) instrument and MasterPlex QT 2.0 (MiraiBio, Alameda, CA) analytical software. All laboratory analyses were performed in the Laboratory for Human Biology Research, Department of Anthropology, Northwestern University. Analytes were considered stable if they remained within ±2 SD of baseline.

The second component of the project compares the effects of spun versus unspun plasma on biomarker concentration. Two capillary tubes (~500 μL) will be collected from each of 10 nonfasting adult volunteers. One sample from each participant will be centrifuged at 500 x g for 5 minutes and plasma transferred into a polystyrene microcentrifuge tube using a disposable pipette. Plasma samples will immediately transferred to -30°C for storage until analysis. The second sample from each participant will be allowed to separate without centrifugation for a period of 60 minutes (at room temperature). Plasma will then be removed using a disposable pipette and transferred into a polystyrene microcentrifuge tube; these samples will be stored at -30°C until analysis. Plasma samples will be analyzed with a commercially available human serum/plasma adipokine kit (Linco Research, St. Charles, MO) for leptin, interleukin-6 (IL-6) and insulin using the Luminex 100 (Luminex Corporation, Austin, TX) instrument and MasterPlex QT 2.0 (MiraiBio, Alameda, CA) analytical software. All laboratory analyses will be performed in the Laboratory for Human Biology Research, Department of Anthropology, Northwestern University.

In the final two components of the project, we will quantify of amount of plasma recovered from capillary blood samples, and document the time needed to collect these samples. Both of these components will rely on the same participant group: Fifty healthy adult volunteers. These participants will be asked to donate blood from a single finger stick. Samples will be centrifuged immediately for 5 minutes at 500x g. Plasma samples will then be transferred into polystyrene microcentrifuge tubes using disposable pipettes. The volume of plasma obtained from each capillary sample will be recorded. The time necessary for capillary blood collection from the 50 volunteers will be recorded.

Findings/Expected Findings

The results of the stability analysis indicate different stability between analytes. IL-6 declined significantly by 2 hours at 4C and 3 hours at RT. Leptin remained stable for 3 hours at 4C and RT, after which it showed a significant decline at both temperatures. Insulin remained stable for 2 hours, after which it declined. The second component is expected to document the ability to obtain at least 100 uL of plasma from EDTA coated capillary collection tubes without centrifugation. Further, we intend to demonstrate that samples left for one hour at room temperature provide similar results, when assayed, to those of the frozen, centrifuged samples. Finally, we intent to demonstrate that an adequate amount of plasma, for use in multi-analyte assays, can be collected from a single finger-stick in a brief period of time. Thus, we intend to document this system of capillary blood collection is a useful alternative to venipuncture in population-based research.