Sections 1 and 2 describe prep and will be excluded from highlighting.

The script for filming should begin as vacutainers are arriving in the lab with a sample collection summary followed immediately by step 2.7:

Samples are collected by phlebotomists in homes of individuals identified as consenting participants by the study coordinator and transported to the lab by a courier (see Supplementary Information (SI) 1 for further pre-processing details).

2.7)Following preparation of the buffers, centrifuge and heat block,document the time of vacutainer delivery on the sample tracking sheet (see Table SI).

Section 3.1 describes the importance of processing within two hours of collection and describes the number of vacutainers per delivery we have based our protocol. This is also mentioned during step 3.4.1, which is highlighted below.

3.2) **Serum Isolation Stage 1 (1 min)**

* + 1. Document the start time on the specimen log (see Table SI 2). Centrifuge (with brake and acceleration **OFF**) the 7.0 ml serum (red) vacutainer (1 per participant) using a swinging bucket rotor with aerosol caps (biosafety level 2; BSL2 certified) for 20 min, 1300 x g, 4 ˚C.

3.3) **DNA Isolation Stage 1 (15 min)**

3.3.1) Document the start time. In a BSL2 cell culture hood, invert the vacutainers containing the ficoll gel 5 times then add 200 µl whole blood from the top of one of the vacutainers into each of two 20 μl aliquots of protease (400 µl total per participant). Leaving the protease + blood microcentrifuge tubes in the hood, continue to the centrifugation of the ficoll containing vacutainers in step 3.4.1.

Note: Use the vacutainer with the largest collection volume, keeping in mind the necessity of balancing the centrifuge (i.e., it may be necessary to remove 200 μl from each of the two vacutainers) when spinning the vacutainers in step 3.4.1. Additionally, to ensure proper separation during the processing of the blue vacutainers, the level of blood remaining in the vacutainer should not be less than 2.5 inches above the ficoll layer.

3.4) **PBMC Isolation Stage 1 (1 min)**

* + 1. Document the start time (must be within two hours of blood draw to avoid an increase in red blood cell contamination and a decrease in mononuclear cell recovery). Invert the ficoll containing vacutainers 8-10 times. Centrifuge (with brake and acceleration **OFF**) the vacutainers (2 per participant) using a swinging bucket rotor with aerosol caps (BSL2 certified) for 30 min, 1600 x g, 22 °C.

3.5) **DNA Isolation Stage 2 (20 min)**

* + 1. Return to the hood and add 200 µl Buffer AL to each of the protease + blood microcentrifuge tubes (step 3.3.1). Cap, remove from hood, vortex 15 sec and flash spin.

Steps 3.5.2 to 3.5.12 describe standard procedure for DNA isolation and will not be filmed. To account for this transition, step 3.5.13 should read:

The following statement is suggested as a transition

“Proceed with DNA isolation through a series of standard procedures until DNA is eluted from the column.”

[visualized with a short clip of the elution column?]

We would like to exclude this from the protocol text however, because it is redundant.

3.5.13) Pool the eluted DNA from the 2 columns per participant. Total yield ~800 µl per participant.

Steps 3.5.14 and 3.5.15 involve quantifying and storing the DNA if needed – not integral to continuing with the next steps, excluded from highlighting.

3.6) **Leukocyte RNA Isolation Stage 1 (30 min)**

3.6.1) Pierce the rubber septum of the K2 EDTA vacutainer with a transfer spike. Retain the sheath and screw cap for use in step 3.6.11. Following BSL2 standard practices, care should be taken to avoid exposure to bloodborne pathogens.

3.6.2 to 3.6.4 discuss simple assembly of equipment – will not be filmed, but summarized at the beginning of 3.6.5:

3.6.5) Following assembly of the K2 EDTA tube system, safely unsheathe the needle (use the end of a metal spatula). Stab the needle into an empty 10 ml evacuated blood collection tube (serum receiver tube) and invert the K2 EDTA vacutainer/filter/receiver tube assembly. Following BSL2 standard practices, care should be taken to avoid exposure to bloodborne pathogens.

Steps 3.6.6 - 3.6.9 involve filtration and a PBS rinse. Will not be filmed but summarized at the beginning of 3.6.10:

3.6.10) Following filtration and a PBS wash, withdraw 3 ml of the RNA stabilization agent using a new 5 ml syringe and the method described in step 3.6.8. Flush the filter as in step 3.6.9. The RNA stabilization agent should remain on the filter. Detach the syringe from the filter without retracting the plunger.

3.6.11) Seal the filter inlet and outlet with the sheath and screw cap retained from the transfer spike leaving the filter saturated with RNA stabilization agent. The filter can be stored at this point. Store the filter at -80 ˚C until time permits (~2 hr) to complete steps 5.3 to 5.4.7.

3.7) **PBMC Isolation Stage 2 (30 min)**

Step 3.7.1 involves transporting the vacutainer in step 3.4.1 from the centrifuge to the BSL2 hood – does not need to be filmed, summarized at the beginning of 3.7.2.

3.7.2) Once the vacutainer is returned to the BSL2 hood, remove the stopper and withdraw 1.5 ml of the top, yellowish, plasma layer (Figure 2) using a serological pipette without getting close to the mononuclear (clear/white) layer. Transfer the plasma to a 5 ml cryovial – (Pool from 2 vacutainers – 1 participant). Log the volume collected. See step 3.7.6 for storage instructions.

3.7.3) Transfer the remaining plasma and the whitish, mononuclear layer (everything above the gel layer – Figure 2) using a serological pipette, to a 15 ml conical tube, pooling the mononuclear layer from each of the two ficoll containing vacutainers per participant into one conical tube.

3.7.4) Add 1X PBS to bring the total volume in the conical tube to 15 ml. Cap tube and invert 5 times. Centrifuge (with brake and acceleration **OFF**) 15 min, 300 x g, 22 °C.

3.7.5) Return to the ficoll containing vacutainers in the hood and collect the red blood cells (RBCs) by using a 5¾” Pasteur pipet to swirl around and loosen the outside of the ficoll gel layer and remove it if possible. Use a serological pipet to collect and transfer the RBCs (~4.5 ml) to a 5 ml cryovial. Log the volume collected.

3.7.6) Transfer both 5 ml cryovials (plasma in step 3.7.2 and RBCs in step 3.7.5) to a controlled rate freezing container and put at -80 ˚C for at least 24 hours after which time they can be transferred to a cryobox and returned to -80 ˚C for long term storage.

3.7.7) Return the conical tube to the hood, when the centrifugation in step 3.7.4 is complete, and aspirate all but ~500 μl of the PBS without disturbing the pellet. PBMC yield is greater if ~200 µl of PBS is left above the pellet at this stage.

3.7.8) Add fresh 1X PBS to bring the volume to 10 ml. Resuspend the pellet gently. Cap tube and invert 5 times. Centrifuge (with brake and acceleration **OFF**) 10 min, 300 x g, 22 °C.

3.8) **Serum Isolation Stage 2 (10 min)**

3.8.1) Aliquot the top serum layer from the serum vacutainer, following centrifugation (step 3.2.1), into 2 ml cryovials as desired. Typical yield is 2.5 ml (Table 1). Log the volume. For example, use 4 cryovials aliquotting 200 µl into cryovial 1, 1000 µl into cryovial 2 and then divide the remaining into cryovials 3 and 4.

3.8.2) Transfer the cryovials to a cryobox and place at -80 ˚C for long-term storage. Document the freezer start time.

3.9) **PBMC Isolation Stage 3 (15 min)**

* + 1. Return to the hood, after centrifugation (step 3.7.8), and aspirate as much supernatant/PBS as possible without disturbing the pellet. Resuspend pellet by pipetting in 2.5 ml PBMC Freezing Medium 1 (see SI 2).
    2. Add 2.5 ml PBMC Freezing Medium 2 (see SI 2) to the cell/medium solution in step 3.9.1. Vortex gently.
    3. Aliquot 10 µl of the cell solution into a 0.65 ml microcentrifuge tube (further dilution may be necessary). Add 10 µl of 0.4% trypan blue stain into the 0.65 ml microcentrifuge tube and mix by pipetting several times. For further details see the manufacturer's manual.
    4. Pipet 10 µl of the mixture into a cell counting chamber slide and place slide into the cell counter within 3 min of mixing. Zoom in and focus the cells. Press the “Count Cells” to obtain PBMC count.
    5. Aliquot, if the viable PBMC number is above 3 million cells per milliliter (mc/ml), as desired into cryovials and continue to step 3.9.9. Store PBMCs in up to 5 cryovials at a concentration of at least 3 mc/ml each.

Steps 3.9.6 - 3.9.8 describe what to do if 15mc are not obtained – not the standard process but rather an alternative that is not required to be filmed for continuity.

3.9.9) Document the cell count per cryovial. Transfer the cryovials to a controlled rate freezing container and put at -80 ˚C for at least 24 hours after which time the cryovials may be transferred to a cryobox and put in a liquid nitrogen tank (vapor phase) for long term storage. Document freezer start time.

Sections 4 and 5 describe buffer preparations for processing on Day 2, which involves the manufacturer's protocol for obtaining RNA from the LeukoLOCK filters, a standard protocol for DNase treatment of the RNA, as well as quality analysis of the RNA. All specimens are stable following the procedures on Day 1 and remain that way for at least 6 months of the initial isolation. Also mentioned is our recommended method for long-term sample storage, but this can be modified according to a lab's standard operating procedures.