VHAS biomarkers

Data collection procedures summary

Order of collection:

Blood pressure (left)

Blood sample collection

- POC tests (HbA1c then CBC)
- collect capillary blood sample (at least 0.4mL, up to 0.6mL), then process blood in centrifuge (3 minutes)
- prepare 1 cryovial plasma (approx 0.25 mL)
- prepare 1 cryovial buffy coat (all remaining volume)

Height

Weight, % Body fat

Circumferences –

- arm (left)
- calf (left)
- waist
- hip

Peak expiratory flow (3 times)

Grip strength (2 times on each hand, alternating)

Hair sample collection

Biomarker supplies checklist

- have these supplies available for each respondent

Equipment

- ❑ Automatic blood pressure monitor
- ❑ Diazyme Smart Assay for HbA1c
- ❑ QBC Star for CBC
- ❑ Stadiometer
- ❑ Scale/body composition analyzer
- ❑ Circumference tape measure
- ❑ Peak flow meter & mouthpieces
- ❑ Grip strength gauge

Blood collection Supplies

- ❑ Clean bench paper
- ❑ 1 capillary collection tube
- ❑ 1 ID label
- ❑ Lancet
- ❑ Gauze
- ❑ Alcohol wipes
- ❑ First aid tape
- ❑ Sharps container

Point of care test supplies

- ❑ HbA1c Reagent kit
- ❑ Fixed volume pipet
- ❑ Pipet tips
- ❑ CBC test capillary tube

Blood processing supplies

- ❑ Gloves
- ❑ 2 cryo vials
- ❑ 2 ID labels
- ❑ Transfer pipets
- ❑ Sample storage boxes

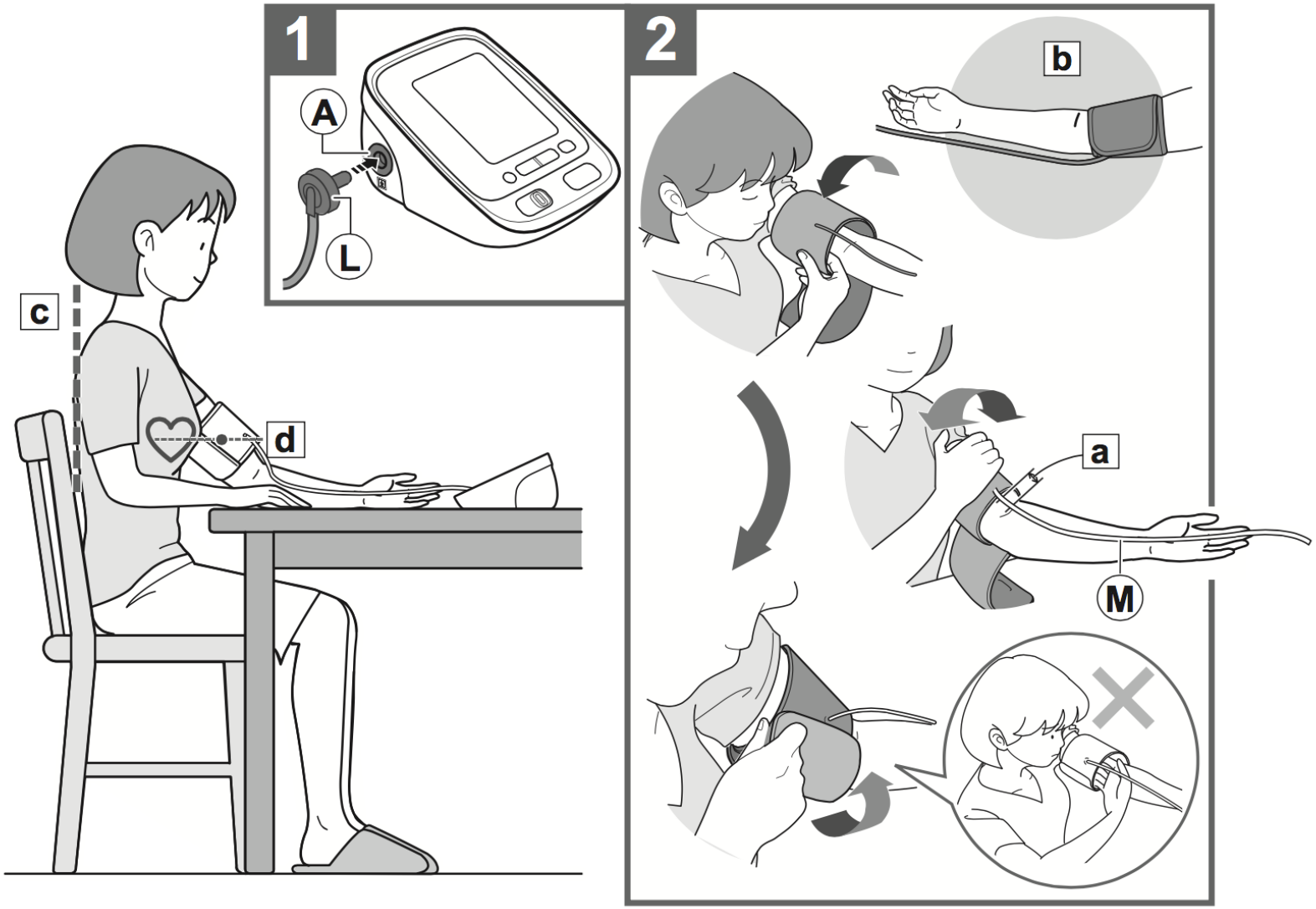
Hair collection supplies

- ❑ Comb
- ❑ String
- ❑ Hair clips
- ❑ Foil
- ❑ Scissors
- ❑ Plastic bags
- ❑ ID label

Miscellaneous supplies: Gloves, paper towels, trash bags, biohazard waste disposal bags, hand sanitizer, pens, extra AA batteries for equipment, cold packs and cooler for sample transport

Blood pressure

Measure on left arm, roll up sleeve



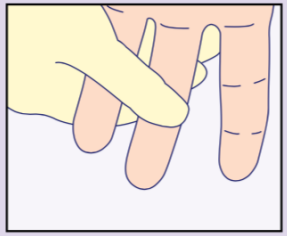
Capillary blood

- Collect blood from finger (don't forget to label tubes)
  - Blood can be collected directly into capillary tubes for point of care test, then continue collecting blood to fill Microtainer blood tubes to between 0.4 and 0.6 mL
  - If the first finger prick doesn't yield enough blood, perform a second finger prick from the same finger or a different one
- First begin to run HbA1c test, then CBC test
- Next, centrifuge the blood and label then prepare cryo vials (1 for plasma, 1 for buffy coat)

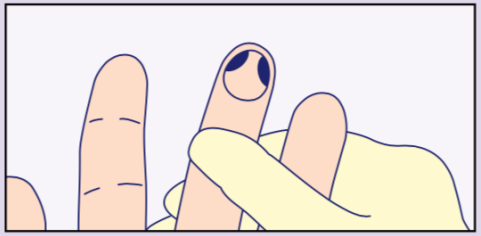
Blood collection

Performing a Fingerstick Collection

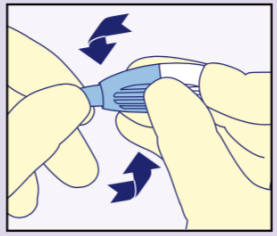
Warm the site.
Use a warm, moist towel or other appropriate warming device (not more than 42°C) for three to five minutes.



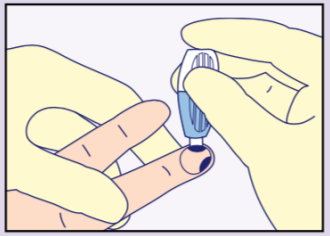
Select the site.
The patient should be sitting or lying down. Have patient hold their hand in a downward position, allowing gravity to help increase blood supply to the hand.



Select finger: middle or ring finger is preferable. Identify the desired puncture site (shaded area) and make sure it is properly cleansed with 70% alcohol according to your facility's established procedures. **The site must be allowed to air dry** in order to provide effective disinfection and to prevent possible hemolysis or erroneous results from residual alcohol.

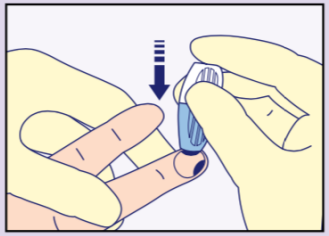


Twist off tab to break the seal and discard.



Position the safety lancet firmly against the puncture site as illustrated.

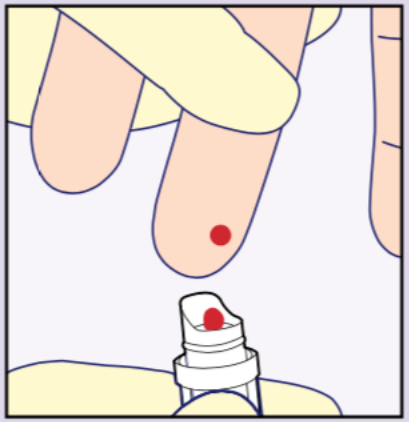
Hold safety lancet between fingers.

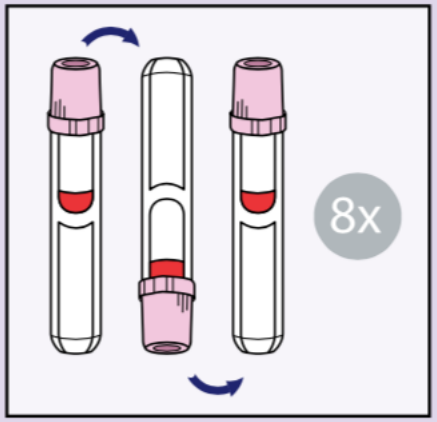


To activate, **press safety lancet firmly against the puncture site.** Do not remove the device from the site until an audible click is heard.

Discard used safety lancet into an approved sharps container.

Collect the specimen.





Gently apply intermittent pressure along finger capillaries up to the puncture site. Hold hand below elbow level to obtain the required blood volume. Strong repetitive pressure (milking) must be avoided.

Wipe away the first drop of blood with a clean dry gauze pad.

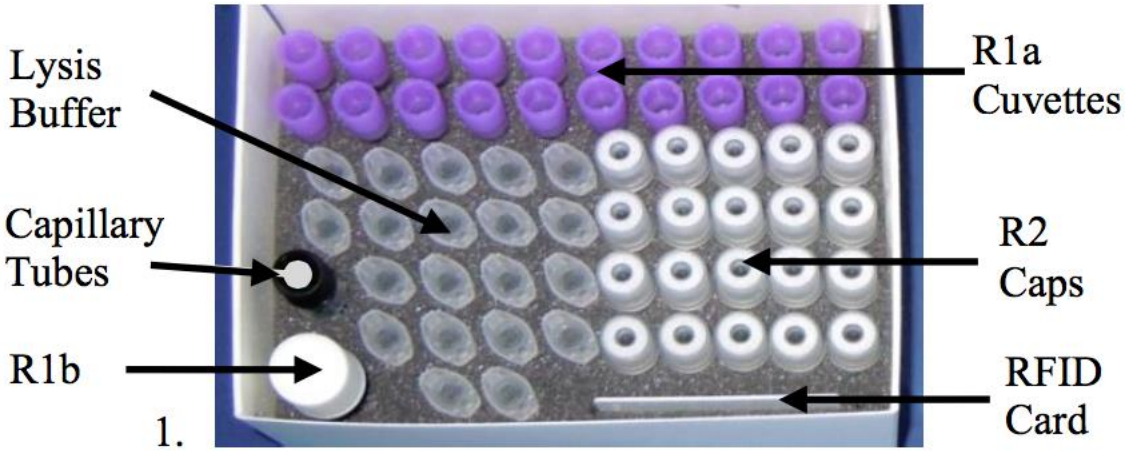
Turn the patient's hand palm down.

Position the BD Microtainer MAP Microtube for Automated Process directly beneath the puncture site. Apply gentle, intermittent pressure along finger capillaries to allow them to refill with blood and to help ensure continuous blood flow. Apply gentle pressure at the ends of the puncture site, opening the puncture site slightly, to maximize blood flow. Ensure that the tube is not scraped over the puncture site. Invert tube according to label instructions.

Complete the procedure. Wipe the site dry and apply direct pressure with a clean gauze pad until the bleeding has stopped. Dispose of all used materials according to your facility's established procedures. Label the tubes and transport them to the lab for processing.

Point of care test procedures

SMART HbA1c Assay



1.

2.

3.

SMART Assay system requires 15 minutes warm-up time. Turn it on before it is needed and leave it turned on through the day. It may be left on through the whole week, but should be plugged into a power strip with surge protection.

Assay Procedure

The step by step assay procedure is described below and illustrated in the accompanying pictures.

1. Power on SMART analyzer. The SMART analyzer is de- signed to be left powered on and does not need to be turned off after each use.

2. Examine Kit contents. Insert the provided lot specific RFID test card into the SMART analyzer for each run. RFID card contains a preprogrammed lot specific calibration curve for the assay.

3.Determine the number of tests to be performed. For example, for a single test, take one lysis buffer (prefilled) micro tube, one DRS Cuvette, one DRS Cap and R1b bottle from the kit box and place on a kit rack. Equilibrate at room temperature for a minimum of 10 minutes before use. Reagents are light sensitive.

4.For venous blood samples, use capillary tube provided to aspirate blood from primary tube until it reaches to the end.. Drop the end-to-end filled capillary tube into micro tube prefilled with lysis buffer and invert sharply 3-4 times and then shake the tube vigorously 30 times (or 15 seconds) to prepare blood lysate. (Make sure that the blood comes out of the capillary tube completely and is dispersed in lysis solution.)

5.Place the micro tube containing the blood lysate on the kit rack and wait for 10 min.

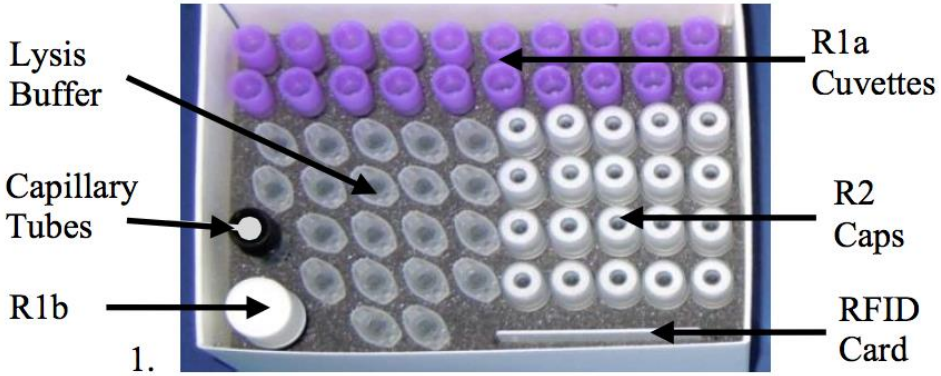
6.Using 100 µL fixed volume pipette with appropriate tip transfer 100 µl of R1b from brown glass bottle into the DRS Cuvette (Reagent R1a). Dispose the tip after use.

7.Using the 100 µL fixed volume pipette with a new tip, transfer 100 µl of the blood lysate from the micro tube from step 7 into the DRS Cuvette from step 8. Make sure there are no bubbles in the tip.

10.Place DRS Cap on top of the DRS Cuvette and snap into place.

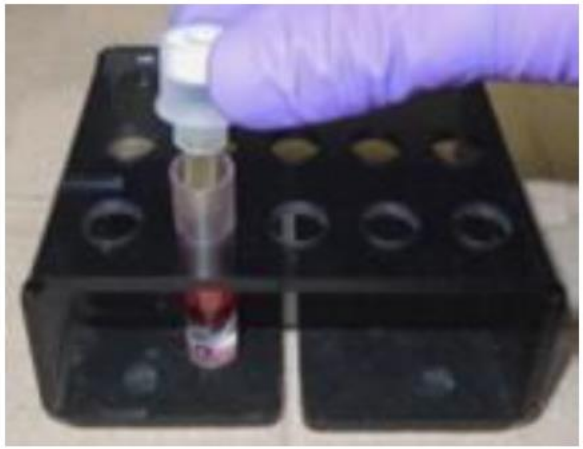
11. Press the touch screen button located on lower left side of display screen to open the door.

12.Insert the capped DRS Cuvette into the cuvette holder of the SMART analyzer door. Do not push the door by hand. To start the assay, close the door by pressing the check button on the screen.



Fill capillary tube with blood.

Drop the end-to-end filled capillary tube into micro tube prefilled with lysis buffer (picture 6) and invert sharply 3-4 times and then shake the tube vigorously 30 times (or 15 seconds) to prepare blood lysate. (Make sure that the blood comes out of the capillary tube completely and is dispersed in lysis solution.) Place the micro tube containing the blood lysate on the kit rack and wait for 10 minutes.



Move 100uL from brown bottle to cuvette, move 100uL from blood lysate mix to cuvette, then place DRS cap on cuvette. Then the cuvette is ready to begin the test. The test will take approximately 8 minutes to run. Record the HbA1c result.

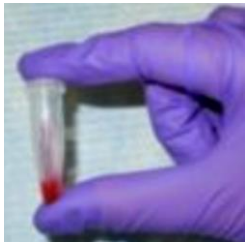
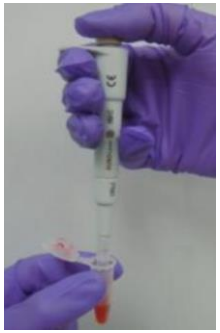
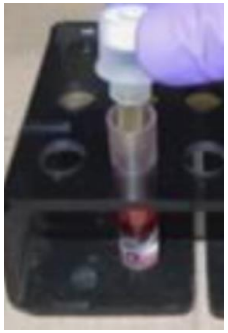
 WAIT 10 MINUTES Fill capillary tube, drop into lysis buffer tube (clear), shake to distribute blood, wait 10 minutes.	 Move 100µL from brown bottle to cuvette (purple cap)	 Move 100µL from blood lysate mix to cuvette	 Place DRS cap (white cap) on cuvette	  Press touch screen button to open door and insert cuvette, then press ✓ button to start test.

Figure 8 – QBC STAR Controls and Indicators

The QBC STAR Tube is preassembled and consists of the following components :

- 3” long precision bore glass tube with dried coatings of acridine orange, heparin, K_2EDTA, potassium oxalate, monoclonal antibody and other reagents
- A vented plug at the end of the tube
- Protective plastic sleeve

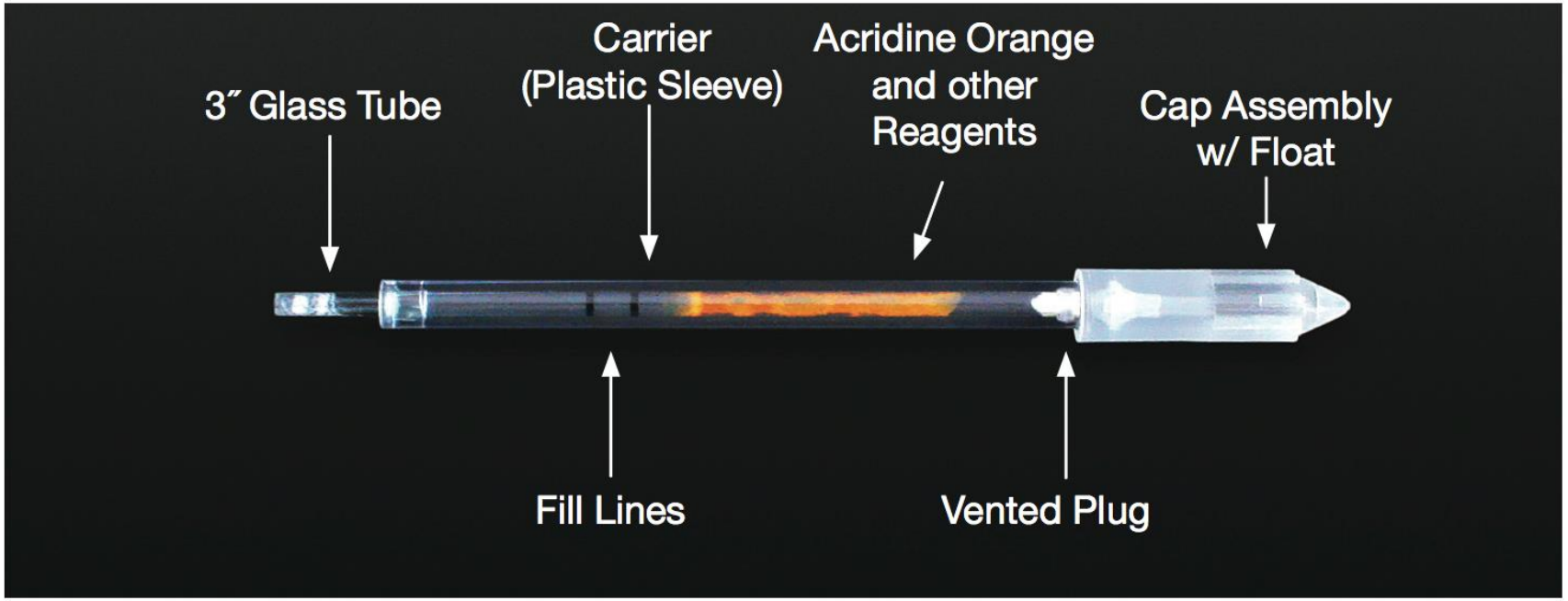


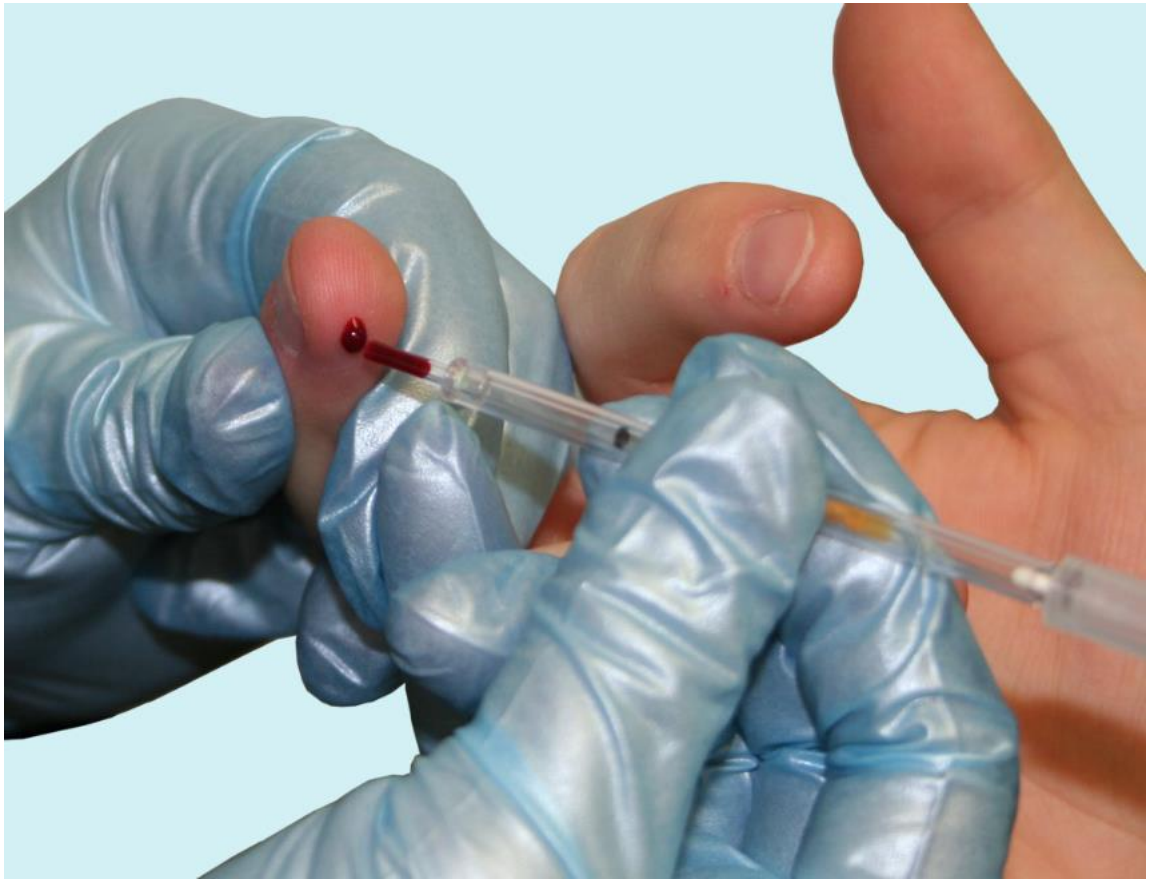
Figure 1 – QBC STAR Blood Collection Tube

Summary of Operation Steps

Routine operation of the QBC STAR Hematology System consists of the following steps:

- Turn the instrument on
- Print out / save electronic controls
- Fill tube with blood
- Mix the tube of blood
- Place the cap onto the tube
- Place the tube into the instrument
- Close door and ensure it is latched
- Push the “STAR” button to start the test cycle
- Obtain the results from the printer
- Dispose of the QBC STAR tube in a biohazard sharps container

Each of these steps is described below.



Fill capillary tube from the finger.

Mix the Tube

Rock the QBC STAR Tube back and forth at least four times to mix the blood with the acridine orange coating. Do not allow the blood to touch the white plug at the end of the tube.

Note: allowing the blood to flow from the collection end toward the plug end of the tube and back is equal to one rock.

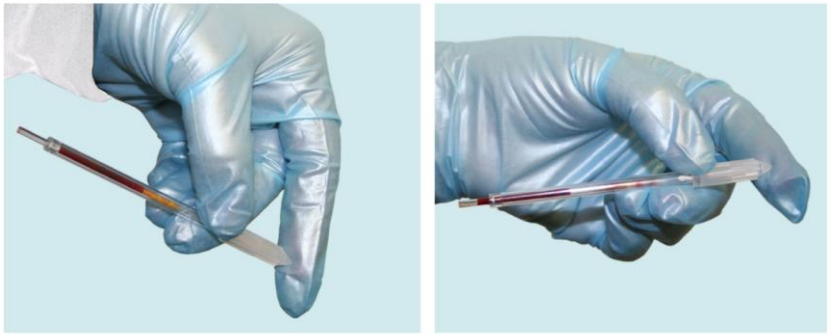


Figure 15 – Mixing the Tube

Cap the Tube

Remove the cap from the tube by pulling it straight off. Ensure that the blood is centered in the tube before placing the cap on the tube. Place the cap over the collection end of the tube by guiding the glass end of the tube into the center of the cap. Push the cap on firmly. The pink float will be guided into the glass tube.See Figures 17 and 18.

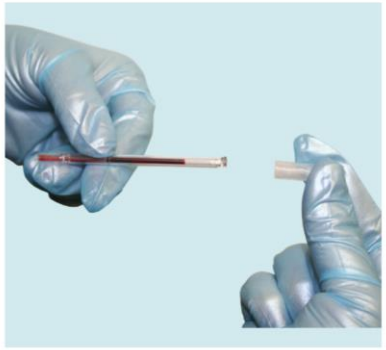


Figure 17– Uncapping the Tube

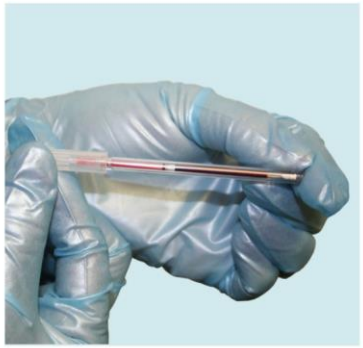


Figure 18 – Seating the Cap and Float

Tilt the Tube

Tilt the QBC STAR Tube as shown, and allow the blood to move down the tube toward the center of the tube. See Figure 16.

Insert the Tube into the Instrument

Insert the tube into the QBC STAR instrument oriented as shown in Figure 19. The tube must be inserted into the instrument within 15 minutes of being prepared and capped.

The best way to insert the tube is to first place the capped end into place, then pivot the uncapped end of the tube down into the recessed area.

If the capped end is not seated correctly the tube will not pivot down into place. If this happens, just lift the tube out, make sure the cap is seated fully, and place the tube back into the instrument as described above.

Close the Instrument Door

Make sure the door clicks into place.

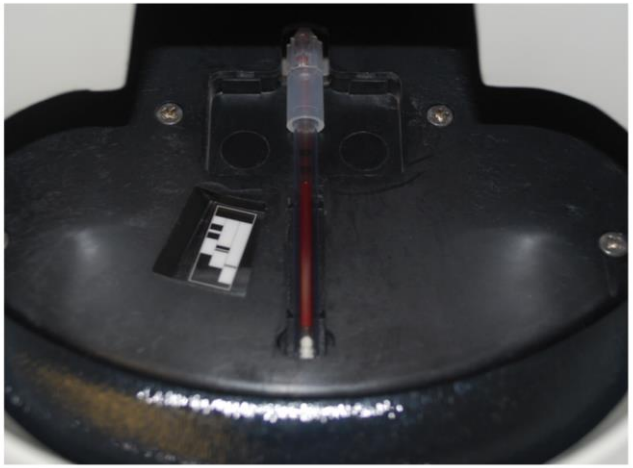


Figure 19 – Inserting the Tube into the Instrument

Start the Test

To start the test, press the “STAR” button. The system automatically locks the door electromechanically.

If there is no tube in the rotor or if the cap is not present, the message, “Sample Not Present (open door)” appears. To proceed, place the tube in the instrument and press the “STAR” button to resume testing.

The system can detect if a sample has already been tested. If this occurs, the message “Press “STAR” button to re-scan sample” appears on the LCD. See below for instructions on rerunning tubes.

The instrument mixes the tube contents for as long as 15 seconds using a low speed spin. The float descends from the top of the tube toward the closure end of the tube. This mixes the blood and reagents in the tube. A countdown displays the time remaining in the cycle.

After mixing is complete, the centrifuge accelerates to high speed to separate and pack the cell populations into distinct cell bands. A countdown displays the time remaining in the cycle.

Finally, a series of readings is taken. The messages “Reading Cycle In Progress,” is displayed while tests are being performed.

If an error message appears on the LCD display, please refer to section 7 (Troubleshooting) for error resolution.

Rerunning Tubes

The QBC STAR will process a tube that has been previously processed. To rerun a tube:

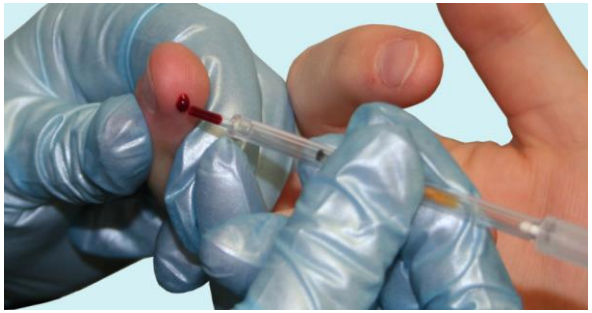
Insert a STAR Tube that has already been run into the STAR.

Close the Lid and press the run button.

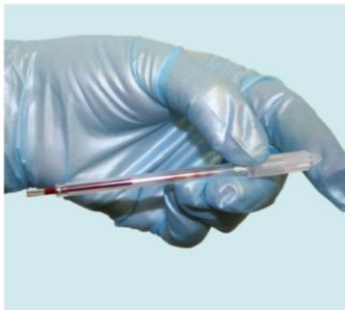
The STAR will check the tube, and display the message “Press “STAR” button to re-scan sample”

Press the “STAR” button and the results are displayed on the LCD screen and internal printer.

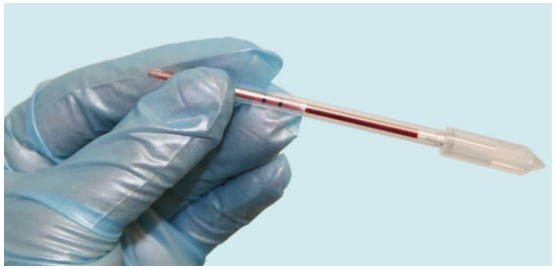
Fill capillary tube from finger. Blood level should be between the two black lines.



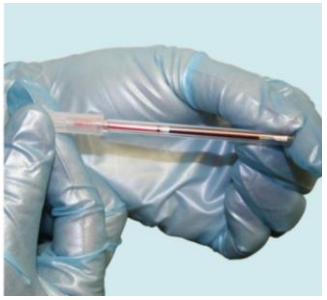
Rock tube back and forth to mix.

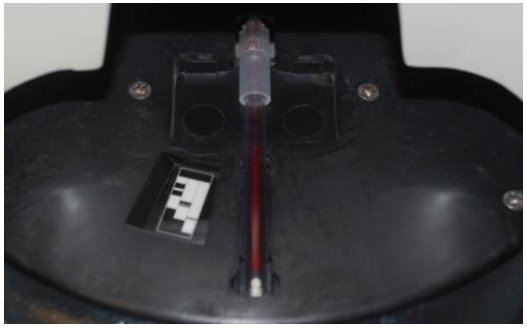


Tilt tube to allow blood to flow to the end with the cap on it.



Remove the cap and put it on the other end of the tube.



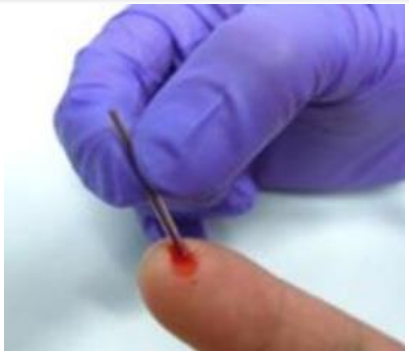


Insert tube into the instrument as shown and close the door, pushing until it clicks.

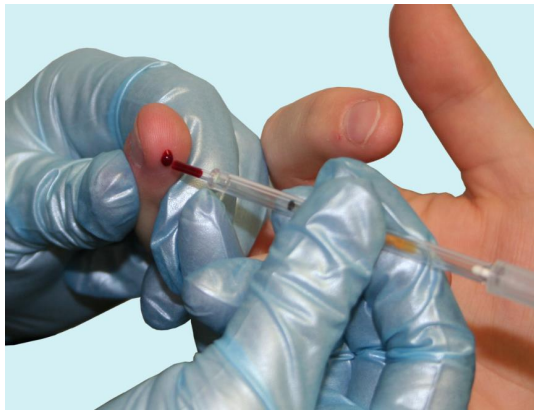
Press the blue STAR button to begin the test.

CBC values:		
Parameter	**Lower Limit**	**Upper Limit**
Hematocrit (Hct)	15%	65%
Hemoglobin (Hgb)	5.0 g/dL	20.0 g/dL
MCHC	25.0 g/dL	37.3 g/dL
White Blood Cells (WBC)	1.6 x 10^9/L	99.9 x 10^9/L
Granulocytes	0.8 x 10^9/L	70.0 x 10^9/L
% Granulocytes	1%	99%
Lymph/Mono	0.8 x 10^9/L	99.9 x 10^9/L
% Lymph/Mono	1%	99%
Platelets	20 x 10^9/L	999 x 10^9/L

Separate the blood components



HbA1c
POC



CBC
POC

~ 0.6mL
whole blood

Centrifuge
3 minutes

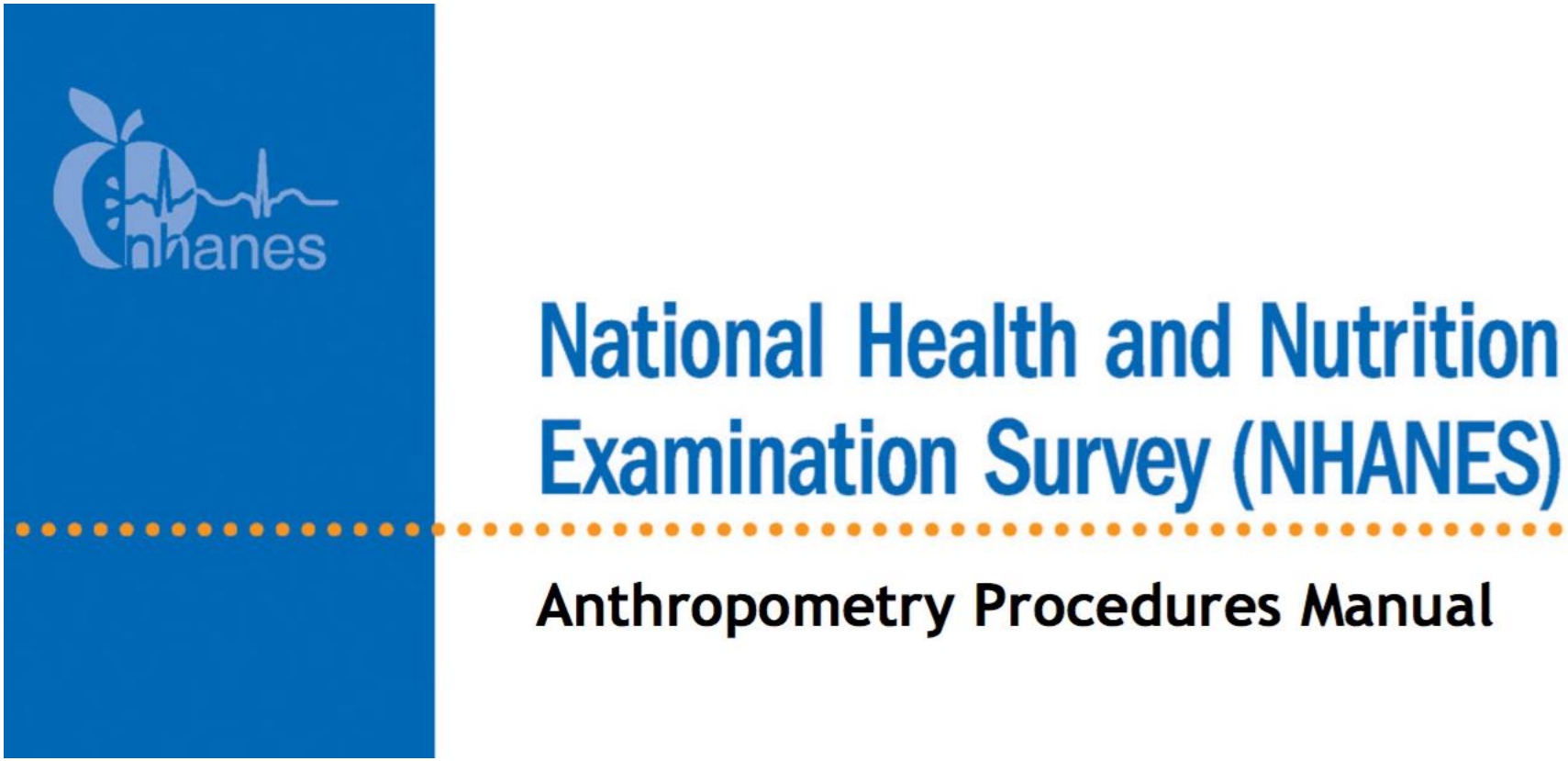
~ 0.25mL
plasma

~ 0.25mL
buffy coat

Confirm that all cryo vials are labeled with respondent ID. Put the cryo vials into the next available slot in the freezer box. Store frozen as soon as possible. Transfer to ultra-low freezer in Hanoi each week.

Anthropometry

Anthropometry procedures from the US CDC NHANES manual



US CDC, January 2017
https://wwwn.cdc.gov/nchs/data/nhanes/2017-2018/manuals/2017_Anthropometry_Procedures_Manual.pdf

Height

Respondent must remove shoes; if necessary, ask them to remove hat or arrange hair to allow accurate measurement.

Exhibit 3-4. Standing height position

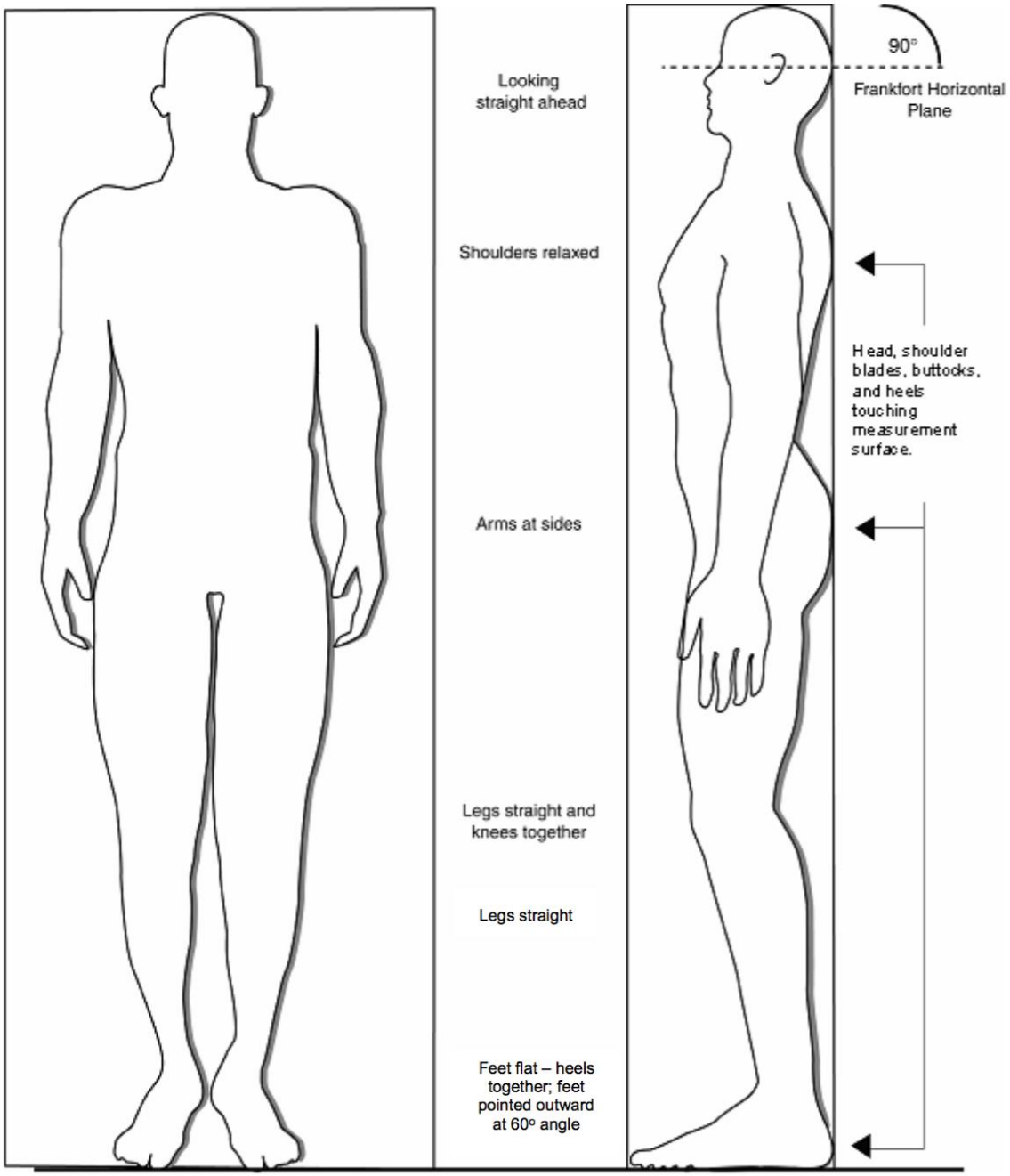
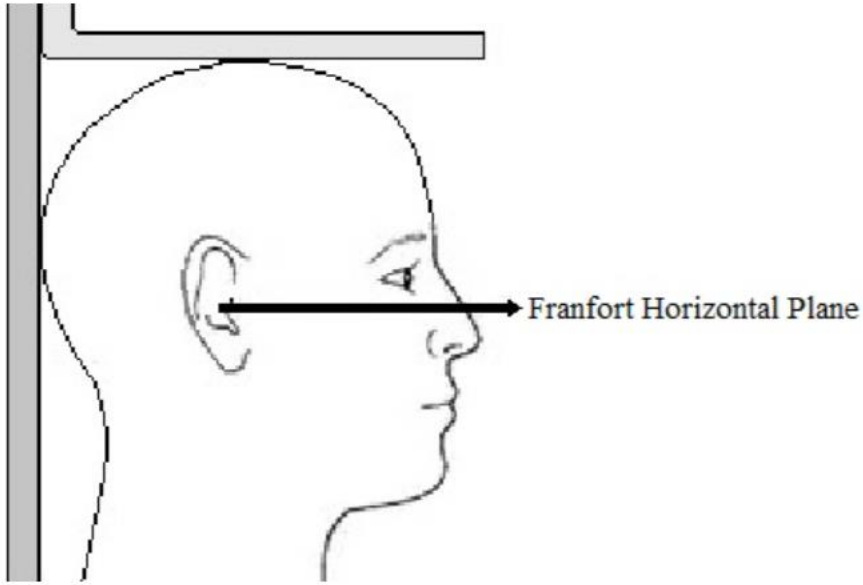


Exhibit 3-5. Frankfort horizontal plane

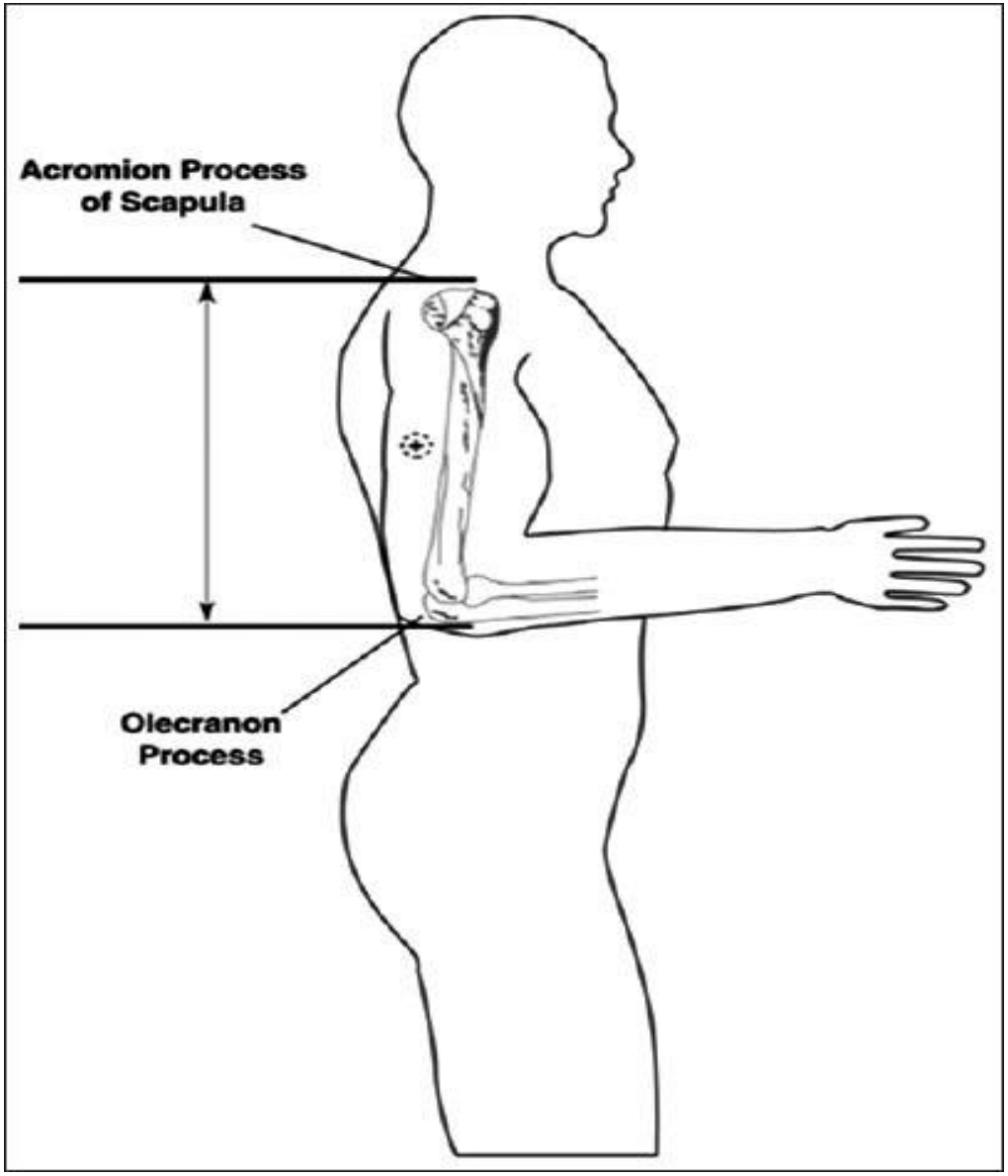


Circumference measures

- Mid upper arm
- Calf
- Waist
- Hip

mid upper arm circumference (MUAC)

- move clothing aside to expose upper arm



From Use of mid upper arm circumference for evaluation of nutritional status of OSMF patients. Yallamraju SR, Mehrotra R, Sinha A, Gattumeedhi SR, Gupta A, Khadse SV - J Int Soc Prev Community Dent (2014)

Exhibit 3-11. Upper arm bony landmarks

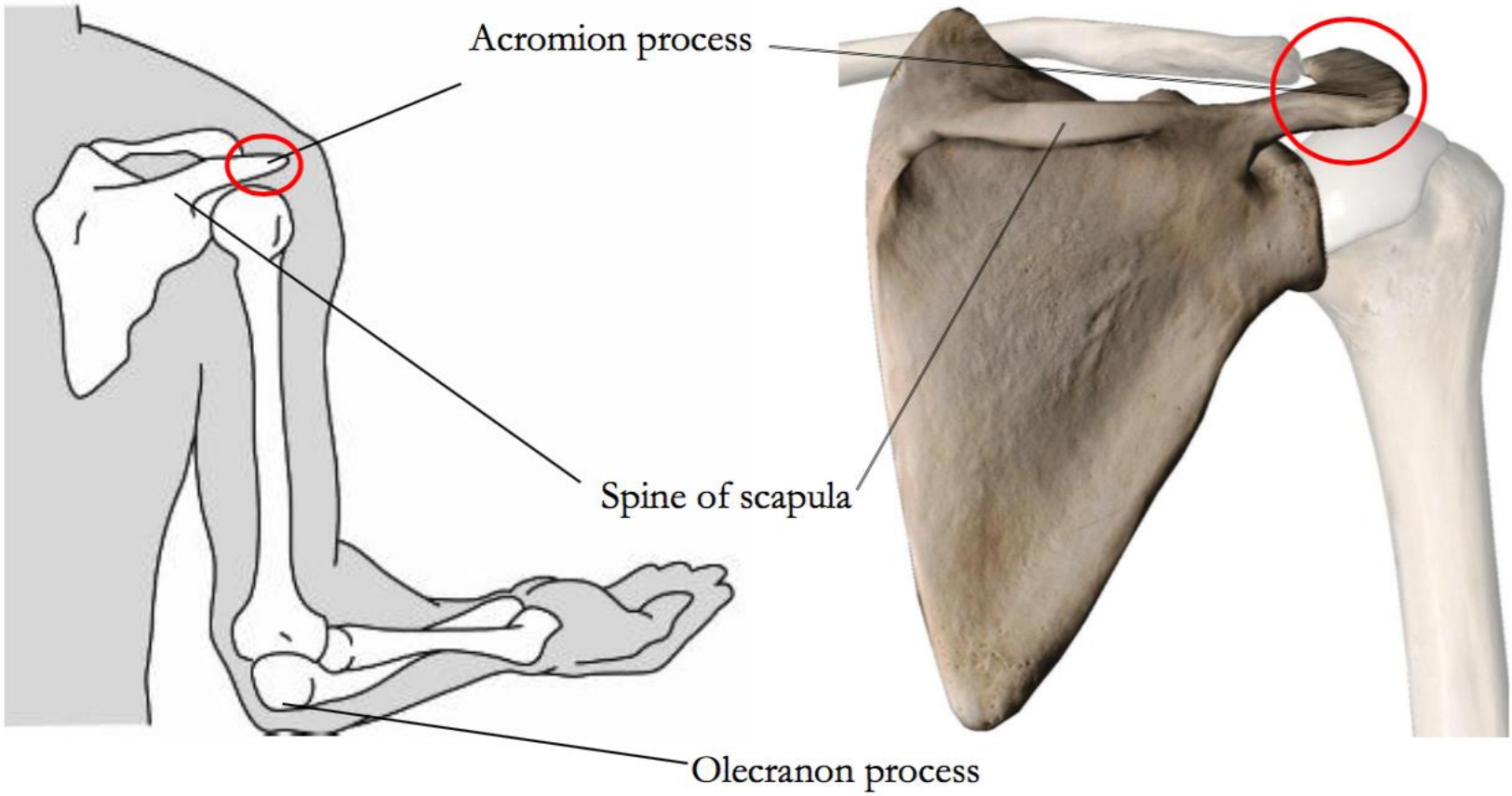
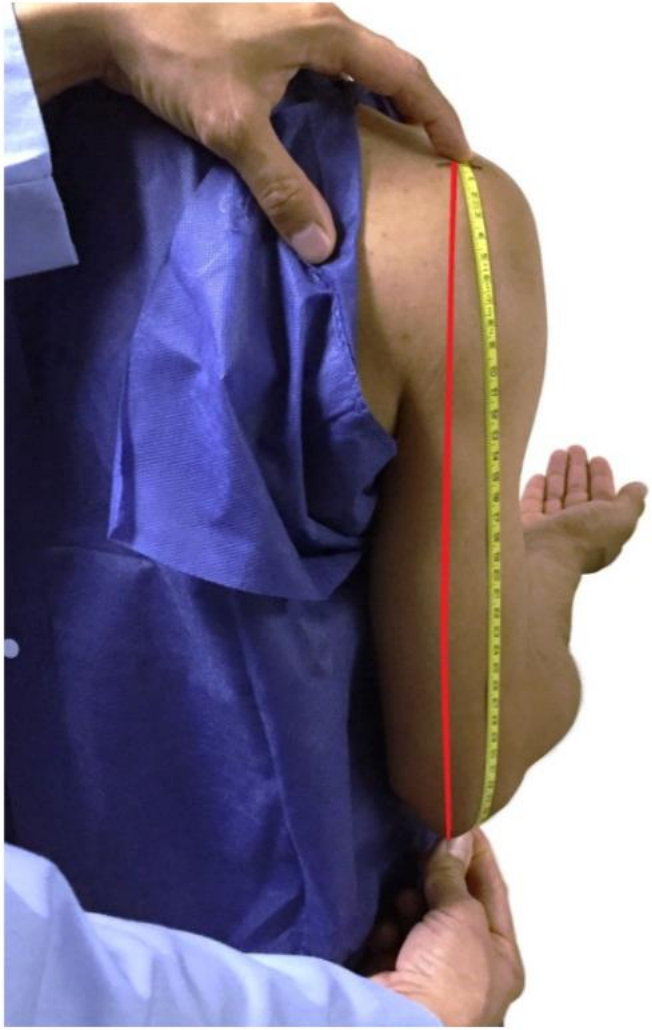


Exhibit 3-13. Marking upper arm length midpoint

Exhibit 3-15. CORRECT tape placement for upper arm length

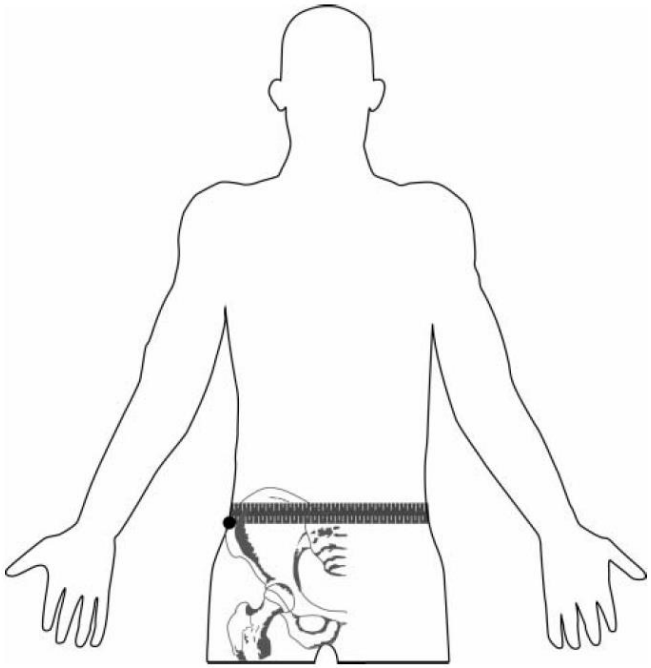
Exhibit 3-16. INCORRECT tape placement for upper arm length



calf circumference

- Move clothing aside to expose the lower leg
- Measure at widest part of the calf

Waist circumference



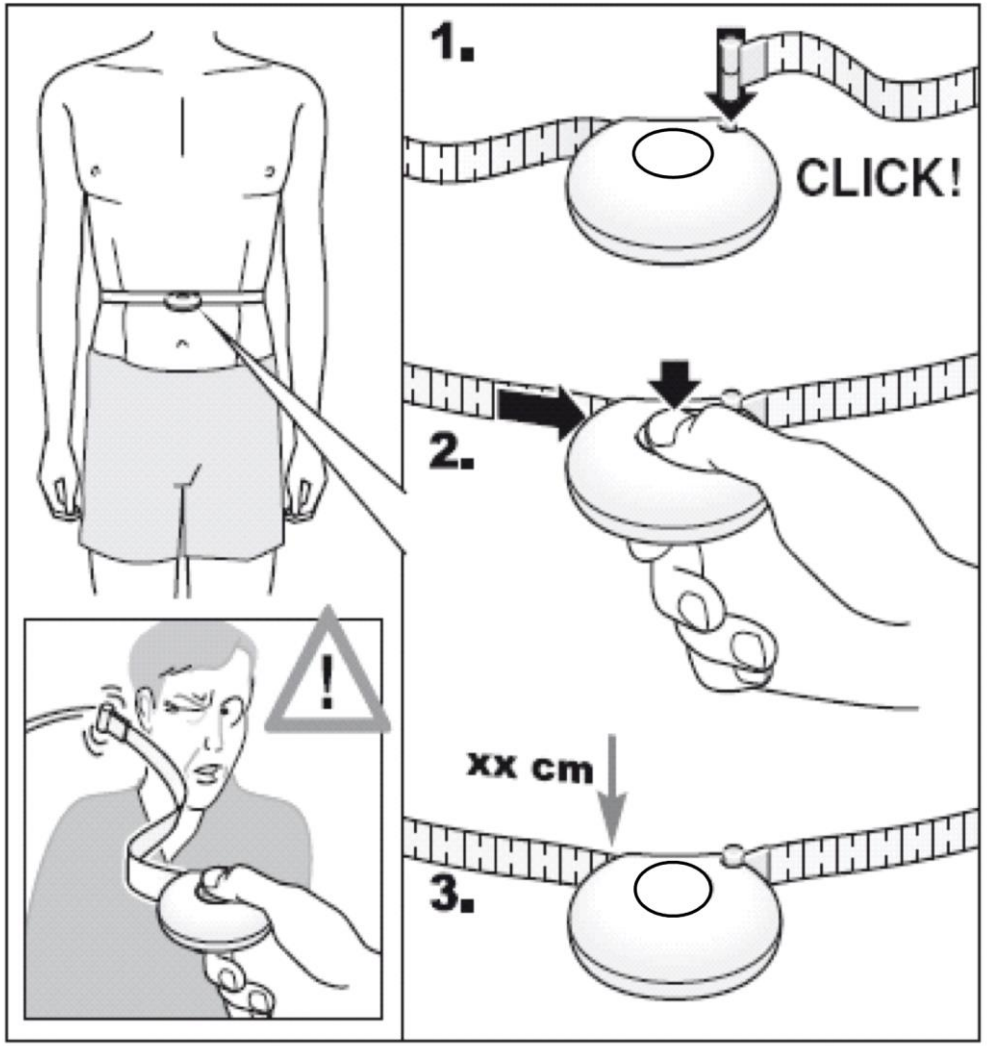


Exhibit 3-19. Waist circumference mark

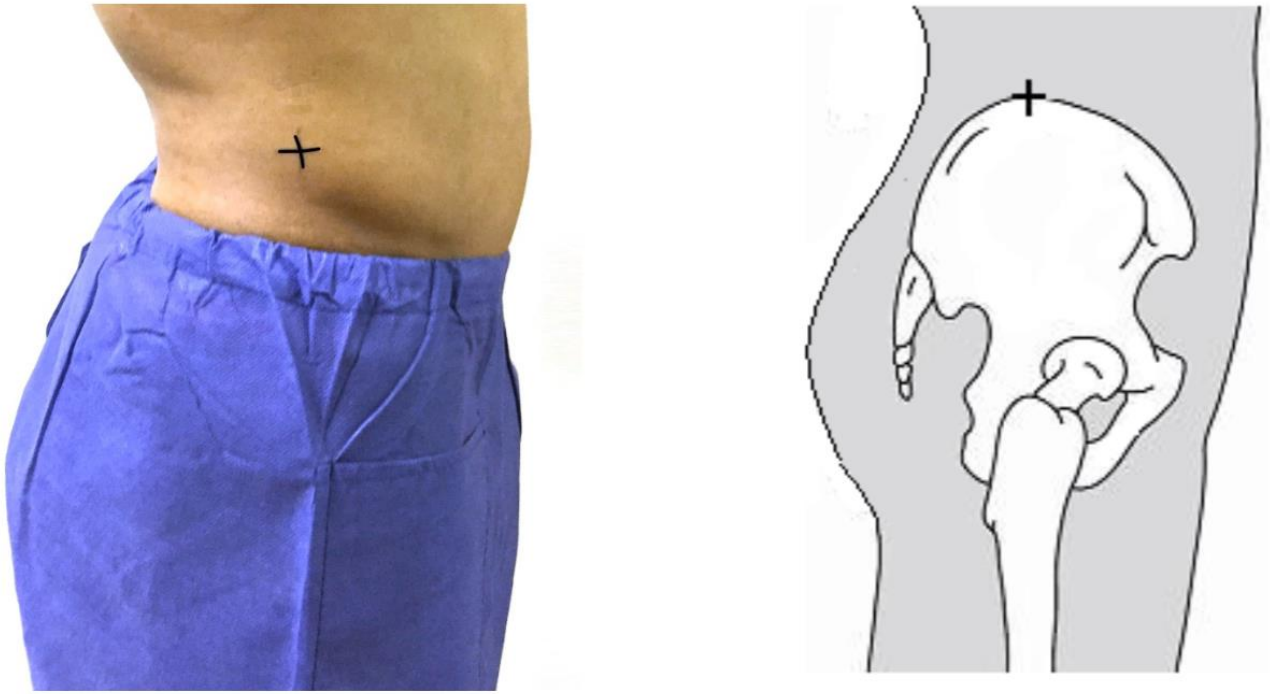
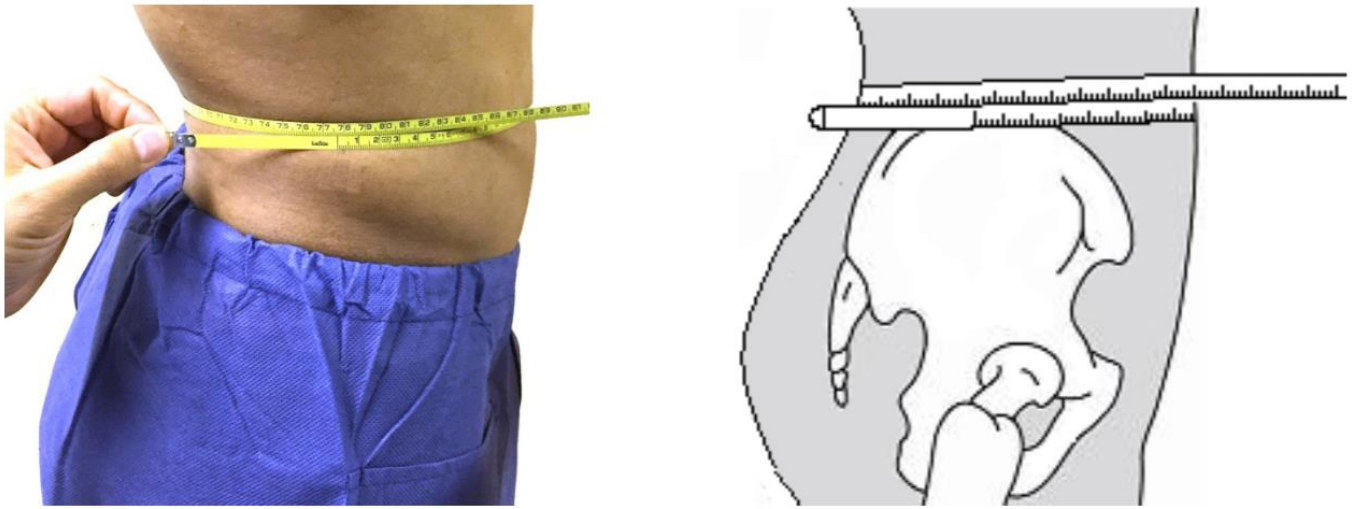
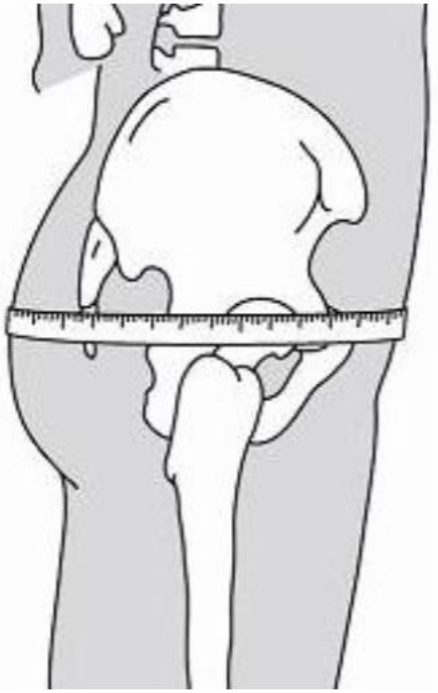


Exhibit 3-20. Measuring tape position for waist circumference



Hip circumference

Exhibit 3-21. Measuring tape position for hip (buttocks) circumference



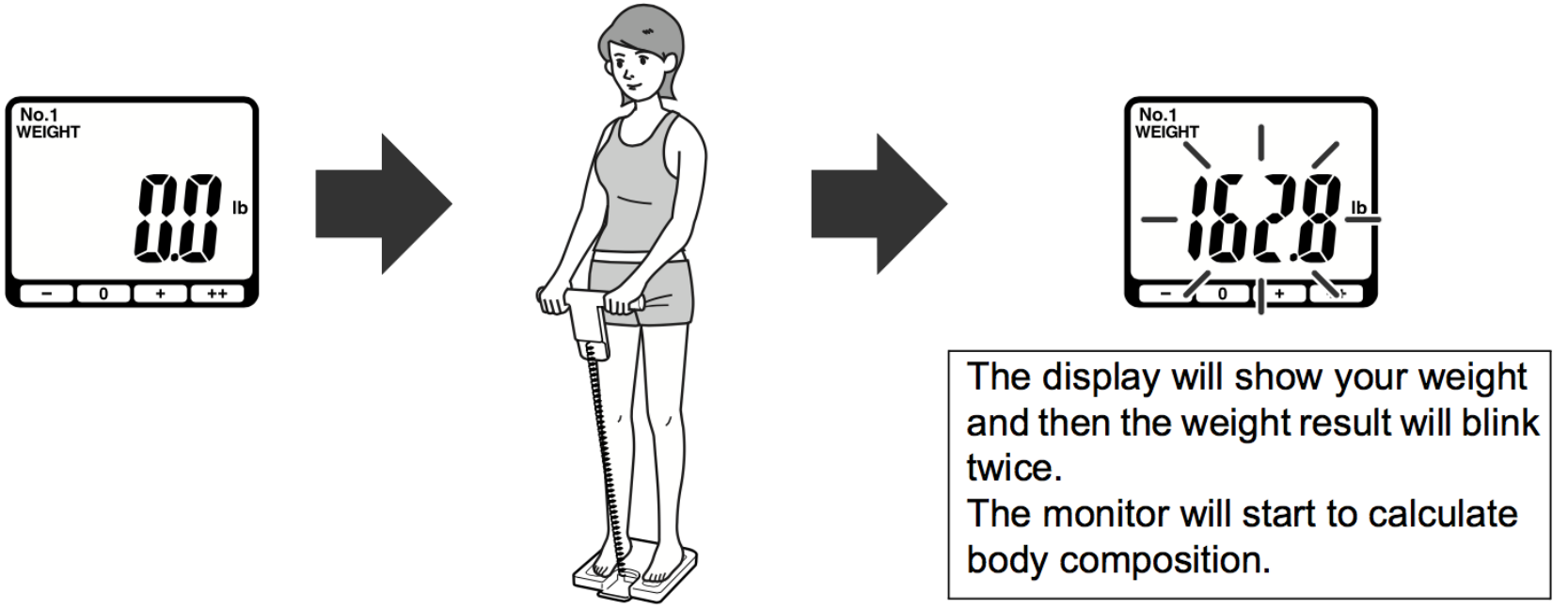
Weight and body fat percent

Respondent must remove shoes and socks

Enter the age, height and sex of the respondent.

4. Start the measurement.

1) Step onto the measurement platform and place your feet on the foot electrodes with your weight evenly distributed.
Remain still and do not move until your weight measurement is complete.

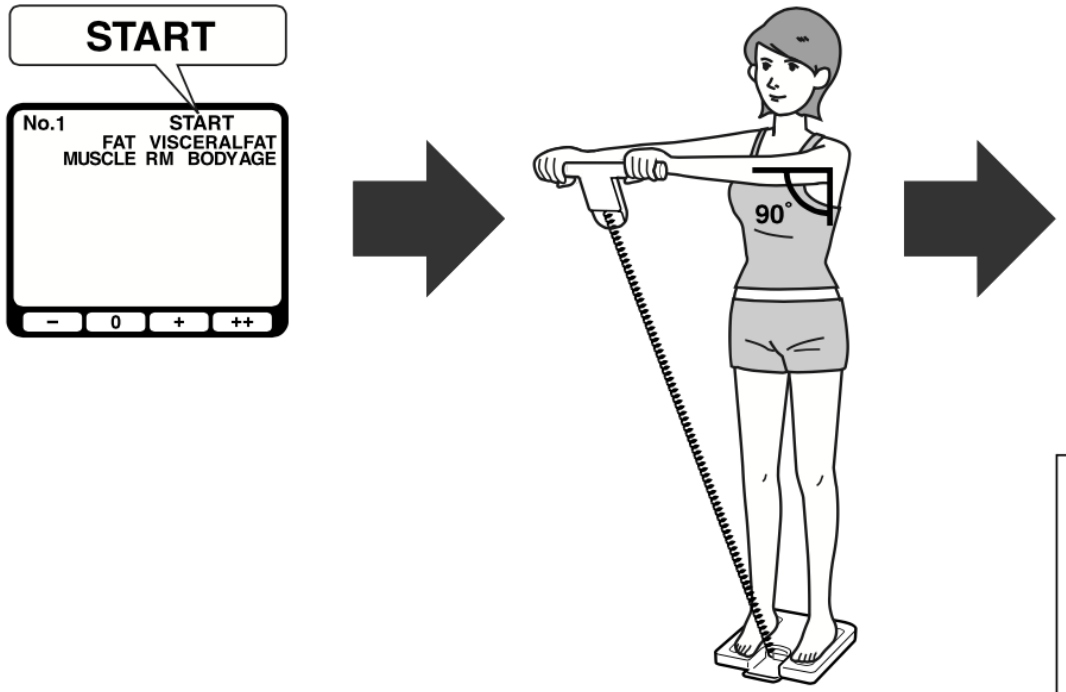


The display will show your weight and then the weight result will blink twice.
The monitor will start to calculate body composition.

Body composition

(shoes and socks off)

2) When “START” appears on the display extend your arms straight at a 90° angle to your body.

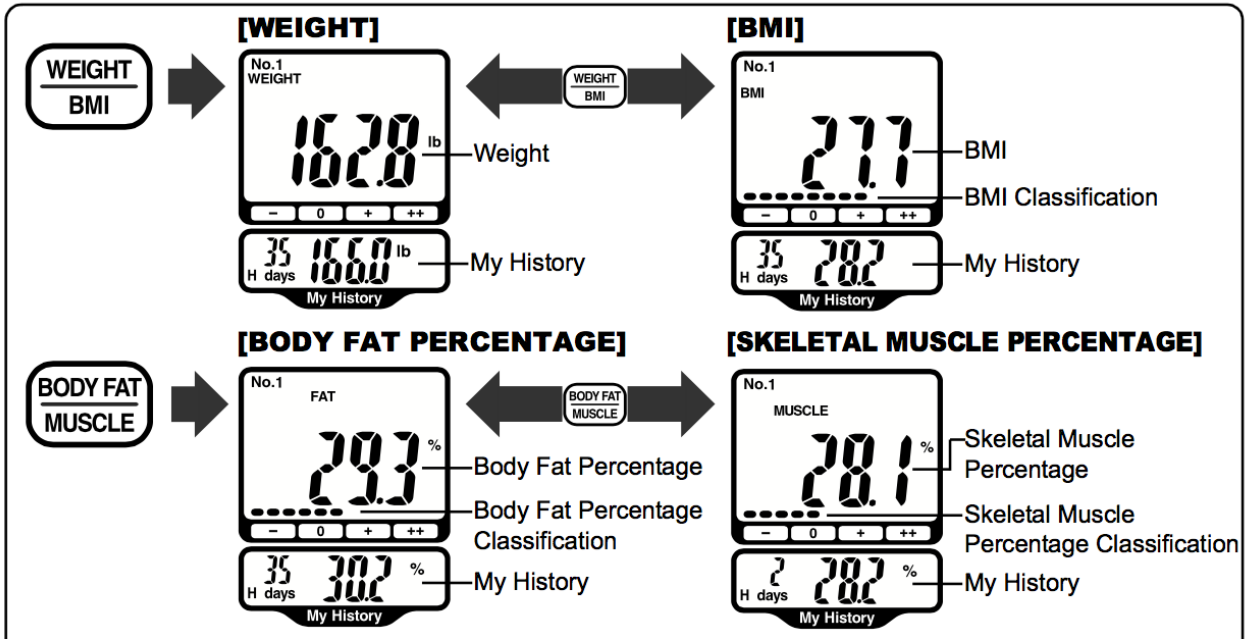


The indicators in the measurement progress bar at the bottom of the display will gradually appear from left to right, based on the measurement being taken.

We will record only weight and body fat percentage.

5. Press the appropriate button to check the measurement results.

For each measurement result, the high reading in memory for that measurement and the number of days ago for that result are shown in the My History area of the display.



Peak flow – 3 readings

- Use a new mouthpiece for each respondent
- Set indicator to zero
- Ask respondent to stand and hold meter by the handle
- Respondent should inhale deeply then seal lips tightly on the mouthpiece and blow out hard and fast
- Record the result, reset meter to zero, repeat test two more times

Grip strength – two readings on each hand

- Ask respondent which is their dominant hand; record answer
- Set hand dynanometer to MAX mode
- Ask respondent to stand with elbow bent, forearm parallel to floor
- Beginning with left hand, have respondent squeeze until instrument beeps; record result
- Reset instrument to zero.
- Test right hand, record result
- Repeat steps and record second measure for each hand

Hair sample collection

- Separate a segment of hair half the diameter of a pencil at the back of the head
- Cut as close to the scalp as possible and wrap in foil indicating which end of the hair came from the scalp
- Label the hair sample with the sample ID and put it in a plastic bag, then write the ID on the plastic bag
- Collection of hair for cortisol testing is shown here

http://gero.usc.edu/CBPH/network/resources/hair.html

and here for short hair:

https://youtu.be/cfZbW_1VDMc

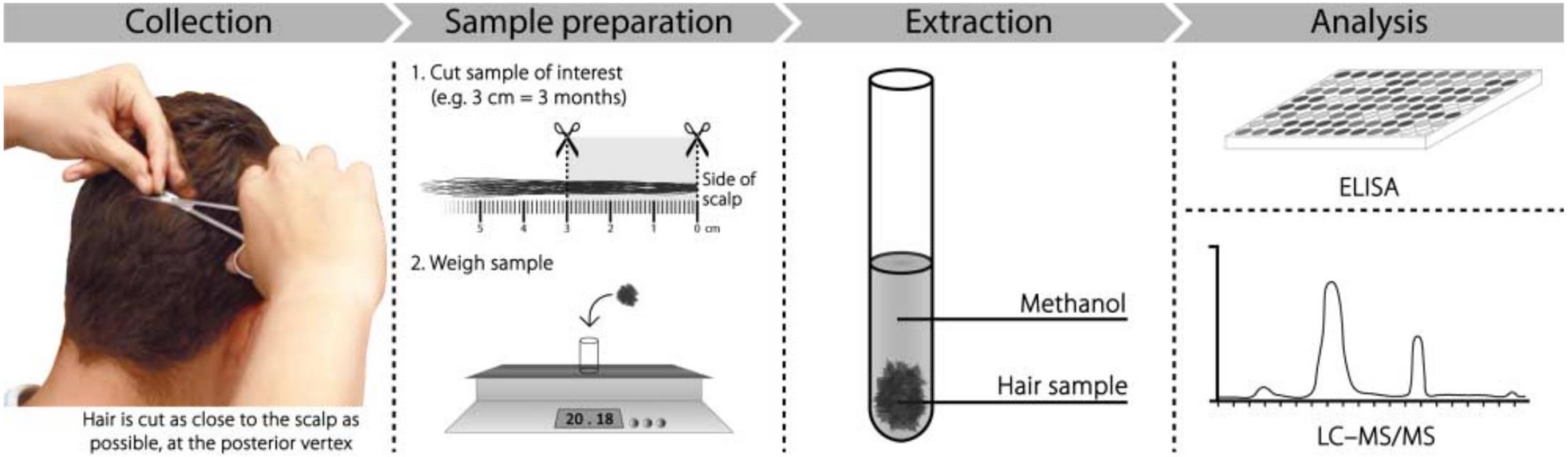


Figure 1

Overview of hair sample collection, work-up and analysis. ELISA, enzyme-linked immunosorbent assay LC–MS/MS, liquid chromatography–tandem mass spectrometry.

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