2012

Instituto Nacional de Salud Pública 2012

MEXICAN HEALTH AND AGING STUDY 2012

(MHAS 2012)

Manual of Procedures

Anthropometrics and Biological Sample

September 2012

Mexican Health and Aging Study (MHAS-II)

Survey Manager: Juan Pablo Gutiérrez (jpgutier@correo.insp.mx)

Development

Manager: Aurora Franco Núñez (afranco@correo.insp.mx)

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INTRODUCTION

The Mexican Health and Aging Study (MHAS II) is a prospective population study on the dynamics of health and aging in Mexico. The overall objective of the study is to contribute to the generation of knowledge about aging and health of older adults in Mexico.

The study includes a longitudinal[1] household survey using a nationally representative sample of people aged 50 or older, which was carried out in 2001 with a follow-up survey completed in 2003. The current MHAS fieldwork represents the third survey of the study.

Study participants are men and women aged 50 years and older, selected since 2001, and their spouses. By 2012 there will be an additional sample of new respondents. In the fieldwork of the survey, the National Institute of Statistics, Geography and Information (Instituto Nacional de Estadística Geografía e Informática, INEGI) was involved in administering the basic survey and the National Institute of Public Health (Instituto Nacional de Salud Pública, INSP) collected data on anthropometric measurements, performance measures, and blood pressure. Likewise, INSP was in charge of taking the tests *in situ* to determine hemoglobin and glycosylated hemoglobin, and to obtain a blood sample for laboratory testing of biomarkers and another biological sample to store for future genetic studies (genetic factors are associated with common diseases, such as diabetes and cardiovascular disease and to the biological aging process).

[1] A follow-up study is one in which a group of randomly selected subjects is usually

It is worth mentioning that while the INEGI administer questionnaires to all study participants, INSP will focus on a subsample of them. The subsample will be comprised of the total sample from four states.

In these states, INEGI staff will conduct the interview at home and notify respondents who agree to participate in the study that in about two weeks INSP health personnel will visit the home to carry out the a second phase of the study.

INEGI will periodically deliver to INSP the list of people who agreed to respond to the questionnaires [participants and spouses (if applicable)]. INSP staff will identify such persons who have provided informed consent and perform the following on the participants:

Anthropometric measurements (height, weight, waist and hip circumference, sitting height and sitting knee height, blood pressure)

Performance tests (balance right and left foot, gait speed and hand grip strength).

Venous blood samples

Capillary blood sample

(See Appendix III "Fieldwork Booklet"

The guidelines in this manual are established to correctly perform anthropometric measurements, performance measures, taking blood pressure and drawing blood samples.

General topics

A suitable data collection depends on the anthropometrist's fulfillment of their roles and responsibilities, the implementation of procedures established for fieldwork with the knowledge and computer skills for data collection, among other aspects.

The quality of the information obtained and the project's success is based on excellent fieldwork performance from the data collector.

Functions and responsibilities of the anthropometrist

The **main function** of the anthropometrist is to apply the established procedures for each measurement in all the selected participants. This function is achieved by taking the following steps:

Ensure the quality of the procedures performed and the complete filling out of the information except the cases where some measurements are omitted.

Use skills and strategies to prevent failure to get successful measurements.

Gather all the information required in the time allotted for each data collection locality.

If necessary, make additional visits to get complete measurements.

Be aware of all the directions provided and be punctual for all work commitments.

Support other work commissioned by the supervisor and attend meetings.

Inform the supervisor on progress as well as mishaps or difficulties that may affect the survey.

Immediately report to the supervisor any unusual or irregular situation present during the fieldwork.

Support their teammates for the conclusion of the planned activities in each household.

When for some reason data cannot be collected from a participant, the data collector should request a signature of a household member on a document including the reason the data could not be collected. The document will then be turned in to the data collection supervisor who will complete the respective review and verify.

If no one is found in the house and the neighbors or another person says that the house is uninhabited or the owners are temporarily away from the home you must indicate on a written document (can be a blank sheet of paper or logbook) specifying the name and address of the person who provided this information.

In case you need clarifications during data collection, the system has a section called “Observations” that you can open at any time to enter additional detailed information.

Moreover, the **primary responsibility** for each anthropometrist is to perform each procedure **excellent quality**. To achieve this goal, you must take into account the following principles:

Quality. The excellent quality is determined primarily by the right application of the techniques, procedures and coverage achieved in accordance with the selected sample.

Productivity. It is very important to comply with the standards of productivity in data collection. This requires collecting all measurements in the set time. Hence, there is the need to meet the workloads within the time given.

Confidentiality. You must maintain strict confidentiality of the information obtained in each household. Through the letter of consent, anthropometrists notify participants that the information obtained will not be disclosed to other people.[2]

Respect. The interviewer should show respect at all times to the traditions of the study area and to the various groups of people who inhabit it.

Another important responsibility of the anthropometrist is to **return all work materials** when fieldwork is completed, in particular the assigned laptop, as well as the equipment for each measurement (stadiometer, scale, and Hemocue, etc). Otherwise, you must submit a written justification, the administrative record, or make the respective payment.

[2] In accordance with the provisions of Article of the Law on Statistical Information and Geography, “The data and reports that individuals provide for statistical purposes or derived from administrative or civil records rerecords will be handled for purposed of this law, under the observance of the principles of confidentiality and discretion and cannot communicate, in any case, institutional or individual, nor will be used against administrative or fiscal authority, nor in or out of court.”

General Guidelines for Data Collection

Procedures for quality data collection

The collection of anthropometric measurements and biological data will be done by applying the procedures to the adults aged 50 and over selected for the study and their respective spouses. To achieve a quality data collection, it is important that the anthropometrists comply with the following:

Be aware of the survey objectives. It is common for interviewed people to ask for information about what the measurements will be used for, so before going into the field it is necessary to know the conceptual background of the project and be able to answer any questions about it.

Perform measurements with informants and enter the data carefully in the computer.

Note: It is extremely important to perform the measurements using all privacy, since the presence of other people can influence the participant and consequently, running the risk of not getting the measurements.

Show credentials as staff of the National Institute of Public Health (INSP) and carry it on a visible spot. Doing so reassures the participant's trust.

Foster a pleasant environment. There are a lot of groups who have different concepts and ways to conceive and organize their life. Along with this, there is the personality of the individual, which requires the anthropometrist's skill and sensitivity to establish an atmosphere of trust and privacy during the data collection.

All anthropometrists must **strictly follow the above procedures**. That way **homogeneity** will be achieved in fieldwork; an essential characteristic to secure **validity** and that the data obtained can be analyzed as a whole, with a high level of **accuracy and precision**.

Blood Pressure

- **Definition**

Blood pressure (BP) refers to the force that blood produces on the arteries when passing through them. Arteries are blood vessels that carry blood from the heart to the body. Blood carries oxygen and nutrients to all organs of the body so they can operate.

Blood pressure consists of two numbers:

The maximum or systolic, which is when the heart pumps blood.
The minimum or diastolic when the heart relaxes.
High blood pressure or hypertension is defined as a continuous or intermittent rise in blood pressure, either systolic or diastolic. When the blood pressure is high, it starts to damage the blood vessels, heart and kidneys. This can cause a heart attack, a stroke, kidney disease, and other problems.

- **Normal levels**

120/80 mmHg is considered a normal value in adults. However, these figures may vary depending on body type, age, and sex of the individual, so to determine a normal level, the participant should be asked what their typical blood pressure level is, as some individuals maintain low blood pressure without problems. There is hypertension when systolic pressure is greater than 140 mmHg and diastolic pressure is greater than 90 mmHg.

- **Equipment and materials**

- Electronic sphygmomanometer (OMRON)
- AA batteries
- Pen or pencil
- Registration log
- Results Card (See Appendix IV)

⇒ **Measurement technique**

- Two measurements are made.
- Prepare the sphygmomanometer, i.e., install the cuff hose to the sphygmomanometer on the left and turn on the blue **ON/OFF** button.
- The systolic and diastolic readings appear at the bottom left of the screen, when turned on 688 shows up for each measurement and 188 shows up for pulse along with the clock with the appropriate time.
- Explain to the participant the procedure you will perform to make the measurement.
- Ask the participant to sit and to put out their left arm, help the participant in case they need help.
- Ask the participant to remove rings, bracelets or watches, etc.
- The participant should be comfortably sitting in an armchair or with a table to allow the arm to be fully extended and supported during measurement.
- Locate the brachial pulse with the index and middle fingers, adjust the cuff so that the hose is not obstructed and stays along the path of the artery.
- Place the cuff around the arm, about an inch above the elbow.

- Ensure that the whole cuff is in contact with the skin but is not too tight.
- Once the cuff is properly placed, press the grey **START** button to inflate the cuff and just before the pressure in the cuff begins to decrease, the blood pressure measurement that is to be recorded will appear on the screen.
- Remove the cuff.

Note: Remember to make the second blood pressure measurement; the adult must be seated for 5 minutes prior to the measurement

Digital sphygmomanometer

Anthropometric Measures

Anthropometry

Anthropometry is a simple, portable, and inexpensive technique applicable globally to assess the size, proportions and body composition of the human body. It reflects nutritional and health status, predicts performance, health and

longevity. As such, it is a valuable tool in guiding public health policies and clinical decisions.

It is the systematic technique to measure and make observations of the human body, which describes quantitative differences in body measurements using appropriate scientific methods. The accuracy of the measurements is limited only by the limitations put forth in context in which the measurements are performed; therefore, the guidelines, measures, and indices can be seen as having a contextual character.

Anthropometry is used to collect measurements such as weight, shoe size, setting height and waist and hip circumferences, knee height, to mention a few.

General note

The anthropometric measures described below will be performed twice, if necessary for reassurance or measurement error, a third measurement may be performed.

Weight

- **Definition**

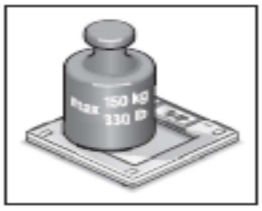
It is the result of the force of gravity (the differences in the measures could be explained by sex, age, and several other individual characteristics).

It is the most widely used measure of nutritional assessment; a measurement needed to calculate one's body mass index (BMI) and used to detect nutritional statuses, such as obesity or malnutrition.

- **Equipment and materials**
  - A standard scale
  - A 5 Kg weight
  - Paper towels
  - Pen or pencil
  - Registration log
  - Measurement log

Electronic portable scales are used with a precision of 100g and a maximum load of 150.0 Kg.

- The operation and scale calibration should be made with the help of the 5 Kg weight to ensure accurate measures.



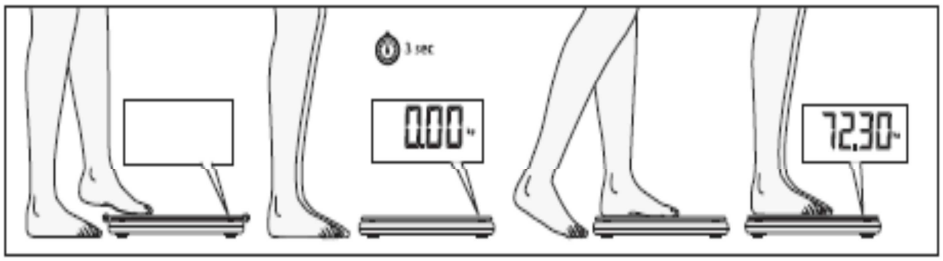
- **General notes on weight**

Explain to the participant the procedure you will perform.

Ask the participant to keep only necessary clothing on and to remove shoes and other items that may overestimate weight, such as keys, coins, thick belts, jackets or sweaters, heavy vests etc. Avoid weighing participants in heavy or wet clothes and women with long wet hair.

Measurement technique

- If wearing pants, have the participant fold the pant leg above the heel before getting on the scale, so that you can ensure the toes and heels remain on the scale
- Have the participant use the restroom before the measurement and ensure the participant did not recently have a big meal
- Ask the participant to take a step unto the scale as shown in the following image



Note

Only help participants with disabilities. If the area is poorly lit, use a flashlight to see the reading on the scale.

Height

- **Definition**

The distance taken vertically from the ground up to the vertex or highest point of the skull.

The sick or healthy status of individuals is closely related to height, which is influenced by the main factors or genetics and nutrition.

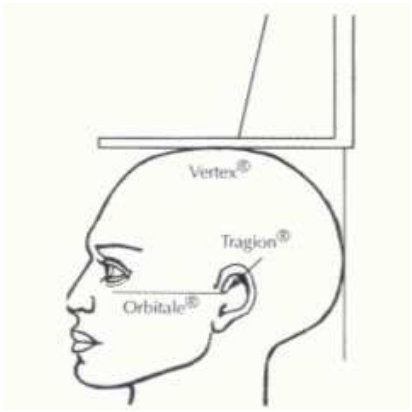
- **Equipment and materials**

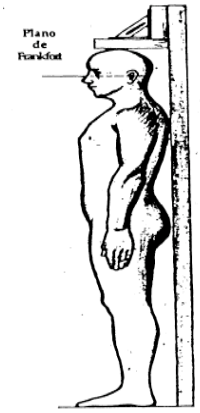
- Stadiometer
- Pen or pencil
- Disinfectant wipes
- Registration log
- Measurement log

Measurement technique

- Explain to the participant the procedure you will conduct.
- Look for a place indoors or outdoors where the floor seems to be most level and there is a 90° angle along a wall where the stadiometer will be placed.
- When attaching the strips of the graduated ruler, check that the strips are in increasing numerical order.
- Make sure that the parts of the stadiometer are securely attached and the geometric figures match, i.e., (with ☼ ☼) (with ◘ ◘) and (with ◙ ◙).
- Insert the top piece and position it so that the indicator with the side arrows is facing the numbered side of the stadiometer.

- Insert the graduated ruler to the plastic base, making sure it is completely in the slot and the numbering is toward the left.
- Have the participant remove their shoes.
- If the participant is wearing long pants, roll them up so that you can see his/her toes and heels.
- In case the participant is wearing high hair accessories, ask the participant to remove them.
- Have the participant place their back against the set up ruler with his/her arms against their sides and their heels, legs, buttocks, back, and neck directly against the stadiometer with feet slightly separated at a 45° angle without leaning.
- The midpoint line of the participant must coincide with the midline of the stadiometer.
- The head should be aligned with respect to the body, straight and against the wall, the reference point being considered the vertex (or the highest point of the skull) and chin should be centered and parallel to the ground.
- With your left hand take the participant's chin to move the head in the Frankfurt plane position (refers to an imaginary line that goes between the lower eye orbital and the medial ear cartilage, with the right hand take the top piece of the stadiometer and lower it until it reaches the top of the participant's head to form a 90° angle.





Head in Frankfurt Angle position Standing Erect Position

- Make sure alignment is correct.
- Examine the reading from by the top piece of the stadiometer as it is shown next to the arrows; the reading has to be done from the left side of the participant and horizontally for a precise measure.
- Record the measurements in the computer, registration log, and measurement log.

Example: Height 165.2=/_1_/_6_/_5_/./_2_/

Centimeters mm

- Feet must be centered in the middle of the base of the stadiometer (Figure 1)

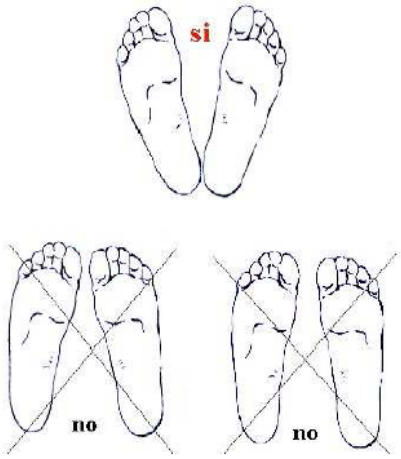
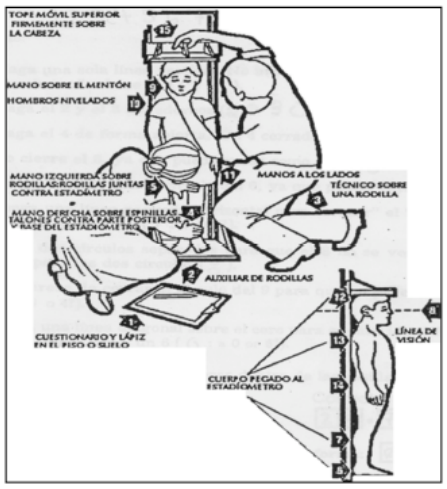


Figure 1



Correct position to measure the height

Waist Circumference

- **Definition**

The waist and hip circumference are widely used as indicators of abdominal obesity in studies on cardiovascular and metabolic risk factors. It is also clear that a large waist circumference is the best indicator of intra-abdominal fat, visceral fat, and the risk factors associated with it.

- **Equipment and supplies**
  - Fiberglass tape measure
  - Pen or pencil
  - Registration log
  - Measurement log

- **General guidelines for measuring circumference**
  - Locate and mark the anatomical reference point
  - Wrap the tape measure around the participant's waist
  - The tape should not be tight and should not be folded
  - The reading should be taken in centimeters and millimeters

Measurement technique

- The participant should be standing relaxed with bare skin showing along the waist, arms crossed and resting on the shoulders, with shoes off.
- Feel along and locate both of the participant's inferior and superior iliac crests on and the last rib and identify the midpoint between the superior iliac crest and the last rib.
- Using the tape measure, measure the midaxillary distance on the right side and again on the left side
- Once the midaxillary distance have been marked with pen in both sides, locate the measuring tape and wrap it around the waist leaving the "0" visible and ensuring there are no folds in the tape, then take the measurement. Remember the measurement should be recorded in centimeters and millimeters.
- Keep your fingers from getting between the tape and the participants waist, which can lead to false readings.
- The measurement is to be made twice for better accuracy and in case there is doubt between the first and second measurement, a third measurement can be completed to verify the result.

Hip Circumference

- **Definition**

It is an indicator, which evaluates the distribution of adipose tissue around the widest part of the buttocks

- **Equipment and supplies**
  - Fiberglass measuring tape
  - Pen or pencil
  - Registration log
  - Measurement log

Measurement technique

- The participant should stand with feet about 20 cm apart, with weight evenly distributed on both bare feet, wearing the least amount of clothing possible.
- The circumference is taken horizontally along the widest area of the buttocks.
- The measurement is made on the widest or bulkiest part of the buttocks.
- The trochanters can be used as a line of reference for the measure.
- The anthropometrist should stand so that they are able to see the widest part of the buttocks to place the measuring tape horizontally along this plane.
- The tape should fit tight on the body but not to the point of squeezing.

- The reading should be taken on the left side, to avoid being improper or making the participant uncomfortable.

Knee Height Measurement

- **Definition**

The steps taken to measure the distance between the heel to the knee in centimeters.

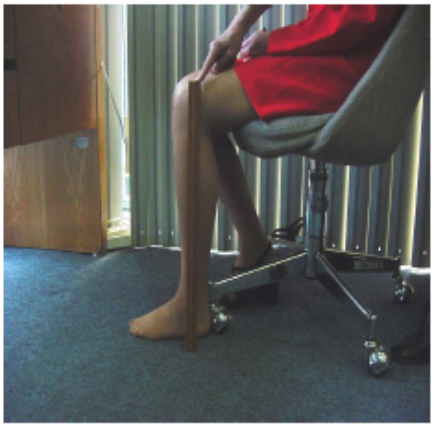
Lateral tibial plateau. It is the most proximal and lateral or external point on the proximal end of the tibia. With this measure it is the possible to determine thigh length, tibial height, mid-thigh circumference.

- **Equipment and supplies**
  - Fiberglass measuring tape
  - Pen or pencil
  - Registration log
  - Measurement log

Measurement technique

Note: Before proceeding with the measurement, you will ask the participant to uncover their leg up three finger widths above the knee. In case there is a physical impediment, help the participant with this step.

- Measure the distance between the heel and the highest part of the knee joint, on the external lateral part, with the participant's leg bent at a 90° angle between the thigh and calf.
- Standing in front of the participant, have the participant bend the knee to from a 90° angle and to sit in a comfortable position.
- The point is first located by first looking with the thumb or index finger the depression at the knee joint, surrounded by three protuberances (femoral epicondyle, anterolateral border of the tibia and fibular head); second, press down on this spot using the lateral thumb of your right hand and locate the border of the tibia and finally, palpate the towards the back until the point coinciding with the external proximal tibial plateau. This point is at least one third of the distance between the anterior and posterior points of the knee.
- Once the anatomical point has been identified, have the participant stand, while keeping the anatomical point always marked.
- To take the measurement, use the left leg if possible with the respondent sitting, with shoes off and with the knee at a right angle (in bedridden people the leg should be bent at a 90° angle).
- Measure the distance between the anatomical point located before and the point where the heel makes contact with the ground. The measure should be made with a straight line passing through the lateral malleolus.
- Round off the measure to every 0.5 cm.
- Record the measure.



Knee height measurement

Sitting Height Measurement

- **Definition**

Distance between the vertex (top of the participant's head) and the surface of the seat where the participant sits.

- **Equipment and supplies**
  - Measuring tape
  - Ruler
  - Pen or pencil
  - Registration log
  - Measurement log

Measurement technique

- It is the distance between the vertex and the lower parts of the pelvis (both ischia), which rest on the seat.
- Normally, this measure should be carried out with participants sitting in a chair with bare feet flat on the floor.
- The participant's head oriented in the Frankfort Plane Position, have them stand in the most erect position, with the upper back and back of the head firmly against the back of the chair, forming a 90° angle with the thighs.
- Record the measurement in the measurement log. Remember to use centimeters and millimeters.
- Have the participant stand up from the chair.
- This procedure is to be performed on the participant twice. Should there be any doubt between the first and second measurement, a third measurement should be made to as a confirmation.

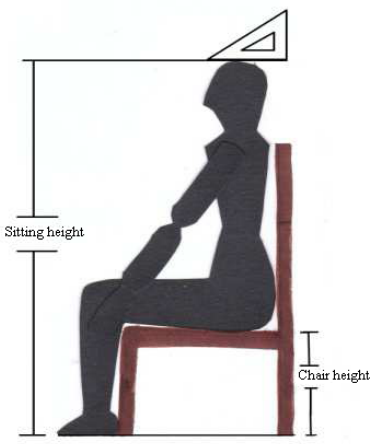


Figure for sitting height measurement

Performance Measures

The estimation of the functional capability is important in evaluating elderly people; usually this capacity is determined in terms of everyday activities such as walking, dressing and eating. Also, there are various functional tests performed like the handgrip strength, gait speed, and the ability to stand on one leg. These tests are good predictors of independence general function and generally relate to body mass and muscle mass.

It is thought that poor nutritional status and changes in body composition are associated with growing problems of balance and gait in the elderly and, therefore, with the risk of falling.

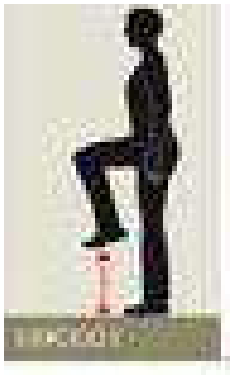
Anthropometrist: It is important that the instructions for the following tests are clear and precise so that the adult can perform them; remember that in no way should the participant feel they are being evaluated by their performance.

Instructions for the study subjects: Now we will do some exercises to measure your mobility. I will show you how to do the following exercise. I would like you to try to do it. If you think you cannot do it or it is too dangerous, please let me know.

Standing, please try to stand on one foot without support or grabbing anything. You can try on any leg and then we will try with the other one.

I will keep the time so that I can tell you when to start and stop (TEN SECONDS). We can stop at any time if you feel you are about to lose your balance.

Let's start with the leg you feel the safest with.



Recording Results

Record the time that the adult is able to remain standing on each foot. In case the participant is not able to complete the test, enter the corresponding code.

Right foot Missing or injured extremity.....94 Tried, but could not do.....95 No attempt was made to be safe.....96 Cannot stand.....97 Refused to do.....99 Passed the test within: Time..... [___|___] Seconds	[ ][ ]	**Left foot** Missing or injured extremity.....94 Tried, but could not do.....95 No attempt was made to be safe.....96 Cannot stand.....97 Refused to do.....99 Passed the test within: Time..... [___|___] Seconds	[ ][ ]

Gait Speed

Instructions for the study subjects: Now I will observe how you walk normally. If you use a cane or other device to walk, you may use it during this time.

We will find a suitable place where you can perform this exercise

- **Equipment and supplies**
  - Three meter strip
  - Stopwatch
  - Registration log

Steps:

First trial of the gait speed test.

This is the walking path. I will ask you to walk to the end of the path with your normal speed, as though walking on the street to go to the store. Show the path to the participant.

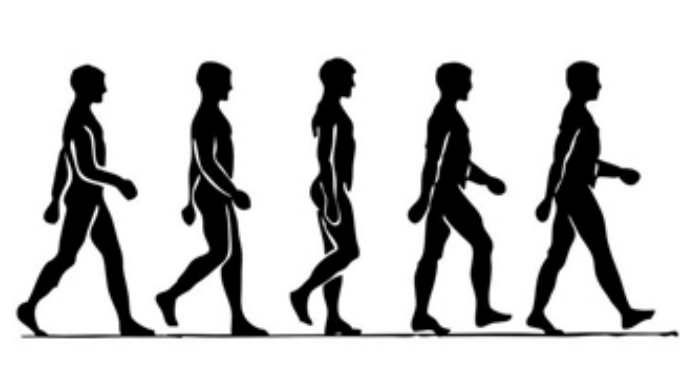
Walk all the way to pass to the other side of the tape before stopping. *Do you feel that this is safe?*

Ask the participant to stand with both feet touching the start line.

When you are ready, I will say "Ready, start." When the participant understands this instruction, say, "*Ready, start*."

Press the start button on the stopwatch while the participant starts walking. Walk behind and to the side of the participant.

Stop taking the time when one foot of the participant is completely across the finish line.



3 meters

Recording Results

Record the time that it takes the adult to cross the first foot across the end of the strip that marks the end of the three meters. In case the test was not completed, the appropriate code should be used when recording the results in question 1.18.

1.17 Time for the first test	Time to walk 3 meters [___|___] . [___|___] → **> Go to 1.19** Min. Sec. If the test was not performed, enter.....00 00	[___][___] . [___][___]
1.18 If the participant did not attempt or failed the test, indicate reason:	Tried, but could not do.....1 The participant could not maintain position without help.....2 No attempt, you did not feel safe.....3 No attempt, the did not feel safe.....4 The participant could not understand instructions.....5 Other (specify)_____.....6 Refused to do.....7 → **Go to 1.23**	[____]
1.19 Aids used during first test	None.....1 Cane .....2 Other.....7	[____]

Comments:_____
_____

B. Second trial of the Gait Speed Test

Instructions for the study subjects: Now I would like to walk the path again. Remember to walk at your normal pace, and continue until you reach the end of the path.

Ask the participant to stand with both feet touching the starting line.

When I want you to start, I will say, "Ready, start." When the participant understands this instruction, say, "*Ready, start*."

Press the start button to start the stopwatch while the participant starts walking.

Walk behind and to the side of the participant. Stop taking the time when one of the participant's foot is completely across the line.

Recording Results

Record the time that it takes the adult to cross the first foot completely across the finish line. If the test was not completed, the appropriate code should be used when recording the results in question **1.21**.

Handgrip Strength Test

Introduction

The handgrip strength affects the functions of daily living such as lifting heavy objects and this strength normally declines with age.

Instructions for the study subjects: Now we will measure your handgrip strength. I will ask you to squeeze an object as hard as you can for and couple of seconds and then release. We will perform the test on both of your hands.

- **Equipment and supplies**
  - Dynamometer
  - Registration log

This equipment is used to measure the strength of the participant in kilograms and is to be performed twice as shown in the figure below:

Instructions

Instructions for the study subjects: I will direct you on how to do it.

- Have the participant remove their rings or other jewelry.
- While the participant is using their dominant hand, adjust the dynamometer for the participant, moving it up and down, so that the bar rests between the index and ring finger.
- In a standing position, hold the dynamometer at a 90° angle and squeeze the handle for a few seconds.
- Ensure that the participant is in the correct position: standing with the arm forming a 90° angle
- Ensure that the dynamometer reads zero.
- Explain the procedure again.
- Allow the participant to practice with their dominant hand. If the participant cannot use their dominant hand, have them practice with the other hand and wait 30 seconds between each test.
- This test should be done twice on each hand.

INTERVIEWER:

Check the answer to question 1.23. If the answer is “1”, perform the test related to 1.26 and 1.27; if the answer is “2”, perform the test related to 1.27 and if the answer is “3” only perform the test related to 1.26

	FIRST MEASUREMENT		SECOND MEASUREMENT	
1.26. We will do two measurements with the left hand.	[____][____].[____] kg Tried, but could not do..... 993.0 Did not try..... 999.0	[__][__].[__] kg	[____][____].[____] kg Tried, but could not do..... 993.0 Did not try..... 999.0	[__][__].[__] kg
1.27. We will do two measurements with the right hand.	[____][____].[____] kg Tried, but could not do..... 993.0 Did not try..... 999.0	[__][__].[__]kg	[____][____].[____] kg Tried, but could not do..... 993.0 Did not try..... 999.0	[__][__].[__] kg

Obtaining Blood Samples

Capillary blood. It is transported through small capillaries, which are interposed between blood vessels and tissues to facilitate the flow of nutrients between the blood and tissues. Cold and cyanotic skin is an abnormal sign and can be avoided by massaging the skin until it becomes pink prior to the blood draw. The puncture is preferably made at the tip of the ring finger because it is more convenient and accessible. The puncture should not be done in an edematous or occluded area. The puncture should be done in a free blood flowing area to collect reproducible results that can be compared with venous blood. Once the puncture is made, rubbing or squeezing the puncture area should be avoided to prevent measurement error.

Equipment and general supplies

- Sanitary wipes
- Gloves
- Cotton balls
- Cotton balls soaked with alcohol and dry cotton balls
- Lancet holder (Softclix)
- Lancets
- Sharps waste container
- Red toxic waste bags (for cotton balls and gloves)
- Black waste bags (for glove packaging, sanitary wipes, etc.)

Glycosylated Hemoglobin Test

- **Definition**

Hemoglobin is a protein that carries red blood cells o RBCs. Blood sugar binds to hemoglobin to form hemoglobin A1 (glycosylated hemoglobin). If the blood contains more sugar, glucosylated hemoglobin rises and stays increased for 120 days. Therefore, the measurement of glycosylated hemoglobin reflects the highs and lows of blood glucose for the past 12 weeks. Hemoglobin A1 provides an average blood sugar level of recent months, while a test for blood sugar (glucose) only indicates the status of diabetes control at a certain point in time.

- **Specific supplies**
  - A1CNOW equipment consisting of:
  - A1CNOW Monitor
  - Package 1: Sample dilution kit
  - Pasteur micropipette
  - Blood collection device
  - Cryotube with solution
  - Cryotube stand
  - Package 2: Test cartridge
  - Cartridge

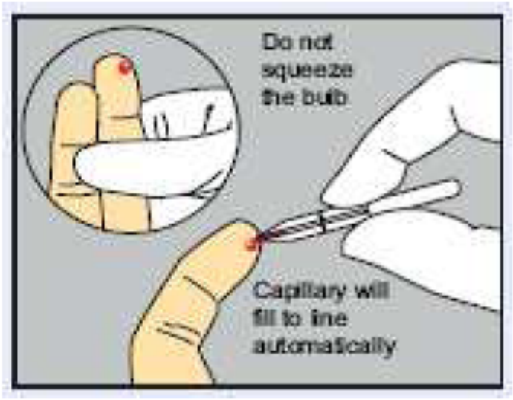
Measurement technique

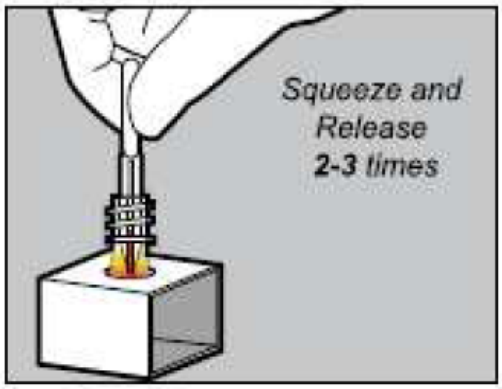
- Place the materials and the A1CNOW equipment so that the sanitary wipes are accessible and on a safe and secure surface.

- Assemble the stand for the cryotube and place it close to the A1CNOW.
- Grab the pipette that will be used to collect the drop.
- Remove the cap from the lancet holder and place the lancet in and press down, replace the cover to the lancet holder and press down until you hear a click.
- In case the puncture site is cold, have the participant rub their fingertips together to warm them; this is to obtain a sufficient blood sample.
- Cleaning the puncture site. Asepsis is performed with a cotton ball soaked in alcohol on the lateral side of the fingertip of the middle or ring finger, preferably on the hand used the least with the purpose to clean the skin surface, remove dead skin, and increase the amount of blood flow to this part of the finger.
- Let the alcohol around the puncture site to dry. Make sure that the puncture site is completely dry, otherwise a blood droplet will not form when it comes through the skin
- Place the lancet on the lateral side of fingertip where the puncture will be made and apply pressure until the lancet is activated, wait for about 2 to 3 seconds before removing the lancet from the puncture site.
- Turn the hand over to facilitate the blood drop to be released without touching or squeezing the finger to prevent altering the composition of the blood sample.
- This drop will be absorbed by placing the pipette so that the tip absorbs the blood drop without touching the skin but ensuring the most blood as possible is absorbed, so that the pipette is filled to the black line and there are no air bubbles for optimal results. If there are air bubbles, the procedure will need to be repeated.

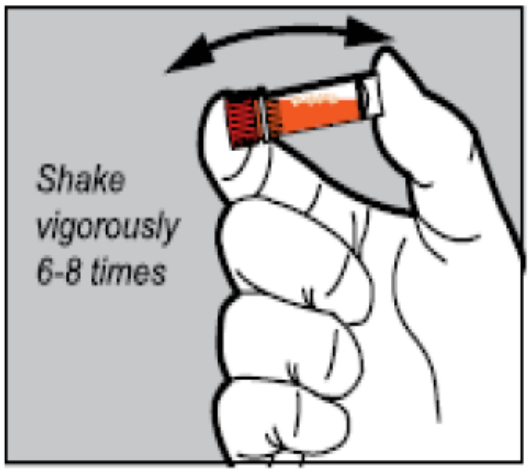
- With a dry cotton ball carefully clean excess blood located around the pipette.
- Insert the pipette into the cryotube and with one single movement, let the drop of blood fall into the tube, remove the pipette, close the cryotube and shake the cryotube vigorously 6-8 times to mix the blood with the solution.
- Once the content in the cryotube have been mixed, place the cryotube in the base and carefully remove the lid.
- Immediately insert the cartridge into the A1CNOW device.
- With the second pipette, proceed to extract the substance, immediately transfer the solution in the A1CNOW device and squeeze the pipette into the white circle located in the center of the cartridge. Remember that at all times, the pipette must be filled to the black line without bubbles. Bubbles can cause measurement error, and the procedure will need to be repeated if they are present in the pipette.
- You should remember that the equipment only has ten reagents; therefore you should use caution necessary to avoid wasting this material.

Note: The equipment has to be kept in room temperature of 18-28C°. In case the temperature is lower, warm the cartridge or store it in a warm place to activate the reagent. If the temperature is higher, a cooler and freezing gel should be used to maintain the appropriate temperature.





Draw the capillary blood sample. Place the sample in the cryotube.



Shake vigorously for 6-8 times. Insert the cartridge and incorporate the sample.

Hemoglobin Test

- **Definition**

Hemoglobin is the main component of red blood cells, from 31% to 34%, is a **protein** in the **blood** that is responsible for transporting **oxygen** from the respiratory organs to tissues. Hemoglobin can be identified as a **heteroprotein** since it is a conjugated protein (combines globular protein subunits and a non-protein heme or prosthetic group)

The Hemocue technique is widely used in field studies, to facilitate in on-site measurements without the need to prepare and store samples. It is very reproducible and the precision and accuracy are very good compared with other hemoglobin measurements made in the laboratory using cytometric flow methods. The hemoglobin concentration is obtained in units of gr/dl.

- **Specific supplies**
  - Hemocue
  - AA batteries
  - Flashlight
  - Reactive microtray
  - Registration log
  - Measurement log

Measurement technique

- Place the materials and the Hemocue within reach of sanitary wipes, all of which should be on a safe and secure surface.

- Show the participant that the equipment you will use is clean and the lancets are new and haven't been used before.
- Load the lancet holder. Uncover the tip of the lancet holder and place a lancet into the holder and press to insert it securely, then replace the cover of the lancet holder and press down until you hear a click.
- Cleaning the puncture site. Asepsis is performed rubbing an alcohol soaked cotton swab over the lateral side of fingertip of the middle or ring finger on either hand to remove dirt, dead skin, and increase the amount of blood flow to this part of the finger.
- Allow the alcohol to dry. Ensure that the puncture site is completely dry, waiting for a few seconds. If the alcohol is not dry, a blood drop will not be able to form.
- Puncture technique. Place the lancet on the area chosen for puncture; the activation of the lancet will be automatic when pressed against the finger.
- The first drop of blood. Turn the hand over to facilitate the blood drop to be released without touching or squeezing the finger to prevent altering the composition of the blood sample.
- Place the second drop of blood in the reactive microtray used to measure hemoglobin in the Hemocue. This drop will be absorbed by placing the tip of the microtray over the drop of blood without touching the skin and within distance from the finger, then collect the blood on the appropriate part of the microtray, ensuring the amount on the microtray is sufficient and no air bubbles are present to obtain an optimal sample.
- With a dry cotton ball, clean any excess blood on the microtray.

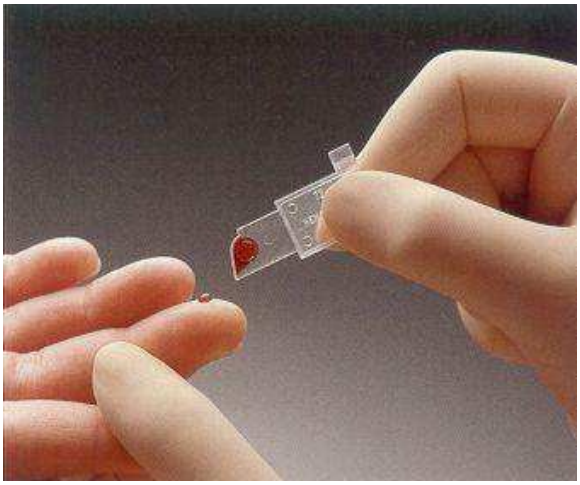
- Insert the microtray into the Hemocue (with the button on the top left of the display turned on before), place the microtray in the slot to hold the microtray in the Hemocue and push inward to obtain the hemoglobin measurement. To avoid faulty measurements, the microtray should remain in position until the result appears on the display, which can take 15-45 seconds.
- Risk of hemoglobin results form. The hemoglobin result that appears on the digital screen of the Hemocue must be registered by the data collector in the registration section of biological measurements.
- Remove the microtray from the slot and turn of the Hemocue.
- Discard the lancet, used cotton balls, gloves and the microtray that was used for the hemoglobin test in the special container intended for each one of them.

Precautions for the hemoglobin test

- Once the puncture is made the microtray is to be brought out, one at a time.
- Never leave the Hemocue container for the microtrays uncovered.
- Never touch the reactive part of the microtray with your fingers.
- When the Hemocue tube has been opened, the date should be written on it and it should be used within 90 days of being opened.
- Do not uncover a Hemocue tube until the previous one has been fully used.
- On a weekly basis clean the display area of the equipment with a cotton ball dipped in alcohol or distilled water, taking care not to leave lint or

debris of any sort, let all the parts dry, replace the microtray holder into the apparatus once the area has dried.

When the three straight lines appear on the meter it indicates that it is in the process of gathering a result.



Microtray blood collection

Place the microtray into the slot to be read

The result will appear after 15 to 45 seconds

Blood Sample Draw

Introduction

Determining the health status of older adults should include laboratory measurements. In this study the following measurement were considered:

Total Cholesterol and High-Density Lipoprotein Cholesterol (HDL). HDL is the so-called good cholesterol because of its “protection” factor of the cardiovascular system.

C-Reactive Protein (CRP). A biomarker of the immune system, most commonly used to measure inflammation and infection. Also has been widely used to study the association CRP with atherosclerosis, type 2 diabetes and cardiovascular disease.

Vitamin D. Besides being an indicator of bone health, in recent years, vitamin D deficiency has been linked to some types of cancer, muscle function and balance among others.

Thyroid Stimulating Hormone (TSH). The most common form of thyroid dysfunction in the elderly is subclinical hyperthyroidism. The potential risks of subclinical hypothyroidism in the elderly include progression to clinical hyperthyroidism, cardiovascular effects, hyperlipidemias, and neurological effects.

Moreover, obtaining an adequate blood sample allows successful analysis of it, so it is very important to perform this procedure as safe, fast, and easy as possible. It is important to ensure the quality of the sample that is collected, that it’s from the right source, and that the procedures of preparation and

storage are strictly observed (setting, centrifuging, pipetting and labeling) and to be preserved properly until it reaches the laboratory, where they will make determinations.

Personnel carrying out this procedure should be aware that the success of the assignment depends on their knowledge, proper dealing with the subjects of study and the ability to perform the job.

Equipment and material

- Latex tourniquet
- Latex gloves
- Vacutainer tube with red top
- Vacutainer needle
- Vacutainer green butterfly needle
- Vacutainer blue butterfly needle
- Cotton ball container
- Cotton balls with and without alcohol
- Vacuum bottle
- Test tube rack
- Congealing gel
- Small sharps disposal container
- Large sharps disposal container
- Black waste bag
- Red biohazard bag
- Labels (blank or printed) for sample identification
- Permanent marker
- Registration logbook

Technique for Venipuncture

- The individual does not necessarily need to fast when the sample is taken, i.e. the sample is drawn at the time the adult is visited.
- Select a suitable, comfortable area, with good lighting inside the home to draw the sample.
- Explain to the individual the procedure that will be performed so he/she will feel safer.
- The individual should remain in sitting position so that he/she feels comfortable and is accessible for the nurse to draw the sample.
- Prepare the workspace and prepare the material necessary to draw the sample, indicate that the material is new, completely sterile and show the individual the needle as the cap is removed.
- Ask the individual to show both of their forearms to check what vein and arm are suitable for the blood draw (see Figure 1), part of the body where it is recommended to obtain the blood sample.

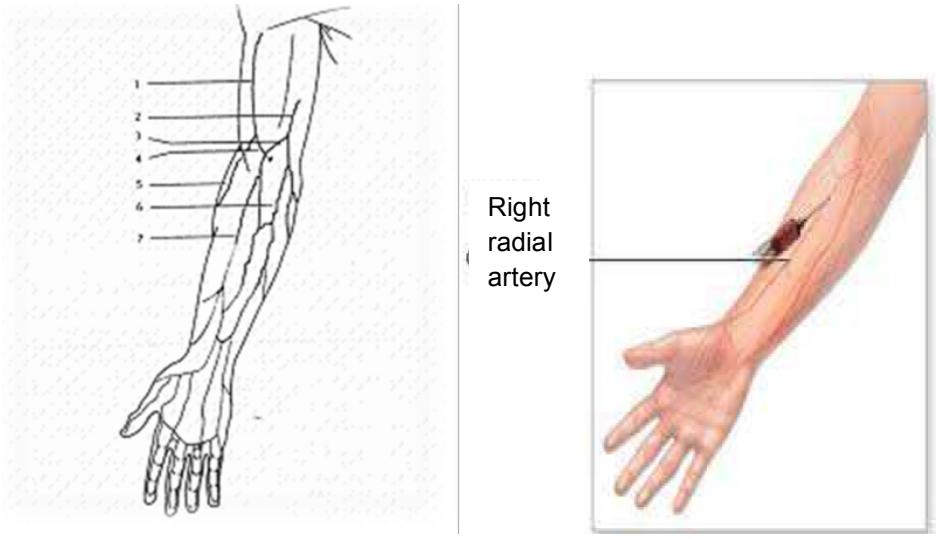


Figure 1. Superficial veins of the arm:

1. Cephalic, 2. Basilic, 3. Median Basilic, 4. Median Cephalic, 5. Radial Accessory, 6. Superficial Cubital and 7. Radial Superficial

- Must feel with the index and middle finger to check the thickness and path of the vein.
- After selecting the puncture site, apply a tourniquet 7 to 8 centimeters (about 3 inches) above the elbow crease. Do not leave the tourniquet on for more than three minutes (this causes hemolysis) and request that the individual close their fist to make veins more visible.
- The area of puncture must be aseptic and must be done from the center to the periphery and from top to bottom, by rubbing the arm with a cotton ball soaked in alcohol. Do not touch the area and let it dry.
- Exert pressure over the vein using the thumb and index finger, distending the skin at the puncture site.
- Venipuncture is performed using the vacutainer system and penetrates through the skin with the needle at an angle of approximately 15º to 30º from the arm and with the bevel up, following the direction of the vein. The tube that will hold the sample is inserted into the vacutainer, when the tube begins to be filled with blood, the tourniquet is removed. Wait until the tube is filled (6 mL of blood).

Note: This step is important because we need a good decision for non-hemolyzed serum. Remember at all times to choose the needle according to

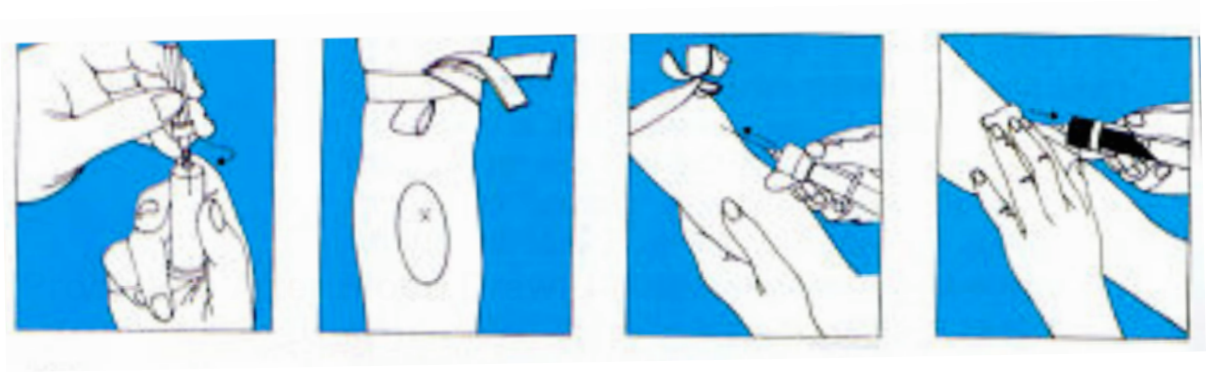
the thickness of the selected vein, also if the individual accepted the DNA sampling, a second tube must be used to draw a second sample.

- Once the procedure is complete, have the individual relax his/her fist and remove the tube.
- Place a dry swab on the puncture site while the needle is gently removed (the swab should remain over the site of venipuncture between one to three minutes).
- Slightly turn the sample upside down 3 to 5 times.
- Must properly label the tube with the corresponding number of the individual.
- You must be aware of the state of the individual at all times. Sometimes the individual can get dizzy at the sight of blood or when the needle is removed, etc.

General Precautions

- The material should always be within reach.
- You must be aware of the individual so that he/she does not move, stand or faint, as these events could cause accidents such as:
- The needle breaks
- The needle comes out and there is bleeding
- A hematoma will form
- Ask the family that no young children be around because they may run into us and disrupt the procedure.

Figure 2. Example of Venous Blood Collection with Vacutainer®



a) Prepare of the necessary equiment.

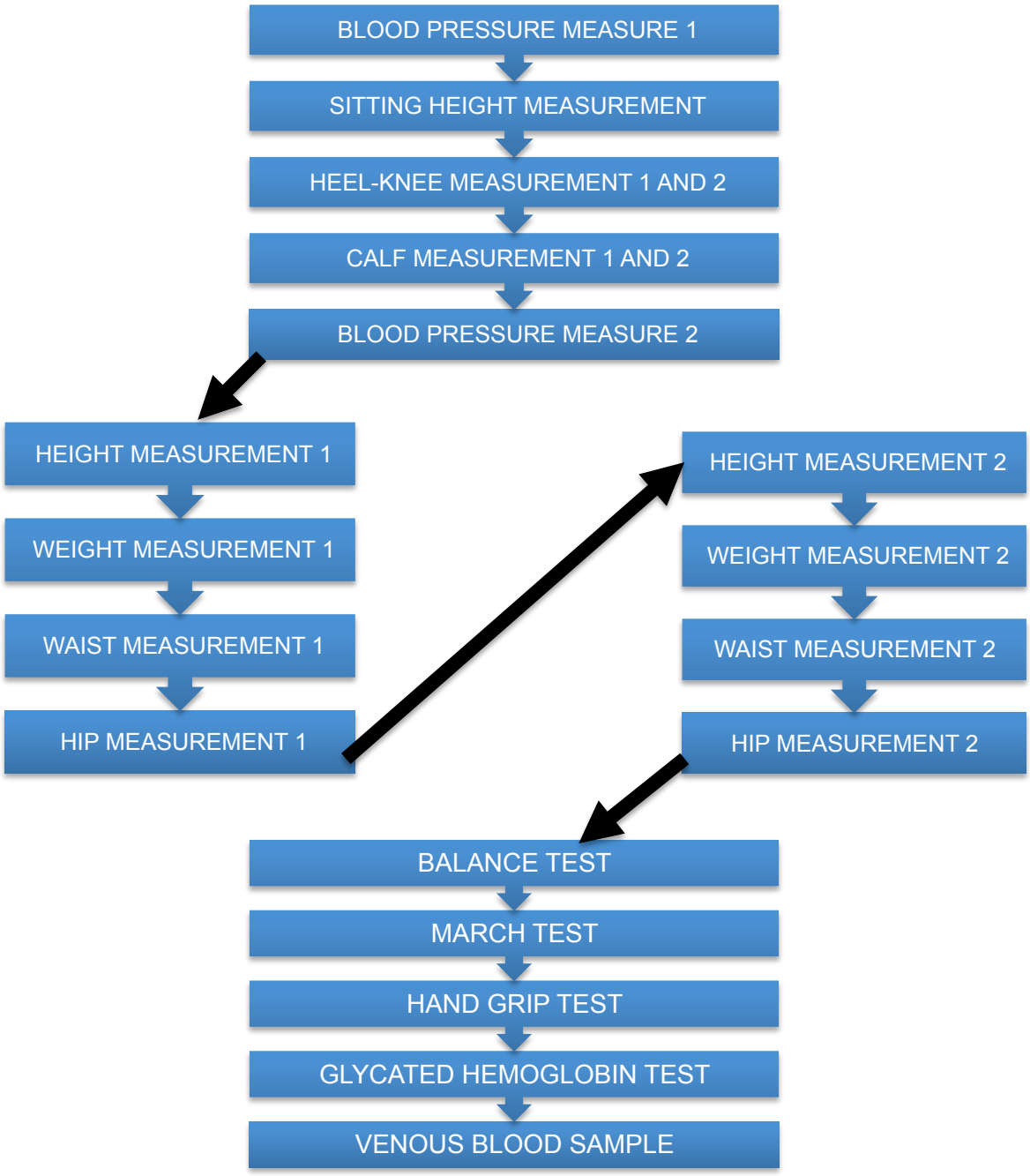
b) Clean the puncture site and apply a tourniquet.

c) Puncture the vein.

d) Obtain the blood and remove the needle.

- The whole blood sample has to be stored at a temperature of 2º to 8º C.
- All collected samples should be centrifuged before 30 minutes and at 2500 RPM for 15 to 20 minutes.
- With this, only the serum from the tube will be obtained, you must check that the serum is not hemolyzed.
- The serum obtained is separated into two measures of 2 ml., each one.
- The hemolyzed blood is to be discarded in the big sharps container.
- Once separated, you must put the serum in the nitrogen tank.
- The nitrogen tank should not be opened continuously since this will cause the nitrogen to leak out.
- When the nitrogen tank is filled to its maximum capacity, it should be transported to the National Institute of Public Health in Cuernavaca or the designated central office as indicated.

Flowchart of Anthropometrics



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Appendix I. Biomarkers

I. Description of samples

A total of 2,016 blood samples, distributed in four states, were analyzed. The raw database with biomarker results includes subjects that are part of the sub-sample previously interviewed by the **Instituto Nacional de Estadística Geografía e Informática (INEGI),** but also includes 13 subjects that only completed blood samples and are not part of the sample interviewed by INEGI.

Table 1.1 includes the household (CUNICAH), sub-household (SUBHOG_12), and individual (NP) identifiers of subjects who completed blood samples but not the complete MHAS interview.

Table 1.1. Subjects who only completed with blood sample

Household ID: CUNICAH (=UNHHID)	Sub-Household ID: SUBHOG_12	Individual ID: NP
3567	1	10
7992	1	20
7995	1	10
8015	1	10
9506	1	20
9513	11	11
10771	11	11
10801	11	10
10943	1	10
13042	0	10
13107	0	10
13110	0	10
14624	0	10

The following descriptive results include the total sample, including these 13 subjects, however they are not included in the final, public data file on the MHAS website www.MHASweb.org.

Table 1.2 shows the distribution of the 13 samples according to condition observed of each sample before they were analyzed.

Table 1.2 Distribution of samples according to blood sample condition

Type of sample	Not Part of MHAS Sample		MHAS Sample		Total	
	Freq	%	Freq	%	Freq	%
Without observations	11	84.62	1,571	78.43	1,582	78.47
Lipemic	2	15.38	384	19.17	386	19.15
Slightly lipemic	0	0.00	1	0.05	1	0.05
Hemolyzed	0	0.00	37	1.85	37	1.84
Slightly hemolyzed	0	0.00	10	0.50	10	0.50
Total	**13**	**100.00**	**2,003**	**100.00**	**2,016**	**100.00**

Advice was requested from INSP, regarding the inclusion of both lipemic and hemolyzed samples in the overall analyses. The following is their opinion regarding these samples:

1. The number of hemolyzed samples is relatively small and does not represent a serious problem. Since the hemolyzed sample are outliers, and represent a small number in such a large sample (less than 2%), it is recommended to consider including the total sample in the analyses.

2. Regarding the analysis of total cholesterol and HDL, it is important to consider that the lipemic serum is a result of hyperlipidemia or inadequate fasting conditions, but it is difficult to establish the actual reason. Furthermore, since total cholesterol and HDL are biomarkers

that can also be measured in non-fasting conditions, lipemic samples can be included in the overall analyses but results must be analyzed with caution.

II. Total Cholesterol (TC) Results

For 2,016 samples analyzed, the average Total Cholesterol was 200.6 mg/dL.

Biomarker	N	Mean	SD	Minimum	Maximum
CHOLESTEROL_mg_dL	2,016	200.65	46.68	78.00	528.00

The *NORMA Oficial Mexicana* (Official Mexican Standard) NOM-037-SSA2-2002[3] establishes the following cut-off points for the assessment of total cholesterol (TC) as a marker in the prevention, treatment and control of dyslipidemia.

	Recommended	Borderline	High risk
TC mg/dL	**< 200**	**200-239**	**≥ 240**

Table 2.1 shows the distribution of total cholesterol using the cut-off points mentioned before. The results indicate that 57.6% of analyzed individuals have recommended total cholesterol levels, 27% have borderline levels, and 15.3% have high-risk values.

Table 2.1 Total Cholesterol (TC) mg/dL

	Frequency	Percentage
< 200	1,161	57.6
200 a 239	546	27.1
≥ 240	309	15.3
Total	2,016	100.0

The results shown in table 2.2 indicate that *State 2* has the highest percentage of people with high-risk total cholesterol levels (19.4%).

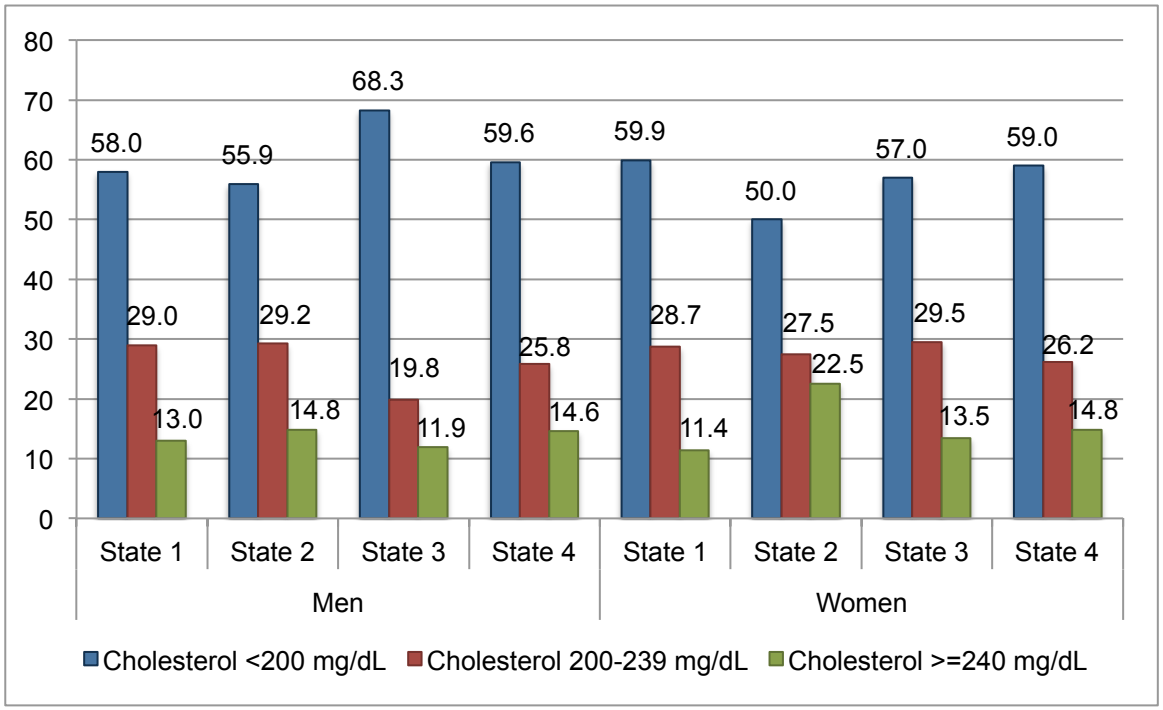
[3] http://www.salud.gob.mx/unidades/cdi/nom/037ssa202.html

Table 2.2 Total Cholesterol Results by State[4]

State	< 200 mg/dL		200 to 239 mg/dL		≥ 240 mg/dL		Total
	Fr	%	Fr	%	Fr	%	
State 1	201	59.1	98	28.8	41	12.1	340
State 2	305	52.4	164	28.2	113	19.4	582
State 3	196	61.4	82	25.7	41	12.9	319
State 4	459	59.2	202	26.1	114	14.7	775
Total	**1,161**	**57.6**	**546**	**27.1**	**309**	**15.3**	**2,016**

In Figure 2.1, the results by state and gender indicate that the percentage of men and women with high-risk cholesterol levels is similar in three of the four states. In *State 2* however, an 8.1 percent difference for women is observed compared to men.

Figure 2.1 Total Cholesterol Results by Sex and State



[4] The name of the 4 states was removed to protect the identity of the respondents.

The descriptive statistics by age group in Table 2.3 indicate that the **youngest cohort** has a higher percentage of people within the recommended levels of cholesterol**.** Moreover, the percentage of people with high-risk cholesterol level among the youngest cohort is 9.1%, this percentage almost doubles among the group aged 51 to 60 years (17.8%), and is also higher for the cohort aged 61 and older (14.5%).

Table 2.3 Total Cholesterol Results by Age Group

Cholesterol mg/dL	Age			Total
	≤ 50	51 to 60	61 and older	
	%	%	%	%
< 200	64.5	51.9	60.7	57.7
200 to 239	26.4	30.3	24.8	27.1
≥ 240	9.1	17.8	14.5	15.3
Total	**100.0**	**100.0**	**100.0**	**100.0**

III. High-Density Lipoprotein Cholesterol (HDL) Results

For the 2,016 analyzed samples, the average HDL was 41.20 mg/dL. Unlike previous values, higher levels of HDL are beneficial and lower levels are detrimental for health.

Biomarker	N	Mean	SD	Minimum	Maximum
HDL mg/dL	2,016	41.20	10.40	17.00	92.00

For the prevention, treatment and control of dyslipidemia, the *NORMA Oficial Mexicana* (Official Mexican Standard) NOM-037-SSA2-2002 establishes the following high-density lipoprotein (HDL) cut-off points.

	Recommended	High risk
HDL mg/dL	**> 35**	**≤ 35**

The results shown in Table 3.1 indicate that *State 3* has the highest percentage of people (37.0%) at high-risk based on the HDL cut-off points.

Table 3.1 HDL Cholesterol Results by State[5]

State	> 35 mg/dL		≤ 35 mg/dL		Total
	Fr	%	Fr	%	
State 1	232	68.2	108	31.8	340
State 2	417	71.6	165	28.4	582
State 3	201	63.0	118	37.0	319
State 4	539	69.5	236	30.5	775
Total	**1,389**	**68.9**	**627**	**31.1**	**2,016**

[5] The name of the 4 states was removed to protect the identity of the respondents.

Figure 3.1 indicates that the percentage of people with high-risk levels of HDL is higher for men compared to women. In *State 1*, the difference is 20.9 percentage points while the remaining states the difference ranges between 11.9 and 13.6 percentage points.

Figure 3.1 HDL Cholesterol Results by Gender and State

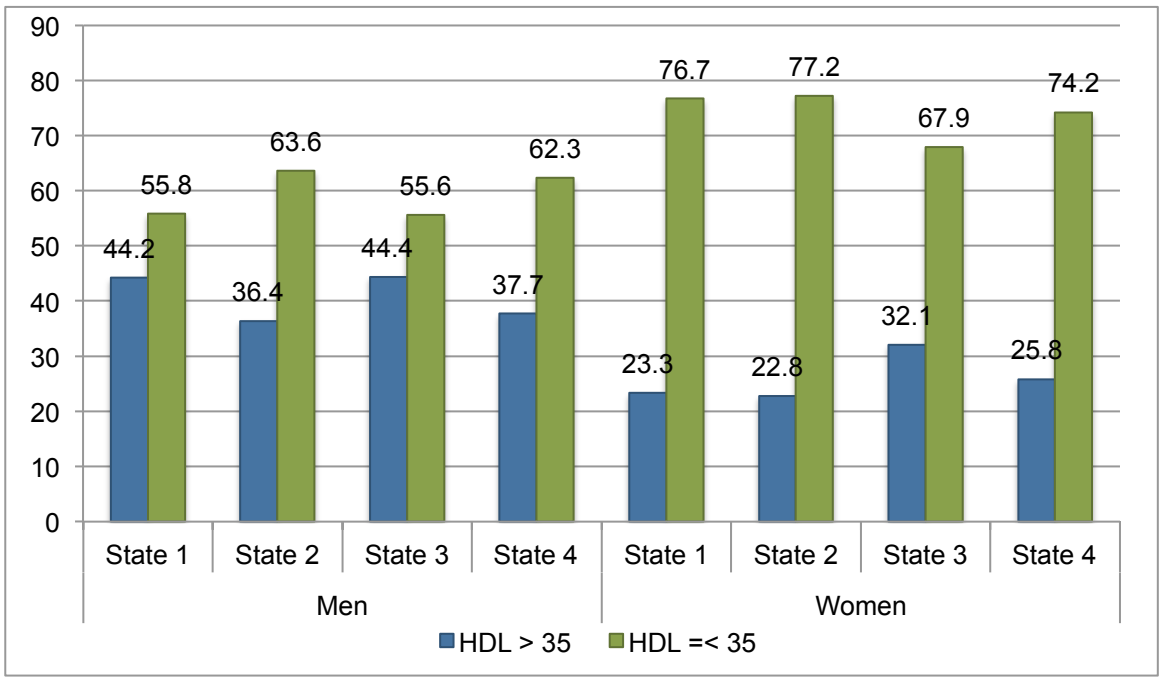


Table 3.2 includes the results of HDL levels by age group. The results indicate that the highest percentage (32.5%) of people with high-risk levels of HDL is among people aged 60 and over.

Table 3.2 HDL Cholesterol Results by Age Group

HDL mg/dL	Age			Total
	≤ 50	51 to 60	60 and older	
≤ 35	29.9	29.7	32.5	31.1
> 35	70.1	70.3	67.5	68.9
Total	**100.0**	**100.0**	**100.0**	**100.0**

IV. C-Reactive Protein (CRP) Results

For the 2,016 analyzed samples, the average CRP was 4.25 mg/dL.

Biomarker	N	Mean	SD	Minimum
C-Reactive Protein mg/dL	2,016	4.26	7.20	0.00

The guidelines of the *American Heart Association*[6] and the LAMARKT[7] laboratory, establish the following for CRP results:

- Individuals are at low risk of developing cardiovascular disease if the CRP level is below 1.0mg/dL
- Individuals have an normal risk of developing cardiovascular disease if their levels are between 1.0 and 3.0 mg/dL
- Individuals are at high risk of developing cardiovascular disease if the CRP level is above 3.0 mg/dL

	Low risk	Normal risk	High risk
CRP mg/dL	**< 1.0**	**1.0-3.0**	**3.0**

The CRP results by state indicate that *State 1* (47.9%) and *State 4* (45%) have the highest percentages of the people with CRP levels representing high-risk of cardiovascular disease.

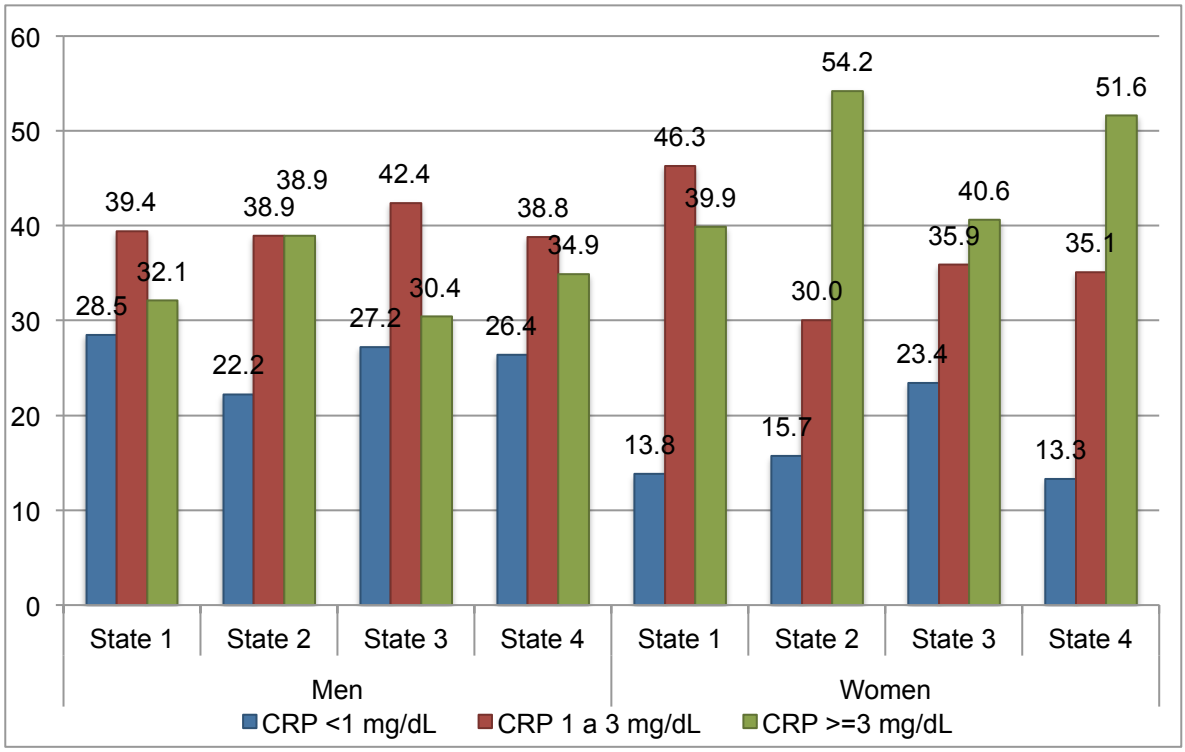
[6] http://www.nlm.nih.gov/medlineplus/spanish/ency/article/003356.htm
[7] http://www.microelisas.com/pdf/PCR%20us%20CLIA%20%20Monobind.pdf

Table 4.1 C-Reactive Protein Results by State[8]

State	< 1 mg/dL		1 a 3 mg/dL		≥ 3 mg/dL	
	Fr	%	Fr	%	Fr	%
State 1	67	19.7	148	43.5	125	36.8
State 2	107	18.4	196	33.7	279	47.9
State 3	79	24.8	124	38.9	116	36.4
State 4	143	18.5	283	36.5	349	45.0
Total	**396**	**19.6**	**751**	**37.3**	**869**	**43.1**

Figure 4.1, shows that the proportion of women with high levels of CRP is systematically higher than the proportion of men. In the *State 4*, the difference is about 16 percentage points, while in *State 3* the difference is 10.2 percentage points, 7.8 in *State 1*, and 5.3 in *State 2*.

Figure 4.1 C-Reactive Protein Results by Gender and State



[8] The name of the 4 states was removed to protect the identity of the respondents.

Table 4.2 includes the results of CRP levels by age group. The results indicate that young adults have the highest percentage (48.2%) of people with high-risk of cardiovascular disease according to CRP levels.

Table 4.2 C-Reactive Protein Results by Age Group

CRP mg/dL	Age			Total
	≤ 50	51 to 60	60 or older	
< 1	23.4	17.7	20.5	19.7
1 to 3	28.4	38.3	38.1	37.2
≥ 3	48.2	44.0	41.4	43.1
Total	**100.0**	**100.0**	**100.0**	**100.0**

V. Thyroid Stimulating Hormone (TSH) Results

For the 2,015[9] analyzed samples, the average TSH was 2.88 uIU/mL.

Biomarker	N	Mean	SD	Minimum	Maximum
TSH uIU/mL	2,015	2.88	5.55	0	100

The following are the reference values for the Thyroid Stimulating Hormone (TSH) set in the Quick Reference Guide[10] for the diagnosis and treatment of primary hypothyroidism in adults. Although the diagnosis of hypothyroidism (primary, secondary or subclinical) requires both the determination of thyroid stimulating hormone and Free Thyroxine (T4), in addition to a clinical evaluation, only TSH values are provided and used for a diagnostic approach.

	Secondary hypothyroidism	Normal	Subclinical hypothyroidism	Primary hypothyroidism
TSH	**<0.1**	**0.1-4.49**	**4.5-10.0**	**>10 & <40**

The TSH results by state, in Table 5.1, indicate that *State 3* has the highest percentages of people with subclinical and primary hypothyroidism, 12.6% and 3.8% respectively.

[9] TSH results were not obtained for one subject because the blood sample was not enough.

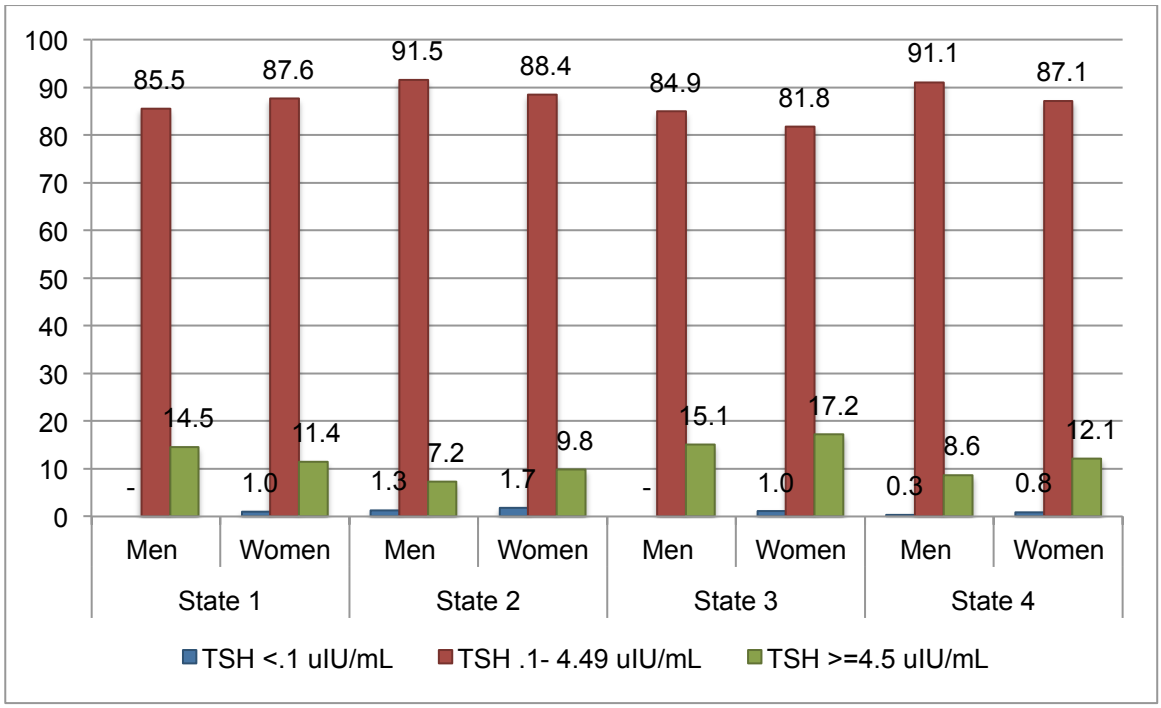
[10] http://www.cenetec.salud.gob.mx/descargas/gpc/CatalogoMaestro/265_IMSS_10_Hipotiroidismo_Primario/GRR_IMSS_262_10.pdf

Table 5.1 TSH Results by State[11]

State	<0.1 uIU/mL		0.1-4.49 uIU/mL		4.5-1.0 uIU/mL		>10 & <40 mg/dL		Total
	Fr	%	Fr	%	Fr	%	Fr	%	
State 1	2	0.6%	295	86.8%	33	9.7%	10	2.9%	340
State 2	9	1.5%	522	89.7%	39	6.7%	12	2.1%	582
State 3	2	0.6%	264	83.0%	40	12.6%	12	3.8%	318
State 4	5	0.6%	687	88.6%	70	9.0%	13	1.7%	775
Total	**18**	**0.9%**	**1,768**	**87.7%**	**182**	**9.0%**	**47**	**2.3%**	**2,015**

In Figure 5.1, the results by state and gender indicate that the percentage of subjects with subclinical and primary hypothyroidism is higher for women compared to men, except for *State 1*.

Figure 5.1 TSH Results by Gender and State



[11] The name of the 4 states was removed to protect the identity of the respondents.

Table 5.2 includes the results of TSH levels by age group. The results indicate that young adults have the highest percentage of people with normal TSH level: 89.80% for adults 50 years or under and 89.22 for adults 51 to 60 years old, compared to 86.17 for adults 60 years or older.

Table 5.2 TSH Results by Age Group

TSH uIU/mL	Age			Total
	≤ 50	51 to 60	60 or older	
<0.1	1.02	0.90	0.87	0.90
.1 to 4.49	89.80	89.22	86.17	87.71
4.5 to 1.0	7.14	7.83	10.35	9.06
>10 & <40	2.04	2.05	2.62	2.61
Total	**100.00**	**100.00**	**100.00**	**100.00**

VI. Vitamin D Results

For the 2,013[12] analyzed samples, the average Vitamin D was 24.21 ng/dL.

Biomarker	N	Mean	SD	Minimum	Maximum
Vitamin D ng/dL	2,016	24.21	8.62	4.7	92.3

The following are the Vitamin D cut-off points from the guidelines for the assessment of the Vitamin D deficiency.

	Deficiency	Normal
Vitamin D ng/dL	**<20**	**≥20**

The results shown in Table 6.1 indicate that *State 1* has the highest percentage of people with Vitamin D deficiency (54.7%), followed by State 2 (36.8%).

Table 6.1 Vitamin D Results by State[13]

State	< 20 ng/dL		≥ 20 ng/dL		Total
	Fr	%	Fr	%	
State 1	186	54.7%	154	45.3%	340
State 2	214	36.8%	368	63.2%	582
State 3	95	30.0%	222	70.0%	317
State 4	154	19.9%	620	80.1%	774
Total	**649**	**32.2%**	**1,36**	**67.8%**	**2,013**

Figure 6.1 indicates that the percentage of people with Vitamin D deficiency is consistently higher among women; the difference ranges between 4.4 (in *State 4*) and 25.7 (in *State 3*) percentage points.

[12] Vitamin D results were not obtained for 3 subjects because the blood sample was not enough.
[13] The name of the 4 states was removed to protect the identity of the respondents.

Figure 6.1 Vitamin D Results by Gender and State

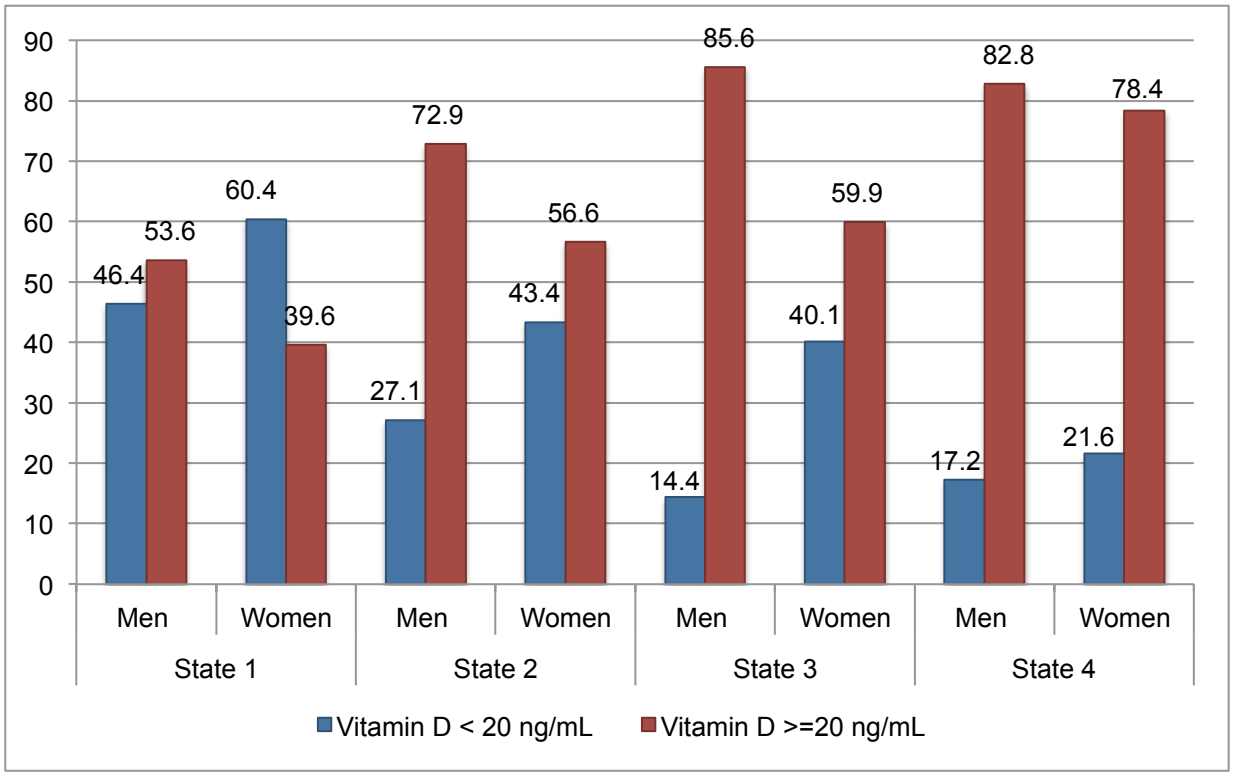


Table 6.2 includes the results of Vitamin D levels by age group. The results indicate that the highest percentage of people with Vitamin D deficiency is among people aged 60 and over (38.0%).

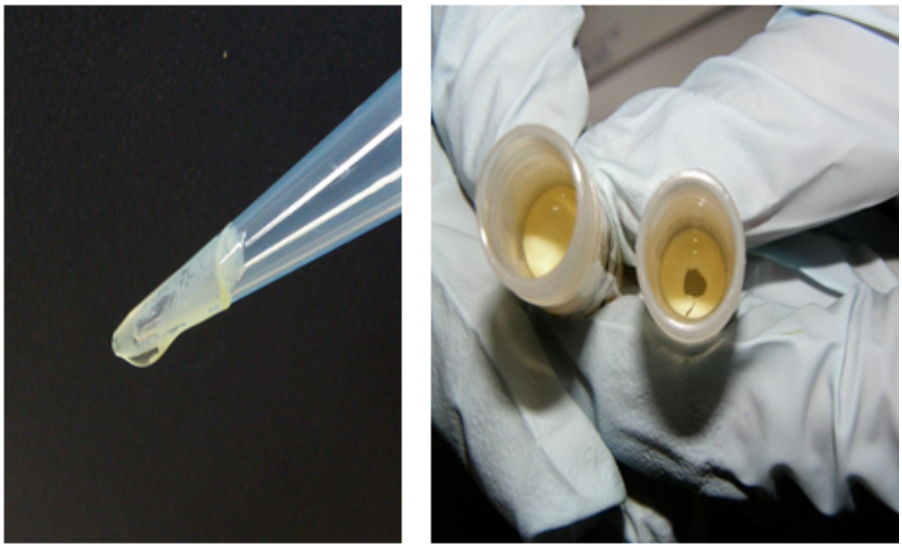
Table 6.2 Vitamin D Results by Age Group

HDL mg/dL	Age			Total
	≤ 50	51 to 60	60 and older	
< 20	27.5	25.8	38.0	32.2
≥ 20	72.5	74.2	62.0	67.8
Total	**100.0**	**100.0**	**100.0**	**100.0**

Appendix II. Technical Note Regarding the Laboratory Analyses Method Used to Determine the Cholesterol Results

A number of serums received were non-fasting blood samples and some presented gross evidence of fat (lipemic samples). Following the norms of the Architect equipment used for analyses, the samples were centrifuged; afterwards, a fat layer formed on the upper part of the pipette (see Figure 1 below). The fat layer varied in size and a portion was absorbed by the pipette from the equipment's robotic arm, this affected the measurements.

Figure 1. Effect of Centrifugation on Lipids and Robot Pipetting

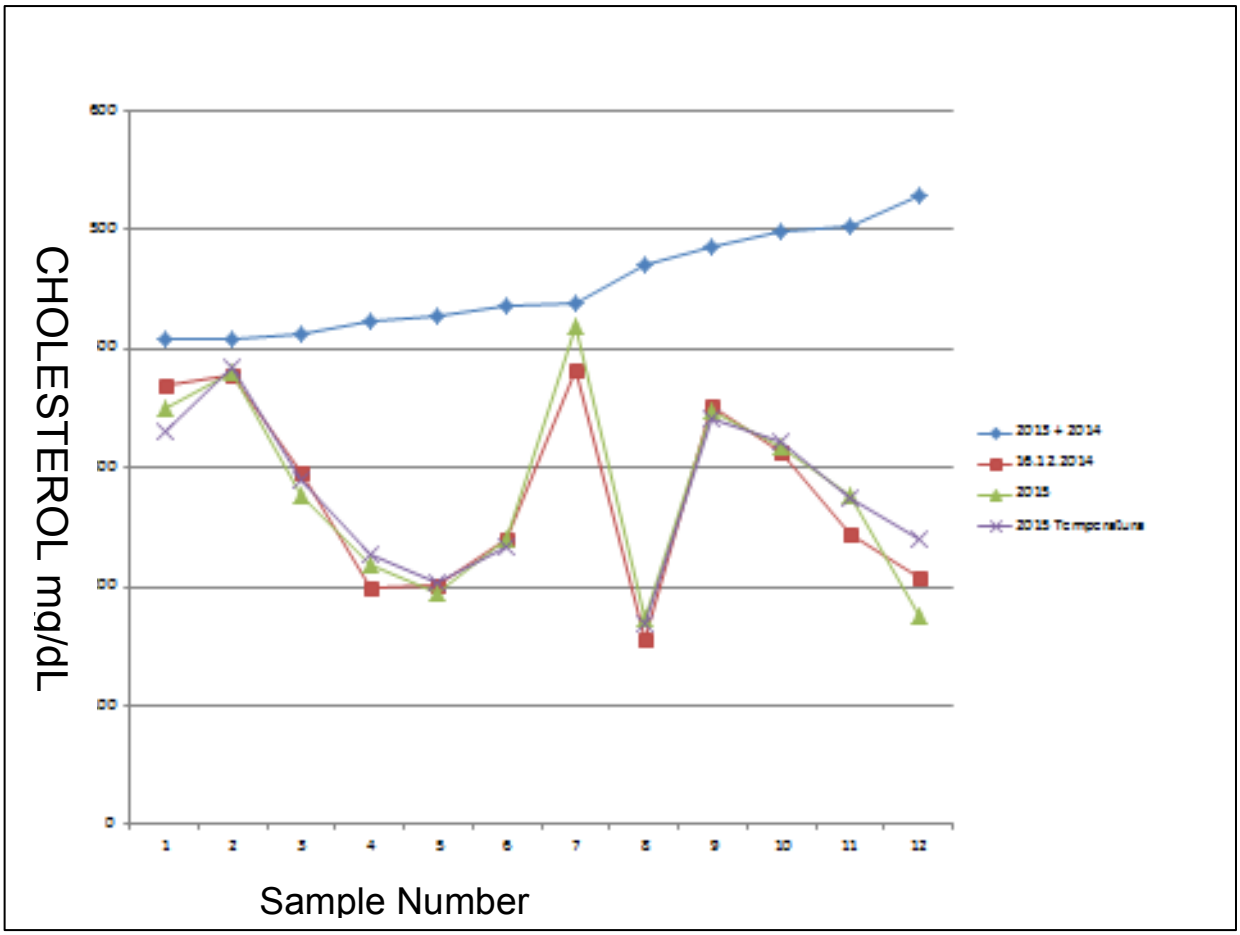


Also, at the request of Dr. Mara Tellez-Rojo, 12 samples with very high levels of cholesterol were centrifuged. Once these results were reviewed, the procedure used for the initial measurement was analyzed. Once these analyses were completed it was decided to repeat the analysis of all samples "with no centrifugation" so that the layer of fat would not appear in the upper

part of the pipette and be mixed within the sample instead. The analysis of the 12 blood samples was repeated, measured one more time, and compared with a second more drastic method that involves heating the sample to 38 °C for 5 minutes to ensure the fat dissolves in the sample and does not interfere with the measurement. This method is not commonly used in serum samples, because it affects other substances that could be measured at a later time.

The blue line in Figure 2 represents the measurements made after centrifugation; it shows higher results and less variability compared to the other three lines. The red and green lines represent the measurements made without centrifugation; and finally the blue line with the ‘x’ represents the measurements made after heating the sample. As observed in the figure, measurements that were not centrifuged are highly comparable and have a lot of variability in contrast to the line that represents the measurements after centrifugation.

Figure 2. Analysis of 12 Samples (sent for verification by Dr. Tellez)



To ensure the quality of the measurements, quality control blood serums were analyzed simultaneously, in double the usual amount. The quality control samples were included at the beginning of the analysis, and were included every 50 samples and at the end of the tests. As indicated in the measurements presented in a Levy-Jennings graph below, the delivered results are of very good quality. We used the Westgard criteria to interpret quality control results, which establishes that the mean determined by the manufacturer should not exceed ± one standard deviation (SD) compared to the sample mean. Figures 3 and 4 show the blood serum results according to the criterion established by Merck Cholesterol 2015 guidelines; the first figure

presents an average concentration of 101 mg/dL and the second presents an average concentration of 242 mg/dL. The results indicate that the difference in the quality control measurements does not exceed ± one SD.

Figure 3. Levy-Jennings Curves. Quality Control Serum for Cholesterol Merck #2015, lot 14431 with theoretical concentration of 101 mg/dL on a series of measures obtained between February 5th and 16th 2015.

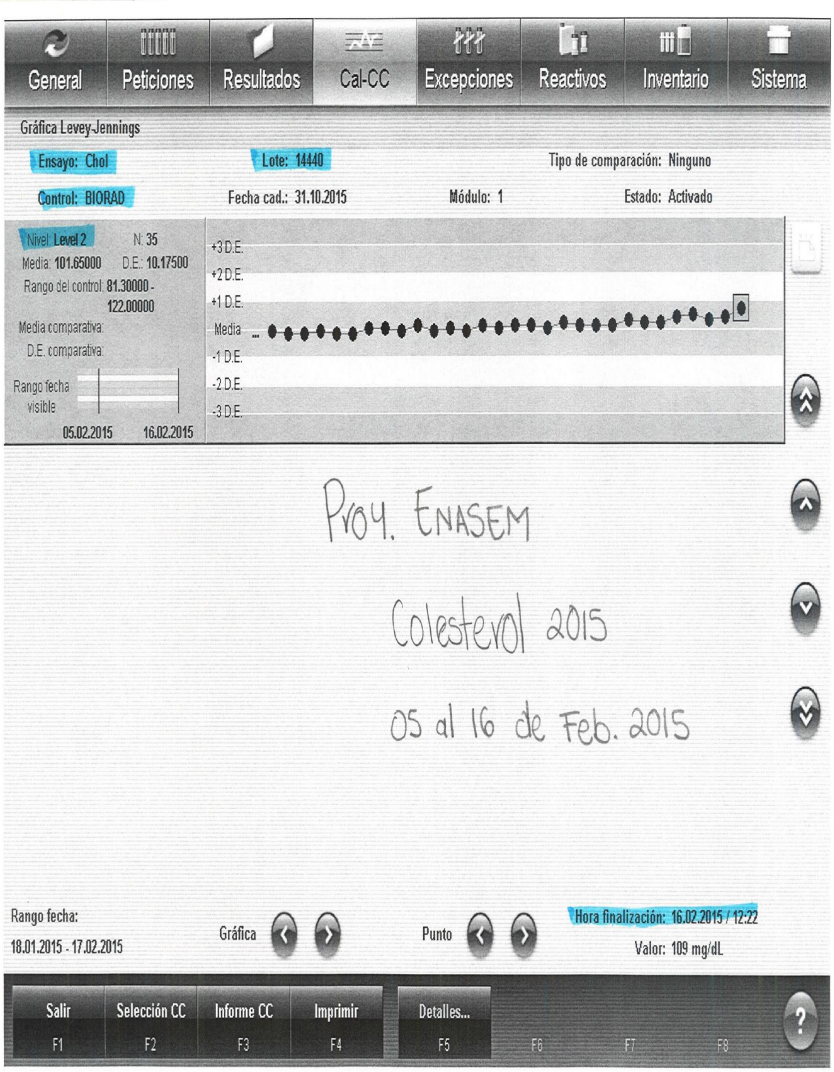
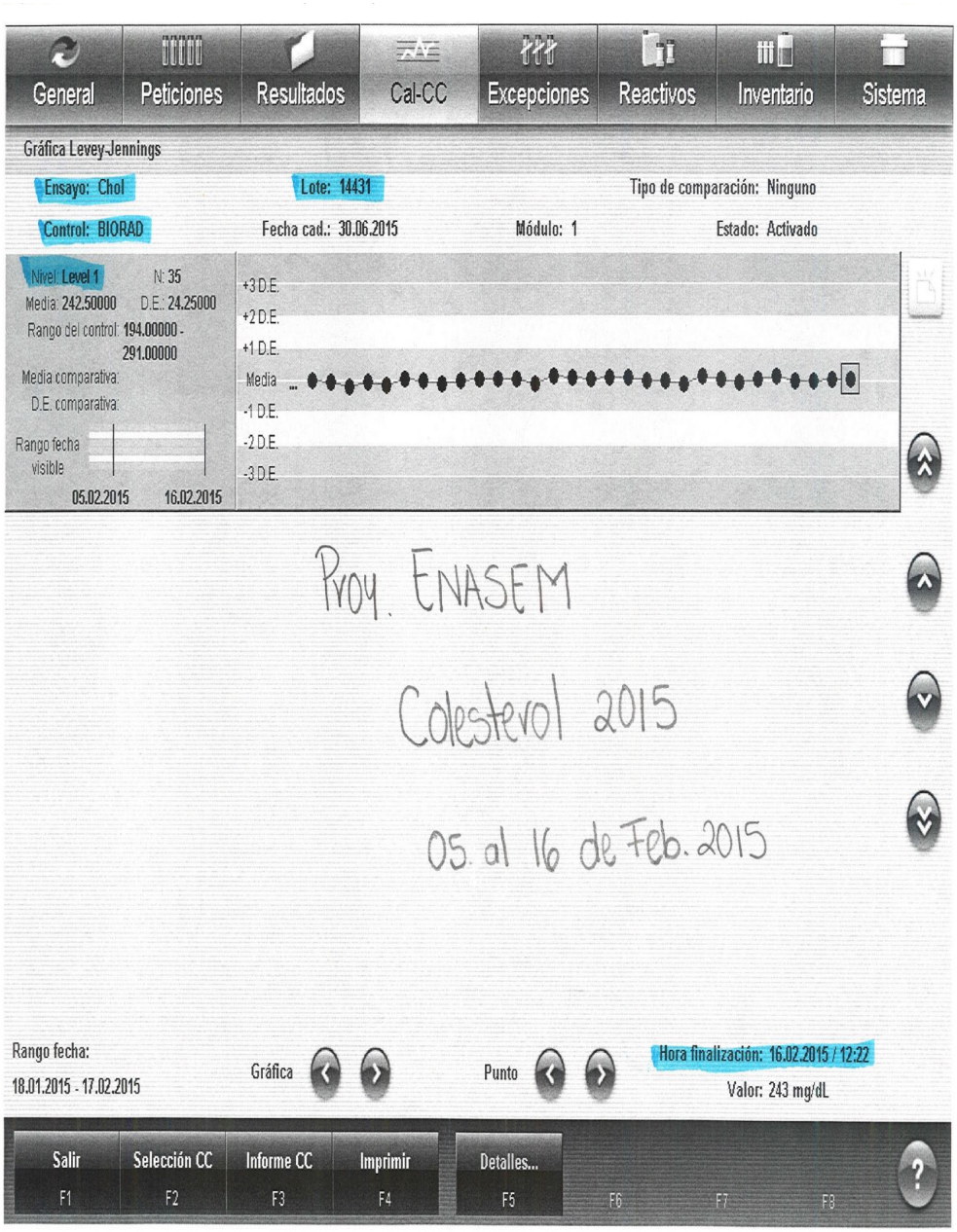


Figure 4. Levy-Jennings Curves. Quality Control Serum for Cholesterol Merck #2015, lot 14431 with theoretical concentration of 242 mg/dL on a series of measures obtained between February 5th and 16th 2015.



Conclusions

All the 2,016 samples were analyzed again without the centrifugation method. The 'corrected' measurements presented here are good measurements as confirmed by quality control results presented above. However, these samples were taken under non-fasting conditions, which does not comply with the recommendation of the ATP II guidelines, that recommends at least 12 hours of fasting and an internationally accepted cut-off point of > 200 mg/dL for the diagnosis hypercholesterolemia.

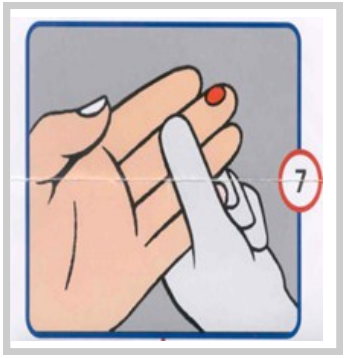
Finally, the table below compares the results of the prevalence of hypercholesterolemia obtained with ENSANUT 2012 and MHAS. As you can see, the results indicate the prevalence of hypercholesterolemia is similar in both studies.

Table 1. Cholesterol Prevalence Comparison, MHAS and ENSANut 2012

Age	Cholesterol > 200 mg/dL (%)	
	ENASEM 2012	ENSANut 2012
<30	23.81	19.7
31-40	36.65	27.9
41-50	39.56	37.9
51-60	34.12	36.7
61-70	45.32	42.1
71-80	38.26	56.8
>81	34.86	33.2

Blood Samples

We will ask you for two drops of blood from one of your fingers, this will help us to know if you have anemia and your blood sugar levels.



We will draw a 5 ml blood sample from the vein of one of your arms, this is equivalent to a tablespoon and will be used to know the amount you have in your blood of the so-called "good fats", cholesterol, and your nutritional status.

Another blood sample of 3 ml (equivalent to a teaspoon) will be sent to the INSP laboratory where it will be frozen and kept for future studies related to its genetic information (known as DNA). Genes are small units that carry the information that is inherited from parents to children, including some diseases. This sample will be used for the purposes of this research only.

We will give you a card with the results of your blood pressure, your height, weight, waist and hip circumference, and your assessment of anemia and blood sugar level. The results of your blood samples will not be released to you because they require further processing in a laboratory.

Thank you for your participation!

Contact Us:

Dr. Martha María Téllez Rojo
Phone Number: 01-777- 3293000
Ext.: 3402 ó 3405

Lic. Aurora Franco
Supervisor responsible for the fieldwork activities
Toll-free number
01 800 838 3409.

Business Hours
Monday-Friday
9:00 am to 5:00 pm.

This study has been reviewed and approved by the INSP Ethics Commission

Mexican Health & Aging Study

INSTITUTO NACIONAL DE ESTADÍSTICA Y GEOGRAFÍA

The National Institute of Public Health and the *Instituto Nacional de Salud Pública* (INSP), The *Instituto Nacional de Estadística Geografía e Informática* (INEGI) are conducting the ***Mexican Health and Aging Study (MHAS)***

The study aims to know various aspects related to the health of the population of 50 years or more in Mexico.

A few weeks ago, staff from INEGI asked you some information regarding your health.

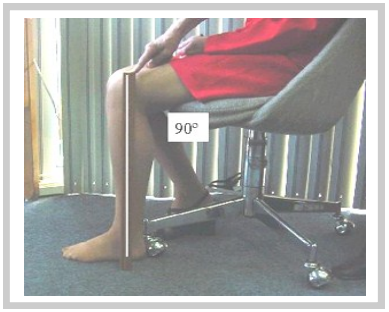
We remind you that your participation is completely voluntary and the information will be kept strictly confidential.

The assessment of your health also includes the following measurements:

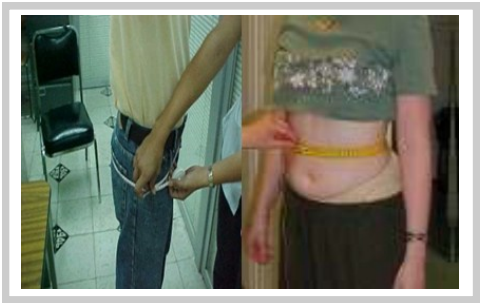
Blood pressure is an important indicator of how well your heart works. It is recommended to maintain a blood pressure of 120/80 or lower. We will measure your blood pressure and pulse with automatic equipment that is placed on the wrist of your arm.

Height can decrease due to osteoporosis and other diseases. When height is combined with other measurements such as ***weight*** or ***waist circumference***, it is possible to know whether or not a person is obese and to determine the nutritional status of a person.

We will also estimate your height by measuring the distance between your heel and the highest part of your knee joint.



The measurement of your ***waist and hip circumference*** will be used to determine the distribution of abdominal fat and we will study how these measurements are related to some diseases.



Muscle strength decreases with age or illness. Measuring your ***grip strength*** is one way to detect your general muscular strength.

Nuestro sentido del ***equilibrio*** se ve afectado con los cambios de la edad y hormonales, con las enfermedades y otros factores.

Le solicitaremos que permanezca en un solo pie por un momento y de ser posible, realizará está prueba con ambos pies.

La velocidad a la que se camina una distancia a paso normal puede indicar su capacidad para llevar a cabo las actividades de la vida diaria.

Le pediremos caminar una distancia corta a un paso normal.

Apéndice IV. Tarjeta de Registro de Resultados

Estudio Nacional de Salud y Envejecimiento en México

FECHA: |__|__| |__|__| |__|__|

NOMBRE : _____

EDAD: _____ SEXO: ☐ ..... MASCULINO ☐ .....FEMENINO

	1a.	2a.
TENSIÓN ARTERIAL	_____/_____	_____/_____
PULSO	_____	_____
TALLA	_____	_____
PESO	_____	_____
CINTURA	_____	_____
CADERA	_____	_____

Resultado de la prueba de hemoglobina (azúcar en la sangre)	|_____|. |____| %
Resultado de hemoglobina	|____|____|. |____| g/dl

RECOMIENDE AL PARTICIPANTE ACUDIR AL CENTRO DE SALUD CUANDO LOS RESULTADOS MUESTREN LOS SIGUIENTES VALORES:

TENSIÓN ARTERIAL.- VALORES POR ARRIBA DE 140 MMHG SISTÓLICA Y/O 90 MMHG DIASTÓLICA

HEMOGLOBINA GLICOSILADA (AZÚCAR EN SANGRE).- VALORES POR ARRIBA DEL 7%

HEMOGLOBINA.-
Hombres adultos: Valores menores a 13.0 g/dL
Mujeres adultas: Valores menores a 11.0 g/d

Estudio Nacional de Salud y Envejecimiento en México

FECHA: |__|__| |__|__| |__|__|

NOMBRE: _____

EDAD: _____ SEXO: ☐ ..... MASCULINO ☐ .....FEMENINO

	1a.	2a.
TENSIÓN ARTERIAL	_____/_____	_____/_____
PULSO	_____	_____
TALLA	_____	_____
PESO	_____	_____
CINTURA	_____	_____
CADERA	_____	_____

Resultado de la prueba de hemoglobina (azúcar en la sangre)	|_____|. |____| %
Resultado de hemoglobina	|____|____|. |____| g/dl

RECOMIENDE AL PARTICIPANTE ACUDIR AL CENTRO DE SALUD CUANDO LOS RESULTADOS MUESTREN LOS SIGUIENTES VALORES:

TENSIÓN ARTERIAL.- VALORES POR ARRIBA DE 140 MMHG SISTÓLICA Y/O 90 MMHG DIASTÓLICA

HEMOGLOBINA GLICOSILADA (AZÚCAR EN SANGRE).- VALORES POR ARRIBA DEL 7%

HEMOGLOBINA.-
Hombres adultos: Valores menores a 13.0 g/dL
Mujeres adultas: Valores menores a 11.0 g/d