



The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

1. **Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.
2. Collect urine before going to bed tonight in the cup labeled “night”. **Please write down the date and time of the sample was collected.** Store the sample in the fridge in the Ziploc bag provided.
3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled “day”. **Please write down the date and time of the sample was collected.** Store your samples in the fridge in the Ziploc bag provided.
4. Please bring the urine samples with you on your day 2 visit. TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!



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Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.
2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.
3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.
4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample.**
5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.
6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.
7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.



Collection Unit



Collection Tube with Spoon

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Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1 (GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines, vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

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Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4 ° C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: <ul style="list-style-type: none"> • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.

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Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Day
Serum	Red/grey SST tube	1 x 4mL	<ol style="list-style-type: none"> Invert 5 times Room temp for 30 min Spin for 10 min @ 1000 x g 	<ol style="list-style-type: none"> Aliquot serum into cryovials¹ with brown² caps (0.5mL/tube) Store at -80°C 	<p style="text-align: center;">Insulin Lipid profile Glucose CRP GLP-1</p>	1,2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	<ol style="list-style-type: none"> Invert tube 8- 10 times Spin for 30 min @1500- 1800 RCF Resuspend by inverting After addition of PBS spin for 15 min @ 300 RCF Aspirate off as much supernatant without disturbing the pellet Repeat wash in 10mL PBS Resuspend pellet in 3mL freezing medium -10% DMSO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco) 	<ol style="list-style-type: none"> Aliquot entire contents above the gel and transfer to 15 mL Falcon tube Add PBS (w/o Ca++ or Mg++) to make 15 mL Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container. 	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @1300 x g 	<ol style="list-style-type: none"> Aliquot plasma into cryovials with green³ caps (0.5mL/tube) Store all fractions at -80°C 	C-reactive protein	1,2
RBC			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot RBC into cryovials with red⁵ caps (0.5mL/tube) Store all fractions at -80°C 	Fatty Acid Analysis	1,2
White blood cells Heparin			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot WBC (buffy coat) in 1 (one) Cryo.s™(RNase and DNase free 	DNA extraction/ Telomere length	1,2

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Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1. Invert 8 times Spin immediately for 10 min @ 1300 x g 2. After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @ 1300 x g	1. Aliquot plasma into cryovials with yellow caps (1.0 mL/tube) ⁵ 2. Add to 1 plasma aliquot (0.5ml), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80°C	Ascorbic acid	1,2
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot plasma in cryovials with purple caps (0.5ml/tube) ⁶ 2. Store all fractions at -80°C	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with purple caps (0.5mL/tube) ⁵ 2. Store all fractions at -80°C	Non-cholesterol sterols	1,2
White blood cells EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free vials) ⁴ 2. Store at -80°C	DNA extraction/ Telomere length	1,2