# 5. VISIT - MOTHER

All consenting mothers will undergo a clinic visit that includes 3 components:

- Physical measurements (blood pressure and anthropometrics)
- Blood sampling, either a 2-hour 75g oral glucose tolerance test (OGTT), non-fasting blood sampling if she has diabetes and is on medication for it (Single Blood Draw), or a Single Blood Draw under special circumstances where she might have been originally scheduled for an OGTT (see Section **5.4.1**)
- Interviewer-administered Questionnaire

Field center staff are responsible for assuring the visit is scheduled and completed, and for making sure that all materials required for the examination are available and appropriately labeled with the mother's HAPO ID. In attaching ID labels to forms and blood and urine samples, field center staff carefully checks that all labels correspond to the same HAPO ID.

Smoking is not permitted during the visit. Mild physical activity (e.g. walking within the clinic area) is permissible during the visit. More vigorous exercise is not allowed.

## 5.1 Forms Used for the Visit – Mother

The data forms used during the Visit – Mother include:

- Test Qualification Form–Mother – (see Section **5.4**)
- Physical Measurements–Mother – (see Section **5.6**)
- OGTT Form–Mother – (see Section **5.7.6**)
- OGTT Sample Processing Form–Mother – (see Section **5.7.8**)
- Single Blood Draw Form–Mother – (see Section **5.8.2**)
- Single Blood Draw Sample Processing Form–Mother - (see Section **5.8.3**)
- Questionnaire – (see Section **5.10**)
- Future Contact Information Form – (see Section **5.10)**

Additional forms that may be needed during the visit include:

- Call Back Register – (see Section **5.3**)

- Phone Call Information
- Blood Pressure Repeat Measurement Form – Mother (see Section **5.6.9**)
- Study Visit Variation – (see Section **5.11**)

**Note:** The Single Blood Draw forms will only be used for mothers scheduled for non-fasting blood samples or those originally scheduled for an OGTT who will instead complete only a single blood draw because of special circumstances. When the Single Blood Draw forms are used, the OGTT forms will not be used.

**Note:** There are 2 additional forms Metformin Use-Mother and Metformin Use Interview-Mother which are only used in the special circumstance where the mother takes Metformin but a diagnosis of diabetes was not reported (see Section **5.4.1** item 5).

## 5.2 Consent

Informed consent must be obtained from all mothers prior to the start of the visit procedures. She should be asked to read and sign the Consent Form-Mother. Each field center has its own consent forms. It is not enough to have her read and sign the form; she must understand what is going to happen to her during the study and the risks involved. Make certain that the participant is actually reading the form and then specifically ask if she has any questions about it. The staff member should be prepared to answer those questions or have someone else available on site to answer them. If it seems appropriate (i.e., she is struggling to read the form), ask if she needs assistance in reading the form. If so, the staff member should read it aloud. **Note:** If a mother is unable to read the Consent Form-Mother, the form should be read and explained to her. If the is unable to write, she should place an "X" in the space on the Consent Form-Mother for her signature, and the form should be signed by a witness other than or as well as the HAPO Follow-Up Study staff member who is obtaining the consent (depending on policies at a given field center).

Included on the Consent Form-Mother is the opportunity to agree to or decline the long term storage of her blood and urine samples and study data at the National Institutes of Health repository for future use by non-HAPO Follow-Up Study investigators. The mother's choice should be marked on the Consent Form-Mother and this should also be noted on the OGTT Visit Form-Mother or Single Blood Draw Form-Mother by marking 'Yes' or 'No'.

Also included on the Consent Form-Mother is the opportunity to have a sample for DNA collected. The mother's choice should be marked on the Consent Form-Mother and this should also be noted on the OGTT Form-Mother or Single Blood Draw Form-Mother by marking 'Yes' or 'No'. If she does not agree to collection of a sample for DNA, a blood sample for DNA should **not** be obtained.

The Consent Form-Mother also offers the opportunity to be recontacted for future research studies. The mother's choice should be marked on the Consent Form-Mother and this should also be noted on the Questionnaire by marking 'Yes' or 'No' for question 2a.

A copy of the signed consent form should be given to the mother and the original filed in her HAPO Follow-Up Study file.

### 5.3 Call Back Register

The Test Qualification Form-Mother will be used to determine readiness for the visit. The Call Back Register will be used to keep track of instances where the participant will need to be called back because of something encountered on the Test Qualification Form-Mother or if the mother and child missed their scheduled study visit.

The Call Back Register includes space to copy the woman's name, her date of birth, and the date of birth of her child from the Recruiting Register or Phone Call Information form. The Call Back Register also has columns to indicate 'Call Back Reason', 'Anticipated Call Back Date', 'Call Back Status', and 'Status Date'. The last two columns are to be completed when the woman is called back.

#### Call Back Reason Codes

'FM-M': If the mother forgot to bring her medications, enter 'FM-M' under 'Call Back Reason'.

'M-M': If the mother is on metformin and needs to confirm reason for use with her doctor's office, enter 'M-M' under 'Call Back Reason'.

'NS': If the mother and child missed their study visit and need to reschedule, enter "NS" under 'Call Back Reason'.

**Call Back Status Codes**

'C': If the call was complete and the issue resolved, enter 'C' for 'Call Back Status'.

'NC': If after 10 attempts following the anticipated call back date the call was not completed and the issue was not resolved, enter 'NC' for 'Call Back Status'.

## 5.4 Completing the Test Qualification Form-Mother

Before proceeding to the collection of the urine sample, physical measurements and the OGTT or single blood draw field center staff must complete the Test Qualification Form-Mother. This set of questions will ensure that the participant meets the requirements and conditions for the BOD POD portion of the physical measurements and, if applicable, the OGTT. The Test Qualification Form-Mother contains questions on illnesses within the past 3 days; diet within the past 3 days; the time the participant last exercised vigorously; use of a steroid inhaler within the past 8 hours; the time the participant last smoked; the time that the participant last ate or drank anything other than water, including taking any medications; the time that the participant last had a drink of water; and questions about the presence of special circumstances that would preclude undergoing an OGTT. In addition, the form contains questions on whether or not the blood drawing was completed.

Before beginning the form, make sure that the participant's HAPO Study ID label has been attached to each page of the form in the space provided.

**Scheduled Visit**

Questions 1-3:

Enter today's date, using the year/month/day format, entering only the last digit of the year. When you begin questioning the participant, enter the time using the 24-hour clock. For question 3, indicate whether she is scheduled for an OGTT or a single blood draw.

**Diabetes**

Questions 4-5:

Ask the participant if she has ever been told by a medical person when she was not pregnant that she has diabetes. If 'No', confirm that she will do the OGTT and skip to question 6. If 'Yes', ask her if she takes oral medication or insulin for treatment of diabetes. If 'Yes', confirm she will do a single blood draw. If 'No' confirm she will do the OGTT.

**Pregnancy**

Question 6:

Ask the woman if she is currently pregnant or breastfeeding. If 'No', proceed to the next question. If 'Yes', stop, cancel the OGTT or single blood draw and record an anticipated call back date in question 7 on the Phone Call Information form and set the form aside. Even though she will not complete the physical measurements and the OGTT or single blood draw, she should still complete the Questionnaire since the child can still complete his/her visit. Enter your HAPO staff ID in question 27 and go to Physical Measurements - Mother and Questionnaire.

**HIV, Hepatitis B or Hepatitis C**

Questions 7-9:

Ask the participant if a medical person has ever told her that she has HIV, hepatitis B or hepatitis C. If 'No', skip to question 10. If 'Yes', due to international shipping regulations her blood samples cannot be shipped. Complete questions 8 and 9 to evaluate her fasting status and readiness for the BOD POD. Then stop, cancel the OGTT or single blood draw. Enter your HAPO staff ID in question 27 and go to Physical Measurements - Mother and Questionnaire.

**Bariatric Surgery**

Question 10:

Ask the participant is she has ever had bariatric or weight loss surgery. If 'Yes', skip to question 25. Even if the participant was originally scheduled for an OGTT, she will now do a single blood draw. If 'No', continue with the next question.

**Medications, Questions 11-12**

'No' to Question 11:

If the mother is not taking any medications regularly, skip to the directions preceding question 13.

'Yes' to Question 11:

Go to question 12 and check to see if any of the medications are oral anticonvulsants, oral glucocorticoids/corticosteroids, or atypical antipsychotics (see the list provided).

'No' to Question 12a:

Go to question 12b.

'Yes' to Question 12a:

Skip to question 25. Even if the participant was originally scheduled for an OGTT she will now do a single blood draw.

Question 12a – forgot medications:

Enter 'FM-M' under Call Back Reason on the Call Back Register with an Anticipated Call Back Date (yy/mm/dd) of one business day after the visit or another date that is convenient for the mother. After the call, correct the form so that the answer is either 'No' or 'Yes'.

'No' to Question 12b:

Proceed to the instructions before question 13.

Question 12b – Metformin for diabetes:

This answer should be marked if one of the medications is Metformin and if answers to question 4 and question 5 are 'Yes'. Skip to question 25.

Question 12b – Metformin, confirm reason for use:

This answer should be marked if Metformin is one of the medications and the answer to either question 4 or 5 is 'No'. Enter 'M-M' on the Call Back Register with a Call Back Date (yy/mm/dd) of two weeks from the visit date. Give her a Metformin Use–Mother Form with her ID label attached.

Question 12b – Forgot medications:

Proceed to the instructions before question 13. An entry of 'FM-M' should already be entered for Call Back Reason in the Call Back Register from question 12a. After the call, send the Metformin Use–Mother form if necessary. Correct the answer to question 12b once the answer is known.

Instructions before Question 13:

If the mother is scheduled for an OGTT, proceed to question 13. If she is scheduled for a single blood draw, skip to question 25.

**Illnesses in the Past 3 Days**

Question 13:

Ask the participant if she has been ill in the past 3 days, indicating that by ill you mean the presence of any of the following: chills; fever; vomiting more than once; or 3 or more episodes of diarrhea.

**Diet for Last 3 Days**

Question 14:

Ask the participant if she has eaten her typical or usual diet for the last three days.

**Time of Last Vigorous Physical Activity**

Questions 15-16:

Ask the woman if she exercised vigorously after 11:00 PM (2300 hours) the night before, including this morning before coming for the visit, indicating that vigorous exercise is any

exercise where she worked up a sweat. If 'No', skip to question 17. If 'Yes', ask her when she finished exercising vigorously. Enter the time, using the 24-hour clock.

**Inhaler use**

Questions 17-18:

Ask the woman if she used an inhaler for asthma or other breathing problems after 11:00 PM (2300 hours) the night before, including this morning before coming for the visit. If 'No', skip to question 19. If 'Yes', ask her when she last used the inhaler. Enter the time using the 24-hour clock.

**Time Last Smoked**

Questions 19-20:

Ask the participant if she has smoked in the last two hours. If 'No', skip to question 21. If 'Yes', ask her what time she last smoked. Wait until at least 30 minutes have elapsed since last smoked before proceeding with the examination. **Note:** Blood pressure should **not** be measured while waiting for 30 minutes to elapse since recent smoking can alter blood pressure.

**Time of Last Eating or Drinking for OGTT**

Questions 21-22:

Ask her if she's had any water in the past 2 hours. If 'No', skip to question 23. If 'Yes' ask her when she last had a drink of water. Wait until 2 hours have elapsed since the last drink of water before proceeding with the visit. If the 2-hour timepoint will mean that the OGTT will not start until after 11:00am, proceed with the OGTT and do the BOD POD at a timepoint that is at least 2 hours after consumption of the Trutol.

Questions 23-24:

Ask the participant if she ate or drank anything other than water after 11:00 PM (or 2300 hours) the night before, indicating that this includes alcohol, candy, chewing gum, as well as medications. If the participant answers 'No', skip to question 27, since the woman has fasted

for at least 8 hours, the minimum fasting time required for the OGTT. If the woman indicates that she ate or drank something after 11:00 PM (2300 hours), complete the follow-up question that asks for the time she ate or drank something other than water. Enter the time using the 24-hour clock. If the time the woman last ate or drank is after 2:00 AM (0200 hours), tell the participant that the test needs to be rescheduled. If the time was between 11:00 PM (2300 hours) and 2:00 AM (0200 hours), the interviewer must determine when 8 or more hours will have elapsed since the woman last ate or drank anything. Ask the woman if she can wait until that time to start the test. If the woman indicates that she cannot wait, the visit needs to be rescheduled. Urinalysis and measurement of blood pressure, height, and weight can proceed while the woman is waiting to start the OGTT. The following suggestions may help the interviewer make the determination of when to proceed with the test:

- If the time of last eating or drinking was before midnight (2400 hours) and the current time is after 8:00 AM (0800 hours), the woman has fasted for more than 8 hours and staff can proceed with the OGTT.
- If the woman last ate at 11:xx PM (23xx hours), the OGTT can proceed after 7:xx AM (07xx hours) where xx represents the minutes after 11:00 PM (2300 hours) that the woman last ate or drank something.
- If the woman ate or drank something after midnight, add 8 hours to the reported time. That time is the earliest time at which the fasting samples can be drawn. Ensure that the woman does not have her fasting blood samples drawn before that time. For example, if the woman last ate at 1:15 AM (0115 hours), the fasting blood samples cannot be drawn before 9:15 AM (0915 hours).

**Note:** If the OGTT needs to be rescheduled because she hasn't fasted for 8 hours and she agrees to reschedule, go to Physical Measurements–Mother and Questionnaire. Only the OGTT should be delayed until the rescheduled date. If the OGTT needs to be rescheduled because she hasn't fasted for 8 hours, but she refuses to reschedule, the rest of the visit should be completed and the samples that are collected at the fasting time-point should be collected. Go to Physical Measurements–Mother, Questionnaire, and Single Blood Draw–Mother (collect the samples that are collected at the fasting draw).

**Time of Last Eating or Drinking for Single Blood Draw**

Questions 25-26:

Ask her what time she had something to eat or drink other than water indicating that this includes alcohol, candy, chewing gum as well as medications. Ask her what time she last had a drink of water. If necessary, wait until 2 hours have elapsed since the last drink of water before proceeding with the visit.

**Form Completion**

Question 27:

Enter your HAPO Staff ID.

For participants doing the OGTT, go to Physical Measurements-Mother, OGTT Form-Mother and Questionnaire. For participants doing a Single Blood Draw, go to Physical Measurements-Mother, Single Blood Draw Form-Mother and Questionnaire. Mothers doing a single blood draw will include those originally scheduled for non-fasting blood samples. It may also include mothers originally scheduled for an OGTT but changed to a Single Blood Draw due to bariatric surgery, interfering medications or unacceptable fasting status.

**Complete After the OGTT Form-Mother or Single Blood Draw Form-Mother If Blood Drawing Not Completed**

Question 28:

This question on the Test Qualification Form relates to the reason why the blood drawing was not completed. **Note:** This question should **only** be completed if the blood drawing (including OGTT or Single Blood Draw) was not completed. If the blood drawing was not fully completed, indicate the reason why. The options include the items that would preclude completing the OGTT or a single blood draw, 'Refused blood samples', 'Fasting glucose sample not obtained', 'Vomited after glucose load', 'Fainted or fell ill after the glucose load'. If the blood drawing was not fully completed for another reason, put an "X" in the box labeled "Other" and write the reason down in the space provided.

**Data Entry Completion**

Question 29:

The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID to indicate the data have been entered, and check the box on the front of the form to indicate data entry is done.

### 5.4.1 Special Situations Precluding the OGTT

During the processes of recruitment and administration of the Test Qualification Form-Mother some questions will be asked about situations that may preclude performing the OGTT but would still allow other data to be collected. Special situations include:

1. Mother reports that she has a diagnosis of diabetes and is on treatment with oral medication or insulin. She will be asked to bring her medication to the visit. The mother with diabetes on medication will be told not to fast and she will have a single blood draw of the samples that are collected at the fasting time-point. The OGTT will not be performed but the rest of the visit will be performed. If a diagnosis of diabetes is reported but there is no treatment, she will be asked to fast and the usual visit will be conducted.
2. Mother is currently pregnant or breastfeeding. If the mother agrees, the child will have the full visit and the mother will complete the Questionnaire. She will be asked to complete the visit at a later date.
3. Mother reports she is HIV positive or has Hepatitis B or C. In this situation due to international shipping regulations, blood sampling will not be performed. She will have the rest of the visit.
4. Mother reports having undergone a bariatric surgery procedure. She will have fasting samples collected (single blood draw) but the OGTT will not be performed. She will have the rest of the visit.
5. The mother takes medication(s) regularly. She will be asked not to take the medication on the morning of the visit and to bring all medications to the visit. If any of the medications are oral anticonvulsants, glucocorticoids/corticosteroids, or atypical antipsychotics fasting samples will be collected (single blood draw) but the OGTT will not be performed. The rest of the visit will be completed. If the medication is Metformin but a diagnosis of diabetes was not reported, the full visit, including the OGTT, will be

performed. She should be given the Metformin Use-Mother form, asked to contact her doctor's office to determine the reason for treatment with Metformin (diabetes, abnormal glucose but not diabetes, polycystic ovary syndrome, weight control, other) and to return the form by mail. If the form is not received within 2 weeks, she should be contacted by phone and questions 4-6 on the Metformin Use Interview-Mother form should be asked. Whichever of the forms is completed should be entered into the REDCap Data Entry System.

## 5.5 Rescheduling the Visit

The visit may need to be rescheduled for any of the following reasons:

1. A mother is unable to keep her original appointment.
2. A mother fails to appear for her scheduled visit.
3. A mother is scheduled to have an OGTT but did not fast for at least 8 hours and agrees to reschedule (in this case only the OGTT needs to be rescheduled).

### 5.5.1 Procedures for Mothers Unable to Keep the Original Appointment

Some mothers will be unable to keep their original visit appointment and will need to reschedule the visit. If a woman contacts field center staff to indicate that she needs to reschedule, the visit should be rescheduled for as soon as possible. Record the rescheduled visit on an appointment book or calendar.

### 5.5.2 Procedures for Mothers who Fail to Appear for the Visit

If a mother is a "no show" at her original appointment, staff should attempt to contact her one business day following the missed appointment or as soon as possible to reschedule the visit. If contact is made, and the mother agrees, schedule the visit for as soon as possible.

Field centers may find that a mother who is a "no show" for a scheduled visit is likely to be a "no show" for a rescheduled visit. Hence, as field centers gain experience with mothers who are "no shows", they may find that the effort needed to reschedule "no shows" is not productive. In

such circumstances, field centers may choose to treat “no shows” as dropouts and not reschedule missed appointments.

### 5.5.3 Procedures for Women not Meeting OGTT Requirements

If the OGTT needs to be rescheduled because a woman does not meet the requirements for the test, the OGTT should be scheduled for as soon as possible. **Note:** When she comes for the rescheduled OGTT, a **new** Test Qualification Form-Mother, OGTT Form-Mother, and OGTT Sample Processing Form-Mother **must** be completed and entered.

If the woman does not wish to reschedule the OGTT, procedures for the Single Blood Draw should be followed (see Section **5.8**).

## 5.6 Physical Measurements - Mother

The Physical Measurements-Mother form is used to record collection of the urine sample, and measurement of blood pressure and anthropometrics.

### 5.6.1 Urine Sample

A urine sample should be collected on all mothers, prior to initiation of physical measurements.

A specimen cup should be labeled with the woman’s urine sample ID (ending with digits 706). Make sure that the ID affixed to the specimen cup matches the ID on the woman’s Physical Measurements-Mother form. She should be asked to provide a clean catch urine sample. The urine sample **should** be collected prior to the blood pressure measurement. **Note:** This reflects the fact that accurate measurement of blood pressure requires an empty bladder. Record the time the sample was collected (even if she can’t void until later in the visit). If the sample is not collected, mark “No” for Question 2.

She should be given the following instructions:

- Holding the labial folds apart with one hand, wipe once with the first wipe from front to back down the left fold and discard the wipe

- Wipe once with the second wipe from front to back down the right fold and discard the wipe
- Wipe once down the center from front to back and discard the wipe
- Void a small amount of urine into the toilet
- Void urine into the cup without allowing the cup to contact anything but the flow of urine
- Quickly cap the cup

The urine sample should be sent to the specimen processing location with the blood samples and aliquotted into 2 clear-top cryovials labeled with the woman's urine sample ID (ending with digits 706) and stored in urine sample freezer boxes (Urine Analysis and Urine Backup) until shipment to the Laboratory Coordinating Center. Enter your staff ID in question 3 on the Physical Measurements-Mother form. **Note:** The urine sample should be sent for processing even if some or all of the blood samples are not collected.

### 5.6.2 Measurement of Blood Pressure

#### 5.6.2.1 Description and Preparation

Blood pressure is measured at the visit after the urine sample is collected. (If the urinalysis specimen cannot be produced, proceed with blood pressure measurement and attempt to obtain the specimen prior to the BOD POD.) Blood pressure is measured three times with the participant seated using the Omron 705 electronic machine. This device cannot measure blood pressures with a systolic > 240 mmHg and cannot measure pressure on participants who have a weak or irregular pulse. A standard manual device (aneroid sphygmomanometer) should be used for such mothers or if the Omron 705 electronic machine is not available. A manual device should also be used for obtaining duplicate measurements for quality control procedures (see Section **10.5.1.1**). **Note:** If the Omron 705 is **not** available, do **not** use another type of electronic machine.

The following equipment is required for blood pressure measurement:

- Omron 705 electronic machine and cuff; or cuff, sphygmomanometer, and stethoscope with bell
- Chair with back
- Table or desk

- Tape measure (Supplied by the Clinical Coordinating Center)

The mother is asked to remove outer garments. The sleeve of the shirt, blouse, etc. is to be rolled up so that the upper right arm is bare for the blood pressure cuff. (If the right arm is missing, injured, or deformed, expose the left arm.) The shirt must not constrict, and the blood pressure cuff should not be placed over the garment. Garments must be removed if obstructing, and a loose fitting gown provided.

Provide a quiet location for measurement of blood pressure. The mother is to be seated. When seated, the mother's right (measured) arm is to rest, palm up, with elbow on the desk or table so that the antecubital fossa is level with the heart. To achieve this, either the position of the mother in the chair is to be adjusted, or the arm raised or lowered on a comfortable support. Incorrect positioning of the mother's arm can introduce systematic error into the measurement. She is not to be placed in an uncomfortable position and legs are not to be crossed.

Before blood pressure is measured, the mother is to rest in the sitting position, with the cuff applied, and with no change in position for 5 minutes. Changes in posture or activity cause blood pressure to change. Conversation is to be limited. A brief explanation of the procedure can be repeated at this time. The mother should be told that when you inflate the cuff, it may feel a little tight on the upper arm for a short time. If this warning is not given, she is likely to respond to the discomfort of the tightness with a rise of blood pressure, due to fright or pain.

The observer should always use the same arm, the right arm. On average, the blood pressure measured from the right arm is slightly higher than that from the left. Therefore, one should consistently measure the pressure from the same arm. Check the appropriate box in question 4 on Physical Measurements-Mother for the arm on which blood pressure is measured. As noted above, the left arm may be used if the right arm is missing, injured, or deformed.

Check the appropriate box in question 5 to indicate if the Omron 705 is being used for blood pressure measurement. If it is not being used, skip to Section **5.6.2.3**.

### 5.6.2.2 Instructions - Omron 705 Electronic Machine:

Field centers should have three cuffs available (small, standard, large) which are provided with the Omron 705 electronic machine. Batteries should be changed every 4-6 weeks to ensure accuracy. Batteries are inserted into the back of the monitor and position contacts (+/-) are indicated by markings in the battery compartment. When ordering new batteries, specify 4 x 1.5 V alkaline batteries (LR or R 1.5 volts, i.e., AA batteries). A tape measure should also be available for measurement of the circumference of the mother's arm.

The procedure for measuring blood pressure using the Omron 705 electronic machine is as follows:

- Follow the general instructions regarding preparation for measurement (see Section **5.6.2.1** above).
- Ensure the appropriate cuff size is used. The standard cuff is 14 x 48 cm and can be used on an arm with a circumference of 22-32 cm. Arms of 32-42 cm require the use of the large cuff which is 16 cm x 65 cm. Arms < 18 cm require the use of the child/small adult cuff. If in doubt, measure the mother's arm.
- Palpate for the brachial artery (just above the cubital fossa, towards the inner side of the arm). Make a mark at the point of maximal pulsation.
- Position the cuff around the arm so that one finger can be inserted under the cuff and the bottom edge is positioned above the elbow joint, with the green mark over the brachial artery. This position ensures that the microphone is pressed against the brachial artery. Make sure that the top edge of the cuff is not restricted by clothing.
- Ask the mother to remain absolutely still and not to talk while her blood pressure is being measured, since the microphone is sensitive to vibration or movement of the arm.
- Connect the air tube to the air jack (on the left side of the device).
- Position the device such that the display is facing away from the mother.
- Press the On/Off power switch so that the machine is on. All display symbols will appear for approximately one second.
- When the machine is ready, the ready-to-measure symbol (♥) will appear on the display.
- Press "START" and the cuff then automatically inflates. The machine automatically determines the target value and when this value is reached, the measurements will be started.
- When the measurement is complete, air is automatically released from the cuff and the reading in the display drops.

- When the measurement has finished the “heart” shaped symbol (♥) appears on the display and the blood pressure and pulse rate values are displayed. The values are displayed for 5 minutes in total.
- Enter the blood pressure and heart rate reading in question 8 on Physical Measurements–Mother.
- Wait 1-2 minutes and repeat the procedure.
- Enter the second blood pressure and heart rate reading in question 9 on Physical Measurements–Mother.
- Wait 1-2 minutes and repeat the procedure a third time.
- Enter the third blood pressure and heart rate reading in question 10 on Physical Measurements–Mother.
- Enter your staff ID in question 11 on Physical Measurements–Mother.
- Turn the machine off by pressing the O/I button. (The machine switches off automatically after approximately 5 minutes, but should be switched off promptly to avoid running down the batteries.)

#### 5.6.2.3 Instructions - Manual Sphygmomanometer:

Field centers should have three cuff sizes available – adult, large adult, and child/small adult. Cuffs should be marked with two “Range Lines” on the inner surface of the cuff, and an “Index Line” at the end of the cuff, to indicate to the user whether the cuff is the correct size for the mother’s arm. The cuff is to be used like a tape measure, to be certain that the cuff is the correct size for the arm: hold the end of the cuff containing the rubber bladder over the upper arm, and pull the other end towards the participant under the arm. The Index Line should lie within the Range Lines for the cuff size; if the Index Line either falls directly on a Range Line or outside the range, select the next smaller or larger cuff. The center of the pocket holding the inflation bag is marked “0”.

Once the correct cuff size has been chosen, palpate for the brachial artery (just above the cubital fossa, towards the inner side of the arm). Make a mark at the point of maximum pulsation. This point is where the bell of the stethoscope should be placed during blood pressure measurement. Apply the cuff so that the “0” lies over the brachial artery. The lower edge of the cuff should be 1” to 1.5” (2-3 cm) above the cubital fossa. This is to allow sufficient

room for the bell of the stethoscope to be placed over the brachial artery immediately below the cuff. Be sure that the top edge of the cuff is not restricted by clothing.

To apply the cuff, place the left hand on top of the cuff and pull the other end with the right hand, underneath the arm in a wrapping movement. The outer edge of the cuff must be kept aligned so that the cuff is wrapped evenly around the arm. The cuff should be applied firmly-not enough to exert pressure on the brachial artery but firm enough to prevent the cuff from slipping. This can be tested by trying to slip two fingers under the edge of the cuff. Only the tip of the fingers should be able to fit between the cuff and the arm.

A standard stethoscope with a bell should be used. Korotkoff sounds are best heard with the bell because of their low pitch. Stethoscope tubing should be about 10-12 inches from the bell piece to the Y branching. When ready to use the stethoscope, check that the ear pieces are pointing downward and forwards, and insert into your ears.

The following procedure is to be followed in measurement of blood pressure with a manual sphygmomanometer:

- Palpate the mother's radial pulse with the fingers of your left hand. Inflate the cuff slowly and note the level (to the nearest 2 mm Hg) on the dial gauge at the point at which the radial pulse disappears. This is the pulse obliteration pressure. Immediately deflate the cuff. Enter the pulse obliteration pressure in question 6 on Physical Measurements-Mother.
- To the pulse obliteration pressure add 30. The resultant value is the peak inflation pressure. Enter this value in question 7 on the Physical Measurements-Mother form. For example, if the pulse disappeared when the dial gauge was nearest to 130, the peak inflation pressure is 160 (130 + 30 = 160). The cuff is to be inflated to the peak inflation pressure for each blood pressure measurement. Occasionally, the Korotkoff sounds may be heard as soon as one places the stethoscope over the brachial artery and begins to listen. If this happens, immediately deflate the cuff, and calculate a new peak inflation pressure by adding 10, and record the new peak inflation pressure in question 7.
- Be sure the stethoscope has the earpieces pointing forward and use the bell of the stethoscope. Ensure that the valve on the stethoscope head is turned to the bell position, or the sound will not be transmitted.

- Place the bell of the stethoscope over the mark for the brachial artery. The bell is not to touch the cuff, rubber tubing, or clothing. Occasionally, blood pressure sounds may be difficult to hear with the bell of the stethoscope. If this occurs, the measurement should be repeated with use of the diaphragm.
- Looking at the manometer with the center of the scale at eye level and the column perfectly upright, or at the dial gauge, inflate the cuff rapidly to a pressure equal to the peak inflation pressure.
- Control the pressure fall with the valve on the hand bulb to allow the column of mercury or the dial gauge to fall at a rate of 2 mm per second. This rate of fall is critical for accurate blood pressure measurement. Listen for the Korotkoff sounds with the stethoscope.
- Record the systolic and 5$^{th}$ phase diastolic blood pressure readings to the nearest 2 mm Hg. The systolic is recorded at the appearance of the first sound in a series of at least two sounds. The Phase 5 diastolic pressure, which corresponds to the disappearance of sound, is recorded at the mercury level 2 mm below the level at which the last sound was heard. (It is NOT at the level of the last sound.) If blood pressure sounds can be heard down to zero, record diastolic pressure as zero. Record the systolic and diastolic blood pressures in question 8 on Physical Measurements-Mother.
- After observing and recording systolic and diastolic blood pressure, release the remaining pressure by opening the bulb valve.
- Wait 1-2 minutes and repeat the procedure.
- Enter the second blood pressure reading in question 9 on Physical Measurements–Mother.
- Wait 1-2 minutes and repeat the procedure a third time.
- Enter the third blood pressure reading in question 10 on Physical Measurements–Mother.
- Enter your staff ID in question 11 on the Physical Measurements–Mother.

#### 5.6.2.4 Omron 705 Electronic Machine:

If you wish to interrupt measurement prematurely, press the “On/Off” button. Inflation is stopped and the air is automatically released from the cuff. If an error has occurred during

measurement, “E” or “EE”is displayed. The following describes failure and possible causes, and rectification.

| Type of Failure and Possible Causes | Rectification |
|---|---|
| Incorrect measurement as indicated by “E” | |
| 1. A correct reading could not be obtained because measurement was disturbed by movement of the body. | 1. Repeat the measurement with the participant keeping perfectly still. Tell the participant not to move or speak. |
| 2. The cuff is not fitted correctly. | 2. Check that the cuff is correctly fitted. Repeat the measurement. |
| 3. Clothing has constricted the blood flow. | 3. Ask the participant to remove the item of clothing which caused the constriction. |
| 4. There is still air in the cuff when the monitor is switched on. | 4. The unit may be defective. Return it to the HAPO FUS Project Manager. |
| The blood pressure values displayed are extremely low or high, or they are implausible. | Repeat the measurement. Use a manual sphygmomanometer if necessary. |
| The display shows a low voltage symbol. | Insert 4 new batteries. |
| The cuff pressure does not rise although the pump motor can be heard. | Check that the air tube is properly connected to the monitor. Push the tube connector firmly into the socket. |

If, after following the trouble-shooting procedures above, your machine is still not working properly, please contact Lynn Lowe, the Project Manager.

#### 5.6.2.5 Training

The centralized training session will include training on operation of the Omron 705. Before a staff member trained locally can begin to take blood pressure measurements on HAPO Follow-Up Study mothers, the Data Coordinating Center must be notified, and HAPO staff ID assigned, if not previously assigned.

#### 5.6.2.6 Blood Pressure Remeasurement

If the mean of the second and third systolic pressures is 140-179 and/or the mean of the second and third diastolic pressures is 90-109, remeasurement is not required. However, at the end of the visit the PI or other health professional is to give the mother a report with her blood pressure

values and is to advise the participant to see her health care provider within 1 month for repeat blood pressure measurement and further evaluation as needed.

If the mean of the second and third systolic pressures is $\geq$ 180 and/or the mean of the second and third diastolic pressures is $\geq$ 110, the blood pressure measurement must be repeated at the end of the study visit, and the Blood Pressure Repeat Measurement Form – Mother completed (see Section **5.13**).

### 5.6.3 Measurement of Height

Height is measured after measurement of blood pressure, and prior to the BOD POD measurements. The mother should be asked to change into the bathing suit needed for the BOD POD prior to the measurement of height and weight, and she should be provided with a robe or other cover-up. Shoes should be removed since they can affect the measurements.

Height is to be measured twice, resetting the height measure between each of the two measurements. If the two measures differ by 1 cm (0.5 inch) or more, do a third measurement.

1. A height rule should be taped vertically to a hard flat surface, with no moulding, with the base at floor level. A stadiometer may also be used, if available.
2. Check to be sure that the floor is level, the wall is at a 90$^{o}$ angle to the floor, the wall is straight and the measuring tape is mounted perpendicular to the floor. Daily stadiometer quality assurance is suggested, using a standard calibration rod (600mm – 800mm is generally used).
3. The floor surface must be hard, flat, and should not be carpeted or have other soft materials. If only a carpeted or variable surface is available, a wooden platform should be laid down to serve as the floor.
4. Heavy outer garments (jackets, coats, etc.) should be removed.
5. Hair ornaments should be re-positioned or removed prior to the measurement.
6. Shoes should be removed. Mark the appropriate box for question 12.
7. To measure height, have the participant stand with her back, head, buttocks and heels touching the back of the wall or stadiometer, feet will be slightly opened out. Note: not all participants will be able to achieve having all body parts connected to the back of the wall or

stadiometer. Ask the participant to hold her head in a position to look at a spot, head high, on the opposite wall, and take a deep breath.

8. The head should be in the Frankfurt horizontal plane. (The horizontal plane is defined by the lower margin of the bony orbit - the bony socket containing the eye – and the most forward part in the supratragal notch - the notch just above the anterior cartilaginous projections of the external ear.) (See figure).
9. Use a bar at right angles to the height rule or a large "L", and slide it down to the head so that the hair is pressed flat. (If a large "L" is used, the "L" can also be used to ensure that the head is in the Frankfort horizontal plane.)
10. Check the appropriate box in question 13 on Physical Measurements-Mother to indicate whether height is measured in centimeters or inches.
11. If using a non-digital stadiometer, reading must be taken at eye level, using a step stool.
12. Record the first height measurement on Physical Measurements-Mother in question 14a to the nearest 0.1 cm or the nearest 1/8$^{th}$ inch.
13. Have the participant step away from the height rule.
14. Repeat steps 6-8 above, and record the second height measurement in question 14b on Physical Measurements-Mother.
15. If the first and second measurements differ by more than 1 cm, perform a third measurement and record the value to the nearest 0.1 cm (1/8$^{th}$ inch) in question 14c on Physical Measurements-Mother.
16. Record the HAPO staff ID number of the person performing the height measurements in question 15.

### 5.6.4 BOD POD Measurements of Weight and Percent Fat

Measurements of weight and percent fat are obtained with the BOD POD equipment. The BOD POD uses air displacement plethysmography to determine the percent fat and fat-free mass. It is a simple 5 minute test which consists of measuring a person's weight using an electronic scale and volume, which is determined by sitting inside the BOD POD chamber. From these two measurements, the person's body composition is automatically calculated. Mark the appropriate box for question 16 on Physical Measurements-Mother to indicate whether the measurement was completed. If the BOD POD was not completed, indicate the reason in question 17 on Physical Measurements-Mother.

1. The floor surface on which the scale rests must be hard, flat, and without carpet or other soft materials.
2. Clothes should be removed and the person should change into a swimsuit (or other tight-fitting clothing like exercise shorts/top) and bathing cap which should fit snugly.
3. Basic information about the person being measured should be entered into the BOD POD computer (HAPO ID, etc.).
4. Calibrate the BOD POD.
5. For entering the height, if the heights recorded in questions 14a and 14b are identical, enter that value for the BOD POD. If the heights recorded in questions 14a and 14b are not identical, but differ by ≤ 1.0 cm or 0.5 in, use a calculator to determine the average of the two measurements, and enter that value for height for the BOD POD. If the heights recorded in questions 14a and 14b are not identical and a third measurement was required, use a calculator to determine the average of the two measurements that are closest together if they differ by ≤ 1.0 cm or 0.5 in, and enter that value for height for the BOD POD. If the differences among all three measurements are > 1.0 cm or 0.5 in, use a calculator to determine the average of all three measurements, and enter that value for height for the BOD POD.
6. Calibrate the BOD POD.
7. Weigh the participant on the digital scale.
8. Have the person enter the BOD POD chamber, and ask her to sit comfortably and quietly and to breath regularly. Tell her that she may hear sounds relating to valves opening and closing.
9. Transcribe the % fat measurement from the BOD POD to the nearest 0.1 % onto question 18 on Physical Measurements–Mother.
10. Transcribe the weight measurement from the BOD POD to the nearest 0.001 kg onto question 19 on Physical Measurements–Mother.
11. Enter the HAPO staff ID of the person completing the BOD POD measurements in question 20 on Physical Measurements-Mother.

#### 5.6.4.1 Measurement of Weight if BOD POD Test Refused:

If the participant refuses the BOD POD test, weight may still be obtained, as follows:

- From the main menu on the BOD POD software select “PRACTICE”
- Select the “MASS” activity

- Follow the instructions on the screen
- Record the mass measurement that is displayed on the screen to the nearest 0.001 kg onto question 19 on Physical Measurements–Mother.

### 5.6.5 Waist Circumference Measurements

Waist circumference measurements are to be obtained at 2 locations:

- Top of the iliac crest
- Midpoint between lowest rib and the iliac crest

The mother should be asked to remove the bathing suit, to put on her underclothing and to keep the robe or cover-up on.

#### 5.6.5.1 Top of the Iliac Crest:

1. Instruct the participant to cross her arms, and place her hands on opposite shoulders (demonstrate the desired position of the arms).
2. If necessary, lower the underclothing to slightly below the waist.
3. Stand on the mother's right side. Palpate the hip area to locate the right ilium of the pelvis. With a non-permanent marker draw a horizontal line just above the uppermost lateral border of the right ilium. Cross this mark at the mid-axillary line, which extends from the armpit down the side of the torso. (see Figure 1).
4. Extend the measuring tape around the waist. Position the tape in a horizontal plane at the level of the measurement mark. Check that the tape sits parallel to the floor and lies snug but does not compress the skin. Pull the tape measure until 1 red ball appears. Measurements are made at eye level.
5. Take the measurement at the end of the mother's normal expiration and record it to the nearest 0.1 cm in question 21a on Physical Measurements–Mother.
6. Repeat the measurement and record the second value to the nearest 0.1 cm in question 21b on Physical Measurements–Mother.
7. If the first and second measurements differ by more than 1.0 cm, perform a third measurement and record the value to the nearest 0.1 cm in question 21c on Physical Measurements–Mother.

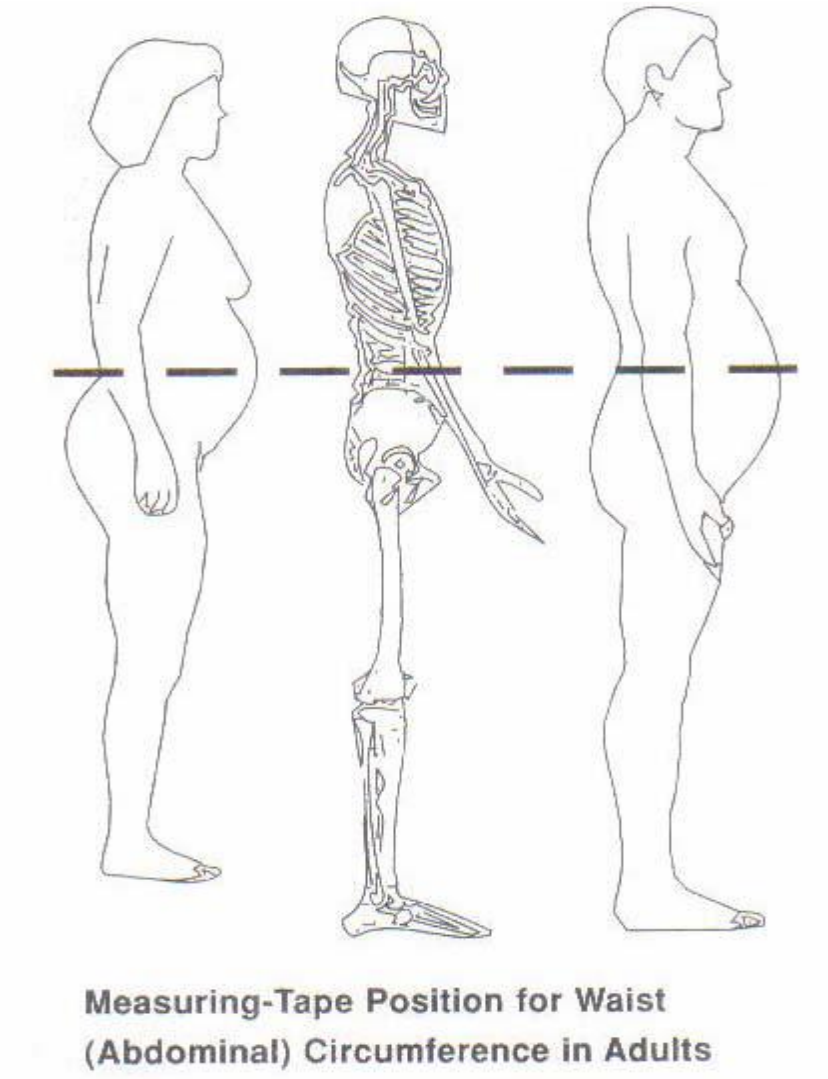


Figure 1. Waist Circumference – Top of Iliac Crest

**5.6.5.2 Midpoint between Lowest Rib and the Iliac Crest:**

1. After performing the measurement of waist circumference at the iliac crest, stand again on the mother's right side and locate the bottom of the rib cage and mark the spot. If it is difficult to palpate the rib, ask her to breathe in very deeply. Locate the rib as she breathes out and follow it as it moves down with your finger.
2. Using a tape measure, determine the midpoint between the top of the iliac crest and the bottom of the ribcage. (see Figure 2).
3. Extend the measuring tape around the waist at this midpoint. Position the tape in a horizontal plane at the level of the measurement mark. Check that the tape sits parallel to

the floor and lies snug but does not compress the skin. Pull the tape until 1 red ball appears. Measurements are read at eye level.

4. Take the measurement at the end of the mother's normal expiration and record it to the nearest 0.1 cm in question 22a on Physical Measurements–Mother.
5. Repeat the measurement and record the second value to the nearest 0.1 cm in question 22b on Physical Measurements–Mother.
6. If the first and second measurements differ by more than 1.0 cm, perform a third measurement and record the value to the nearest 0.1 cm in question 22c on Physical Measurements–Mother.

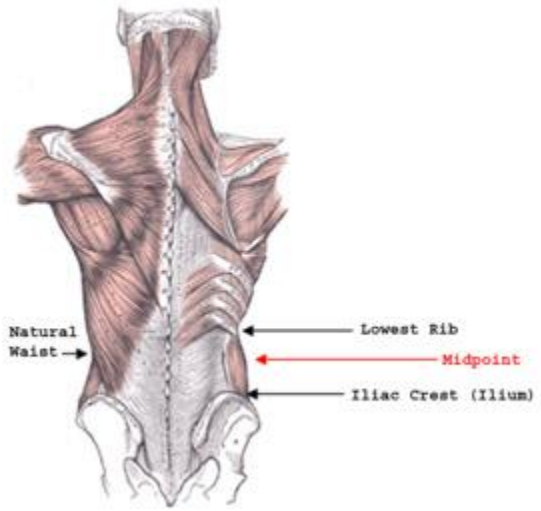


Figure 2. Waist Circumference – Midpoint (www.phenxtoolkit.org)

### 5.6.6 Hip Circumference Measurement

Hip circumference measurements should be done on the participant's right side (rather than in front) with her feet together.

1. Instruct the mother to stand erect but relaxed, with weight distributed equally over both feet.
2. Locate the level of maximal protrusion of the gluteal muscles (hips). (see Figure 3).
3. The measuring tape is placed horizontal around the body while the person obtaining the measurement is at eye level with the tape measure. Record the measurement to the nearest 0.1 cm in question 23a on Physical Measurements–Mother.

4. Repeat the measurement and record the second value to the nearest 0.1 cm in question 23b on Physical Measurements–Mother.
5. If the first and second measurements differ by more than 1.0 cm, perform a third measurement and record the value to the nearest 0.1 cm in question 23c on Physical Measurements–Mother.
6. Record the HAPO staff ID number of the person performing the measurements in question 24.

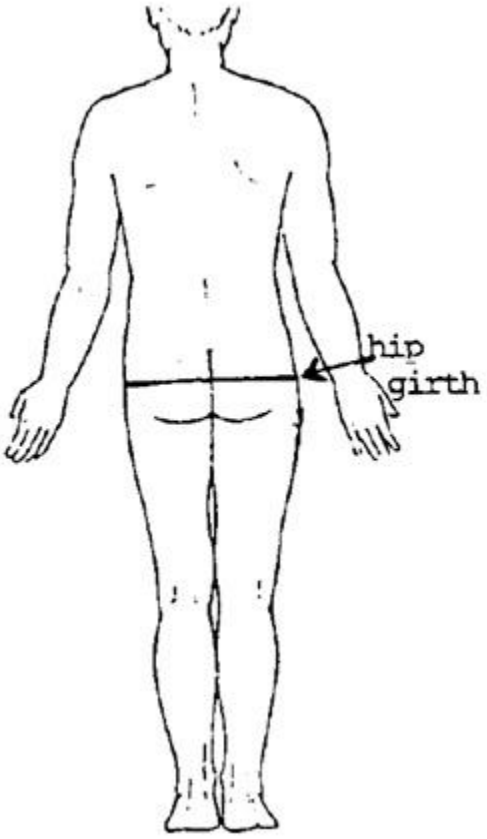


Figure 3. Hip Circumference (www.phenxtoolkit.org)

### 5.6.7 Data Entry Completion – Physical Measurements - Mother

The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID in question 25 on Physical Measurements–Mother to indicate the data have been entered, and check the box on the front of the form to indicate data entry is done. This form is to be re-entered (preferably not by the same person). After the data are re-entered, the person

doing the re-entry should check the box on the front of the form to indicate data have been re-entered.

### 5.6.8 Training

Field center staff will be trained in methods of measurement of height, weight/BOD POD, waist and hip circumference at the centralized training session. If field center staff who did not attend centralized training are to perform these measurements, they must be trained locally. This process involves review of the training materials that were presented at the centralized training.

Before a staff member trained locally can perform these measurements on HAPO Follow-Up Study participants, the Data Coordinating Center must be notified, and a HAPO staff ID assigned, if not previously assigned.

### 5.6.9 Measurement Results

Results from the measurements of blood pressure, height, weight, waist and hip circumference should be recorded on Physical Measurements-Mother. These measurements should also be provided to the participant along with BOD POD results.

With regards to the results of blood pressure measurement, if the mean of the second and third systolic pressures is 140-179 and/or the mean of the second and third diastolic pressures is 90-109, remeasurement is not required. At the end of the visit the PI or other health professional is to give the mother a report with her blood pressure values and is to advise the participant to see her health care provider within 1 month for repeat blood pressure measurement and further evaluation as needed.

If the mean of the second and third systolic pressures is $\geq$ 180 and/or the mean of the second and third diastolic pressures is $\geq$ 110, the blood pressure measurement must be repeated at the end of the study visit, and the Blood Pressure Repeat Measurement Form – Mother completed (see Section **5.13**).

## 5.7 OGTT Procedures

The OGTT is administered in the morning after at least three days of usual diet and usual physical activity. The test should be preceded by an overnight fast of 8-14 hours, during which only water may be consumed. The OGTT consists of a fasting blood sample followed by consumption of a standard glucose-containing drink, and a 2-hour sample taken 2 hours after the start of consumption of the drink.

All mothers who weigh 42.6 kg or more receive a standard 75g glucose load as a flavored 10 oz (296 ml) drink (Trutol) to be served chilled. Those weighing less than 42.6 kg will receive 1.75 g/kg. This should be consumed in its entirety in 5 minutes or less.

The presence of specific factors that could influence interpretation of the results of the test must be recorded. Such factors include: illness within the past 3 days, overnight fast < 8 hours, vigorous physical activity within the past 8 hours, or use of an inhaler within the past 8 hours. Illness is defined as the presence of any of the following: chills, fever, vomiting > 1 time, or diarrhea $\geq$ 3 times. Seasonal allergies would not be considered an illness.

A snack should be given to the participant soon after the 2-hour samples are drawn.

### 5.7.1 75g Glucose Drink

Each mother will be given a 75g dose of glucose (Trutol) as a drink. The pre-bottled drink contains a flavored solution which should be chilled prior to serving. The entire contents of the bottle (10 oz or 296 ml) should be emptied into a cup and served to the participant. **Note:** If the mother weighs less than 42.6 kg, refer to the Trutol Volume by Weight Chart (see end of chapter) to determine the volume she should be given. The volume should be precisely measured using a 250 ml graduated cylinder for doses equal to or less than 250 ml. For doses greater than 250 ml but less than 296 ml (296 ml is the amount in a full bottle), the amount over 250 ml should be precisely measured using an additional 50 ml graduated cylinder. **Note:** The Trutol solution should **not** be served on ice since this will alter the volume of solution. **Note:** Nothing, including water, should be consumed by the participant during the OGTT because this could change the rate of gastric flow and thereby affect OGTT results. **Note:** If it appears that there is < 296 ml in the bottle, do NOT top off.

### 5.7.2 Blood Drawing Procedure

Blood samples for glucose analysis will each be collected in 4 ml (grey top) tubes containing sodium fluoride and centrifuged to separate the plasma into two aliquots. One aliquot will be sent to the Laboratory Coordinating Center in the next shipment for determination of glucose concentration, and the second will be held temporarily at the field center as a backup and sent in a later shipment. Backup samples will be shipped to the Laboratory Coordinating Center for long-term storage no sooner than 4 weeks after they are frozen. Samples for insulin/C-peptide, lipids, storage, and A1c will also be obtained at the time of the fasting glucose sample. A sample for DNA will also be obtained, if the participant has specifically provided consent for this sample. At 2-hours samples for glucose and storage will be collected.

To obtain the blood samples:

1. Follow general instructions for blood drawing described in Section **4.2 General Instructions**. **Note:** Blood should be obtained from the antecubital vein, if possible, to minimize hemolysis of samples.
2. Draw the fasting blood samples. Record the time the fasting draw is completed on the OGTT Form-Mother.
3. Serve the glucose drink; instruct the participant to consume it in its entirety within 5 minutes. Record the time the participant begins to consume the glucose drink and the time the participant finishes the drink on the OGTT Form-Mother (using a 24-hour clock, e.g. 09:15).
4. Draw the 2-hour blood glucose and storage samples two hours after the glucose drink is started. These samples should be drawn as close to the 2-hour time as possible and within 10 minutes of the 2-hour interval. Record the clock time the 2-hour sample is drawn, e.g. 11:15.

### 5.7.3 OGTT Stopped – Not Rescheduled

- If the test is stopped and not completed because the participant vomited or fell ill, the test should be stopped and not rescheduled. Return to question 28 on the Test Qualification Form-Mother and check the appropriate box for the reason the test was stopped and not completed. If fasting samples were collected, place an “X” in the box at the top right of the OGTT Form-Mother to indicate that the OGTT was

incomplete. Then go to the Questionnaire. The forms from the incomplete OGTT should be entered.

- If the 2-hour glucose sample is not collected, try to reschedule the OGTT. If she refuses to reschedule, these same steps should be followed. Mark "Other" for question 28 on the Test Qualification Form-Mother and indicate that the 2-hour glucose was not collected.

### 5.7.4 OGTT Stopped – Rescheduled

If the 2-hour glucose sample was not collected and she agrees to reschedule, the Questionnaire should still be administered and the forms from the incomplete OGTT should be entered. **Note:** When she comes for the rescheduled OGTT, a **new** Test Qualification Form-Mother, OGTT Form -Mother, and OGTT Sample Processing Form -Mother **must** be completed and entered.

### 5.7.5 OGTT Blood Handling

Blood sample tubes and tube labels required for each of the samples are described below. After all of the blood samples for the OGTT have been drawn, they should be sent for processing accompanied by the OGTT Sample Processing Form-Mother. Make sure that the correct HAPO ID label has been affixed to each page of this form prior to sending it for processing.

#### 5.7.5.1 Fasting Blood Samples:

Blood sample tubes required:

- Fasting Glucose – 1 x 4 ml Grey top(sodium fluoride) tube
- Insulin/C-peptide – 1 x 6 ml Red top tube
- Lipids – 1 x 6 ml Red top tube
- Storage – 1 x 6 ml Red top tube
- DNA – 1 x 4 ml Purple top tube (if consented)
- A1c – 1 x 4 ml Purple top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 700 (Fasting Glucose tube)

- 1 x Bar-code label ending with digits 701 (Insulin/C-peptide tube)
- 1 x Bar-code label ending with digits 702 (Lipids tube)
- 1 x Bar-code label ending with digits 703 (Storage tube)
- 1 x Bar-code label ending with digits 704 (DNA tube) (if consented)
- 1 x Bar-code label ending with digits 705 (A1c tube)

Procedure:

- Before drawing blood ensure the correct tubes are available.
- Check that the correct labels have been affixed to the tubes and that the labels correspond to the participant's HAPO ID.
- Draw blood according to general procedures described in Section **4.2 General Instructions**.
- Gently invert the Grey and Purple top tubes 6 times to ensure mixing. Do not mix the Red top tubes (insulin/C-peptide, lipids and storage).
- Place the Grey top (glucose) and Purple top (DNA, A1c) tubes in ice (either ice cubes with a small amount of water or crushed ice). **Note:** Do **not** place Red top (insulin/C-peptide, lipids and storage specimen) tubes in ice, but let them sit at room temperature for clot retraction and release of serum prior to processing. These samples may, however, be placed on ice for transfer to the field center laboratory, but should then stand at room temperature for 60 minutes prior to processing if clot retraction has not already taken place during the OGTT.
- Transfer samples to the location where they will be processed, accompanied by the OGTT Sample Processing Form-Mother. **Note:** Samples must be processed within 2 ½ - 3 hours of when the fasting samples were drawn.

**5.7.5.2 2-Hour Blood Samples**:

Blood sample tubes required:

- Blood Glucose – 1 x 4 ml Grey Top tube
- Storage – 1 x 6 ml Red top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 720 (2-hour glucose)

- 1 x Bar-code label ending with digits 723 (2-hour storage)

Procedure:

- Follow the procedure described above for the Fasting Blood Samples.
- Gently invert the grey top tube 6 times to ensure mixing. Do not mix the Red top tube.
- Place the Grey top tube, in ice. **Note:** Do **not** place the Red top tube in ice, but let it sit at room temperature for clot retraction and release of serum prior to processing.

### 5.7.6 OGTT Form Completion

The OGTT Form-Mother is used to record the drawing of OGTT, insulin/C-peptide, lipids, storage, DNA (if consent obtained) and A1c blood samples. Make sure that the correct HAPO ID label has been affixed to the top of each page of the form. The following should then be completed on this form:

1. Enter the date of the visit, using year/month/day format, entering the last digit of the year.
2. Enter the weight measurement from the BOD POD scale.
3. Indicate whether the participant consented to having her blood and urine samples stored at the NIH.
4. Indicate whether the participant consented to having a DNA sample drawn.
5. Indicate in the checkbox whether the fasting glucose sample was drawn. **Note:** If the fasting glucose sample was **not** drawn, stop and cancel the OGTT, reschedule, and leave the remaining items on the form blank, except the checkbox at the top right of the form indicating that the test was incomplete. Return to and complete question 28 on the Test Qualification Form-Mother.
6. Indicate in the checkbox whether the fasting sample for insulin/C-peptide was drawn.
7. Indicate in the checkbox whether the fasting sample for lipids was drawn.
8. Indicate in the checkbox whether the fasting sample for storage was drawn.
9. Indicate in the checkbox whether the fasting sample for DNA was drawn.
10. Indicate in the checkbox whether the fasting sample for A1c was drawn.
11. Enter the time that the drawing of the fasting samples was completed, using 24-hour clock format.

12. Determine the volume of the glucose load (Trutol) in milliliters for the mother's OGTT and record it.
13. Enter the time the participant began to consume the glucose drink, using 24-hour clock format.
14. Enter the time the participant completed consuming the glucose drink, using 24-hour clock format. **Note:** If the woman vomited or fell ill prior to the 2-hour blood draw, stop the OGTT, and leave the remaining items on the form blank, except the checkbox at the top right of the form indicating the test was cancelled/incomplete. Return to and complete question 28 on the Test Qualification Form-Mother.
15. Indicate in the checkbox whether the 2-hour glucose sample was drawn.
16. Indicate in the checkbox whether the 2-hour sample for storage was drawn.
17. Enter the time the 2-hour samples were drawn, using 24-hour clock format.
18. Indicate in the checkbox whether there were any blood draw side effects observed during the visit or reported later. If the mother reports side effects after the visit, the answer on the form may need to be changed.
19. Indicate in the checkbox if all samples were sent for processing. **Note:** If blood drawing was not completed for any reason, answer question 28 on Test Qualification Form – Mother.
20. Enter the HAPO staff ID of person completing OGTT test progression portion of this visit. Check that the form has been completely filled out. Place the completed form in the participant's HAPO folder for entry into the REDCap Data Entry System.
21. The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID at the bottom of the form after the data have been entered and check the box at the top of the form to indicate data entry is done.

### 5.7.7 Aliquotting/Labeling

#### 5.7.7.1 General Instructions:

Specific instructions for handling each type of specimen are described according to the type of specimen. However, there are general instructions that must be followed for all blood specimens:

- Before filling specimen vials, make sure that the correct Bar-code label has been affixed vertically to the correct vial and that the Bar-code label corresponds to the HAPO ID on the blood sample tube.
- Blood specimen tubes must be processed within 2 ½ - 3 hours of when the fasting samples were drawn.
- Specimen vials should be filled such that the aliquot for analysis is made first, followed by the backup aliquot.

Blood and urine specimen vials, specimen vial labels, specific blood handling procedures required, and disposition of vials for each sample are as follows:

#### 5.7.7.2 Urine Sample:

Specimen vials required:

- 1 x Corning Cryovial with Orange screw top, without white insert. (Urine)
- 1 x Greiner Cryovial with Orange screw top, with white insert. (Urine)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 706 (Urine)

Procedure:

- Pipette 1.8 ml into each of the cryovials. Do not fill it above the 1.8 ml mark.

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store one vial (706) without white insert in a "Urine-Analysis" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 706 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (706) with white insert in a "Urine-Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 706 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

#### 5.7.7.3 Fasting Blood Glucose Sample:

Specimen vials required:

- 1 x Greiner Cryovial with Yellow screw top, without white insert. (Glucose)
- 1 x Greiner Cryovial with Yellow screw top, with white insert. (Glucose)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 700 (Glucose)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 700, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the plasma from the blood tube into 2 aliquot vials with yellow colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store one vial (700) without white insert in a “Blood Analysis” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 700 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

- Store the vial (700) with white insert in a “Blood Backup” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 700 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

#### 5.7.7.4 Fasting Insulin/C-peptide Sample:

Specimen vials required:

- 1 x Greiner Cryovial with Green screw top, without white insert. (insulin/C-peptide)
- 1 x Greiner Cryovial with Green screw top, with white insert. (insulin/C-peptide)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 701 (insulin/C-peptide)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 701, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the serum from the blood tube into 2 aliquot vials with green colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.

- Store the vial (701) without white insert in a “Blood Analysis” plastic freezer box at -20° C or colder, and attach one Bar-code label ending in digits 701 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.
- Store the vial (701) with white insert in a “Blood Backup” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 701 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

#### 5.7.7.5 Fasting Lipids Sample:

Specimen vials required:

- 1 x Greiner Cryovial with Clear screw top, without white insert. (lipids)
- 1 x Greiner Cryovial with Clear screw top, with white insert. (lipids)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 702 (lipids)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 702, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the serum from the blood tube into 2 aliquot vials with clear colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store the vial (702) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach one Bar-code label ending in digits 702 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (702) with white insert in a "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 702 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

**5.7.7.6 Fasting Storage Sample:**

Specimen vials required:

- 1 x Greiner Cryovial with Blue screw top, without white insert. (Storage)
- 1 x Greiner Cryovial with Blue screw top, with white insert. (Storage)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 703 (Storage)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 703, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the serum from the blood tube into 2 aliquot vials with blue colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store the vial (703) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 703 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (703) with white insert in a "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 703 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

#### 5.7.7.7 Fasting DNA Sample:

**Note:** This sample should **only** have been obtained if a separate consent form for this sample was signed.

**Note:** This sample is **not** centrifuged or aliquotted. The plastic purple top tube used for the DNA sample with Bar-code label ending with digits 704 should be inverted 6 times to ensure mixing and should then be placed in a cardboard freezer box at -20° C or colder prior to dispatch to the Laboratory Coordinating Center, and a Bar-code label ending with digits 704 attached to the box's Shipping Grid, corresponding to the tube's location in the cardboard box (see Section **7.1.5 Storage of DNA Samples**). Place the tube in the box so that only every other slot in the box is filled.

#### 5.7.7.8 Fasting A1c Sample:

Specimen vials required:

- 1 x Greiner Cryovial with Red screw top, without white insert. (A1c)
- 1 x Greiner Cryovial with Red screw top, with white insert. (A1c)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 705 (A1c)

Procedures:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Do **not** centrifuge blood tube labeled with digits 705.
- Suspend red blood cells uniformly by inverting the tube 6 times to ensure mixing.
- Place the whole blood from the blood tube into 2 aliquot vials with green colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store the vial (705) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 705 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (705) with white insert in "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 705 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

### 5.7.7.9 2-Hour Blood Glucose Sample:

Specimen vials required:

- 1 x Greiner Cryovial with Yellow screw top, without white insert. (Glucose)
- 1 x Greiner Cryovial with Yellow screw top, with white insert. (Glucose)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 720 (Glucose)

Procedures:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 720, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the serum from the blood tube into 2 aliquot vials with yellow colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store one vial (720) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 720 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (720) with white insert in "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 720 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

**5.7.7.10 2-Hour Storage Sample:**

Specimen vials required:

- 1 x Greiner Cryovial with Blue screw top, without white insert. (Storage)
- 1 x Greiner Cryovial with Blue screw top, with white insert. (Storage)

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 723 (Storage)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 723, preferably in a refrigerated centrifuge at +4° C, at 3000 RPM for 10 minutes.
- Place the serum from the blood tube into 2 aliquot vials with blue colored tops, one without white inserts and one with.
- Do **not** fill vials above the 2.0 ml mark. Place 0.5 ml into the Analysis cryovial (the one without the white insert). Place the remainder into the Backup cryovial (with white insert). If there is any excess, it should be placed in the Analysis vial and it should not be discarded. **Note:** The Analysis cryovial is the first priority. If there is less than 0.5 ml available in total, place whatever is available into this cryovial and do not put anything into the backup cryovial.
- **Ensure that no cells are transferred to sample vials.**

Disposition of vials:

- Follow the general instructions described in Section **4.6 Storage and Shipping** and the specific instructions described in Section **7 Storage and Shipment of Specimens**.
- Store the vial (723) without white insert in a “Blood Analysis” plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 723 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.
- Store the vial (723) with white insert in a “Blood Backup” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 723 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

### 5.7.8 OGTT Sample Processing Form Completion

Details of the processing and analysis should be recorded on the OGTT Sample Processing Form-Mother.

The following items on this form should be completed by the person processing the samples:

1. Enter the HAPO staff ID of the person who processed these specimens.
2. Check the appropriate box to indicate if glucose and any DNA samples (if a DNA sample was drawn) were received on ice.
3. Check the box to indicate if all samples were processed according to HAPO protocol. If 'Yes', skip to question 6.
4. Check the box to indicate if the fasting and 2-hour glucose samples were processed according to HAPO protocol. If 'Yes', skip to question 5.
    a. Check the appropriate box to indicate the number of aliquots (yellow cryovials) made from the fasting glucose sample. If no aliquots were made, stop and return the form to the HAPO Follow-Up Study as soon as possible.
    b. Check the appropriate box to indicate the number of aliquots (yellow cryovials) made from the 2-hour glucose sample.
5. Check the box to indicate if the insulin/C-peptide, lipids, storage, A1c and urine samples were processed according to the HAPO Follow-Up Study protocol. If 'Yes', skip to question 6.
    a. Check the appropriate box to indicate the number of aliquots (green top cryovials) made from the fasting insulin sample.
    b. Check the appropriate box to indicate the number of aliquots (clear top cryovials) made from the fasting lipids sample.
    c. Check the appropriate box to indicate the number of aliquots (blue top cryovials) made from the fasting storage sample.
    d. Check the appropriate box to indicate the number of aliquots (red top cryovials) made from the fasting A1c sample.
    e. Check the appropriate box to indicate the number of aliquots (orange top cryovials) made from the urine sample.
    f. Check the appropriate box to indicate the number of aliquots (blue top cryovials) made from the 2-hour storage sample.
6. Enter the time the samples were separated or aliquotted, using 24-hour clock format. **Note:** If samples were processed at different timepoints, the time of the final sample processing should be used, e.g., if the fasting samples were processed at timepoint A and the 2-hour samples were processed at timepoint B, timepoint B should be entered.

7. Enter the date the samples were separated or aliquotted, using year/month/day format, entering the last digit of the year.
8. The person performing the aliquotting should enter their HAPO staff ID for question 8.

The person who enters the data into the Data Entry System should complete question 9:

9. The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID after the data have been entered and check the box on the front of the form to indicate data entry is done.

### 5.7.9 OGTT Blood Flow Chart

The procedures for obtaining and processing specimens at the visit are summarized in a flowsheet (see the form: MOO-Processing-02-01-13.doc)

### 5.7.10 Quality Control

Data from the OGTT Form-Mother will be checked by the Data Coordinating Center for deviations from protocol. Specific items to be checked include timing of glucose administration and venipunctures.

## 5.8 Single Blood Draw Procedures

Women who are on oral medication or insulin for treatment of diabetes are not scheduled for an OGTT and are not asked to fast overnight. They will have a non-fasting single blood draw of all of the samples that are drawn prior to the start of the OGTT. Other circumstances that would lead to a single blood draw without an OGTT include having had bariatric surgery, treatment with medications that alter glucose metabolism (interfering medications), or unacceptable fasting status (originally scheduled for an OGTT but period of fasting insufficient and woman will not reschedule).

A blood sample for glucose analysis will be collected in a 4 ml (grey top) tube containing sodium fluoride and centrifuged to separate the plasma into two aliquots. One aliquot will be held up to two weeks prior to shipment to the Laboratory Coordinating Center for determination of glucose

concentration, and the second will be held temporarily at the field center as a backup. Backup samples will be shipped to the Laboratory Coordinating Center for long-term storage no sooner than 4 weeks after they are frozen. Samples for insulin, lipids, storage, and A1c will also be obtained. A sample for DNA will also be obtained, if the participant has specifically provided consent for this sample.

To obtain the blood samples follow general instructions for blood drawing described in Section **4.2 General Instructions**. **Note:** Blood should be obtained from the antecubital vein, if possible, to minimize hemolysis of samples.

### 5.8.1 Single Blood Draw – Blood Handling

The samples collected at the single blood draw should be handled as outlined for the fasting OGTT samples in Section **5.7.5.1**. Aliquotting and labeling should be performed as described in Sections **5.7.5.1** and **5.7.7.2 through 5.7.7.7**.

### 5.8.2 Single Blood Draw Form Completion

The Single Blood Draw Form–Mother is used to record the drawing of the glucose, insulin, lipids, storage, DNA (if consent obtained) and A1c blood samples. Make sure that the correct HAPO ID label has been affixed to the top of each page of the form. The following should be completed on this form:

1. Enter the date of the visit, using year/month/day format, entering the last digit of the year.
2. Indicate whether the participant consented to having her blood and urine samples stored at the NIH.
3. Indicate whether the participant consented to having a DNA sample drawn.
4. Indicate in the checkbox whether the glucose sample was drawn.
5. Indicate in the checkbox whether the sample for insulin/C-peptide was drawn.
6. Indicate in the checkbox whether the sample for lipids was drawn.
7. Indicate in the checkbox whether the sample for storage was drawn.
8. Indicate in the checkbox whether the sample for DNA was drawn.
9. Indicate in the checkbox whether the sample for A1c was drawn.

10. Enter the time that the drawing of the samples was completed, using 24-hour clock format.
11. Indicate in the checkbox if there were any blood draw side effects observed at the visit or reported later. If the mother reports side effects after the visit, the answer on the form may need to be changed.
12. Indicate in the checkbox if all samples were sent for processing.
13. Enter the HAPO staff ID of person completing the single blood draw. Place the completed form in the participant's HAPO folder for entry into the REDCap Entry System.
14. The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID at the bottom of the form after the data have been entered and check the box at the top of the form to indicate data entry is done.

### 5.8.3 Processing-Single Blood Draw Samples

Details of the processing should be recorded on the Single Blood Draw Sample Processing Form-Mother.

The following items on this form should be completed by the person processing the samples:

1. Enter the HAPO staff ID of the person who processed these specimens.
2. Check the appropriate box to indicate if glucose and any DNA samples (if a DNA sample was drawn) were received on ice.
3. Check the box to indicate if all samples were processed according to HAPO protocol. If 'Yes', skip to question 4.
    a. Check the appropriate box to indicate the number of aliquots (yellow top cryovials) made from the glucose sample.
    b. Check the appropriate box to indicate the number of aliquots (green top cryovials) made from the insulin/C-peptide sample.
    c. Check the appropriate box to indicate the number of aliquots (clear top cryovials) made from the lipids sample.
    d. Check the appropriate box to indicate the number of aliquots (blue top cryovials) made from the storage sample.

e. Check the appropriate box to indicate the number of aliquots (red top cryovials) made from the fasting A1c sample.
f. Check the appropriate box to indicate the number of aliquots (orange top cryovials) made from the urine sample.

4. Enter the time the samples were separated or aliquotted, using 24-hour clock format. **Note:** If samples were processed at different timepoints, the time of the final sample processing should be used, e.g., if the fasting samples were processed at timepoint A and the 2-hour samples were processed at timepoint B, timepoint B should be entered.
5. Enter the date the samples were separated or aliquotted, using year/month/day format, entering the last digit of the year.
6. The person performing the aliquotting should enter their HAPO staff ID for question 6.

The person who enters the data into the Data Entry System should complete question 7:

7. The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID after the data have been entered and check the box on the front of the form to indicate data entry is done.

### 5.9 Storage of Specimens

Details of specimen storage are described in Section **7 Storage and Shipment of Specimens**. Specimens should be placed in freezer boxes immediately following processing. As each specimen is placed in a freezer box, an ID label containing the identical Bar-code label should be placed on the Shipping Grid for the specific freezer box, indicating the position of the specimen in the freezer box.

Cryovials with white inserts should be stored in plastic freezer boxes that are specifically labeled as either “Blood Backup” or “Urine Backup” storage boxes. Cryovials without white inserts should be stored in separate plastic freezer boxes specifically labeled as “Blood Analysis” or “Urine Analysis” storage boxes for Laboratory Coordinating Center analysis. Samples should be placed in the plastic freezer boxes according to the instructions provided in Sections **7.1.3, 7.1.4** and **7.1.5**.

Purple top tubes containing DNA samples should be placed in cardboard freezer boxes. These boxes should be filled according to the instructions provided in Section **7.1.6**.

**Note:** It is very important that the correct HAPO ID Bar-code label is affixed to the correct location on the Shipping Grid, as this is the only efficient means of identifying the location of specific specimens.

### 5.10 Questionnaire

The Questionnaire is an interviewer-administered questionnaire that collects data on the participant's demographics, family history of diabetes and hypertension, smoking, alcohol, medication and medical history of the mother, HAPO child, and the child's biological father. (The Questionnaire must be translated into the local language for centers in which English is not the primary language, or field centers that expect to have > 10-15% participants who prefer conversing in a language other than English and where local HAPO staff are fluent in that language, with back translation by the Data Coordinating Center to ensure uniformity of meaning.) In administering the questionnaire, it is important to follow instructions precisely and to use the scripts provided. It is critical that the questionnaire be handled in a standardized manner. There must be a quiet, private space provided for meeting with the participant and completing the questionnaire. The data collector must remain neutral. Interpretation of questionnaire items for participants should be kept to a minimum.

#### 5.10.1 Instructions for Administering the Questionnaire

The Questionnaire is to be administered to the mother during the visit, between collection of the fasting and 2-hour OGTT samples or after collection of the single blood draw samples for those with special circumstances (treatment with oral medication or insulin for diabetes, bariatric surgery, interfering medications, inadequate fasting).

**Note:** If the mother has already completed the Questionnaire at a prior visit when she was pregnant or breast-feeding and the child had his/her visit, ask her to review it to see if any of the information she provided has changed and, if yes, update the Questionnaire accordingly.

Prior to administering the questionnaire, make sure that the correct HAPO ID has been affixed to each page of the form.

In addition, prior to administering the questionnaire, the staff member should state the following to the participant in a supportive conversational manner:

- "I will read each question and tell you how you can respond to the question. I will repeat each question as often as necessary. And I will read each possible response. For some questions, I can also explain what a response means, if the meaning is not clear to you."
- "When you are clear about the question I have read to you, and you have decided how to respond, tell me which response you have selected."

Read the Introduction section of the Questionnaire to the participant prior to asking the questions on the questionnaire.

Question 1:
Enter the current date, using year/month/day format, entering the last digit of the year. (**Note:** This question is **not** asked of the participant but should be completed before you begin to ask the participant the rest of the questions.)

Question 2:
Mark the appropriate boxes to indicate whether the mother gave agreement to be recontacted for future research studies for herself and for her child (copy the answers from their consent forms.)

**Sociodemographics - Mother**

Question 3:
Enter the participant's date of birth using year/month format, entering the last two digits for year.

Question 4:
Marital status or living arrangement means the participant's most current status. All of the responses should be read before the participant responds in order that all options are heard. It

is important for the participant, not the interviewer, to determine the appropriate classification. An explanation of the options is summarized below.

Check the one response which describes the participant's current marital status:

- Now married—Those individuals who consider themselves as married, whether or not the spouse currently resides in the household (e.g. spouse in the armed forces, spouse in different city due to job, etc.) unless separated.

- Living together in a marriage-like relationship—Those individuals who live with another individual in a long-term relationship that the person does not consider a marriage. Long-term roommates who are not intimate partners do not qualify for this designation. These relationships can be either homosexual or heterosexual in nature.

- Separated—Those individuals who have been deserted or who have parted because they no longer want to live together, but have not obtained a divorce. This classification applies whether or not the separation is legal.

- Divorced—Those individuals who have been legally divorced and whose marital status has not since changed; however, someone who is divorced and remarried is considered "married".

- Widowed—Those individuals who are a widow whose marital status has not changed since the death of the spouse.

- Never married—Those individuals who have never been married or whose only marriage was annulled.

Question 5:

Current employment status refers to the participant's current employment. Employment means working for pay at a job, running one's own business or profession, or working without pay in a family business. Accept the participant's definition of full-time and part-time work. Although a participant may, for example, both work part-time and be a student, the participant is asked to

select the one category that **best** describes her current status. The determination of best is left to the participant.

Question 6:

Read the list of possible response options for ethnic origin for your center and ask the participant to choose only one. Enter the number corresponding with the selected option.

Question 7:

Ask her how many years of school she has completed and enter the number. **Note:** This includes kindergarten through post-secondary education).

**Family History of Diabetes - Mother**

Questions 8-11:
These questions pertain to a history of diabetes in first degree **blood** relatives (mother, father, siblings, children). If a person is adopted and knows nothing about a history of diabetes in her birth parents, select 'Don't know'. For questions pertaining to sisters and brothers, only information for full blood (natural) sisters and brothers should be reported. Information for half-brothers/sisters should **not** be reported. If the participant is unsure about whether a first degree blood relative had diabetes, 'Don't know' should be selected.

**Family History of Hypertension - Mother**

Questions 12-15:

These questions pertain to a history of hypertension (high blood pressure) in first degree **blood** relatives (mother, father, siblings, children). If a person is adopted and knows nothing about a history of hypertension in her birth parents, select 'Don't know'. For questions pertaining to sisters and brothers, only information for full blood (natural) sisters and brothers should be reported. Information for half-brothers/sisters should **not** be reported. If the participant is unsure about whether a first degree blood relative had hypertension, 'Don't know' should be selected.

**Smoking - Mother**

Question 16-19:

These questions refer to current smoking. The mother is asked to select the category that is most representative of the average number of cigarettes smoked on a typical day. **Note:** Do **not** include 'Refused' and 'Don't know' in the list of possible categories read to the participant. Check 'Refused' only if the participant indicates that she is unwilling to answer the question. Check 'Don't know' if the participant has smoked some cigarettes but is unsure of the quantity. Ask her whether she uses any other kind of tobacco regularly. Then ask whether her HAPO child smokes and whether anyone else in the household smokes.

**Alcohol - Mother**

Question 20:

Drinks of alcohol refers to alcohol current alcohol consumption. A drink refers to, for example, a glass of wine, a bottle of beer, or a drink containing hard liquor. The participant is asked to select the category that is most representative of her average consumption on a typical day. **Note:** Do **not** include 'Refused' and 'Don't know' in the list of possible categories read to the participant. Check 'Refused' only if the participant indicates that she is unwilling to answer the question. Check 'Don't know' if the participant has consumed some alcohol but is unsure of the quantity.

If the participant does not know the average number of drinks per day, but can report a total number per week, then the total should be divided by 7 to determine the average per day. Similarly, if the participant indicates that her use varies such that she might have 5 drinks on Saturday night and none the rest of the week, ask her for the total per week and, again, divide by 7 to determine the average number per day.

**Medical History and Medication Use - Mother**

Questions 21-34:

This series of questions refers to the participant's current menstrual status, use of birth control pills or other hormonal contraceptives, treatment with hormone replacement therapy for menopause (define in accordance with local terminology), medication for treatment of hypertension or high cholesterol, history of heart attack or stroke, intentional weight loss of 10 pounds (4.5 kg) or more in the past year. **Note:** For question 21, if the mother indicates that she's not having periods because she has an IUD or uses some other type of contraceptive that prevents periods, the answer should be "No".

**Medical History and Medication Use – HAPO Child's Father**

Questions 35-45:

This series of questions refers to the HAPO child's father's weight and height, medication for treatment of diabetes, hypertension or high cholesterol, and history of heart attack or stroke.

**Pregnancy and Breastfeeding - Mother**

Questions 46-52:

This series of questions refers to the number of subsequent pregnancies, breastfeeding history of the HAPO baby, and presence during the HAPO pregnancy of any major life stressors.

**Physical Activity and Sleep - Mother**

Questions 53-57:

This series of questions refers to the frequency of vigorous activity as well as typical hours of sleep.

**Medical History and Medication Use – Child**

Questions 58-65:

This series of questions refers to the HAPO child's date of birth, gender, and for girls, menstrual history. It also includes questions that refer to whether the HAPO child has had any health problems that could result in slow growth. Problems absorbing food may include conditions such as celiac disease, Crohn's, inflammatory bowel disease, cystic fibrosis, ulcerative colitis. Stomach problems may include conditions such as irritable bowel syndrome, ulcers. Intestinal problems may include Crohn's disease, irritable bowel syndrome. Liver problems may include hepatitis.

**Physical Activity and Sleep - Child**

Questions 66-71:

This series of questions refers to the amount of time the HAPO child spends watching TV or playing computer games not requiring physical activity, as well as typical hours of sleep.

**Note to Interviewer:** Check the answer to question 2a and 2b on the first page of this form. If the answer to question 2a or 2b on the first page of this form is "yes" ask the participant to complete the Future Contact Form.

**Form Completion**

Question 72:

Enter your HAPO staff ID.

Question 73:

This question is to be completed by the person entering the data from the Questionnaire into the Data Entry System. After the data have been entered, check the box at the top of the form to indicate data entry is done. This form is to be re-entered (preferably not by the same person). After the data are re-entered, the person doing the re-entry should check the box on the front of the form to indicate data have been re-entered.

You have come to the end of the Questionnaire. To complete the interview:

- Review the form to ensure that all questions have been answered, that a response has been marked for each question that was not skipped, and that the correct HAPO ID has been affixed to each page.
- Thank the mother for taking the time to answer the questions and remind her again that her responses will be kept confidential.

### 5.10.2 Entering Data from the Questionnaire

At each field center, the information from completed questionnaires will be entered via the REDCap System provided by the Data Coordinating Center.

### 5.10.3 Training

Field center staff will receive training in general questionnaire administration and on the Questionnaire at the centralized training session.

## 5.11 Study Visit Variation

To record any variation during the study visit, the Study Visit Variation Form should be completed. This might include consumption of water or something else during the visit which should not occur until the conclusion of the blood drawing. Or, it might include instances where all aspects of the visit were completed up to the point of the blood drawing but blood drawing was not successful and blood drawing is rescheduled. Or, it might include instances where the urine sample is collected but the blood samples aren't collected until a rescheduled visit. This form should NOT be used to record a special circumstance encountered during recruitment (use the Special Circumstances Form instead). And, it should NOT be used to record reasons why the OGTT was not completed (use the last page of the Test Qualification Form instead). The recorded data should be entered into REDCap. **Note:** When a visit variation occurs, an email should be sent to the Data Coordinating Center describing the variation.

## 5.12 2-hour Glucometer Measurement

At the end of the visit, a small sample of blood collected at the 2-hour time-point should be measured using a glucometer. If the result is greater than 270 mg/dl or 15 mmol/l the mother is

to be given the result and referred to her health care provider or sent to the Emergency Room for immediate evaluation and treatment as needed.

### 5.13 Blood Pressure Remeasurement

If the mean of the second and third systolic pressures is ≥ 180 and/or the mean of the second and third diastolic pressures is ≥ 110, the blood pressure measurement must be repeated at the end of the study visit, and the Blood Pressure Repeat Measurement Form – Mother completed and the recommended follow-up given according to this form.

#### 5.13.1 Completing the Blood Pressure Remeasurement Form

For question 1 enter the time at which the first repeat blood pressure was measured after completion of the study visit.

Have the participant sit quietly for 5 minutes. Measure the blood pressure and record it in question 2.

After the participant sits quietly for 1-2 additional minutes, measure the blood pressure and record it in question 3.

After the participant sits quietly for 1-2 additional minutes, measure the blood pressure and record it in question 4.

The staff person doing these repeat measurements should record their staff ID for question 5.

For question 6 mark the recommendation for follow-up. If the mean of the second and third systolic blood pressures is < 140 and the mean of the second and third diastolic pressures is < 90, mark “none”. If the mean of the second and third systolic blood pressures is 140-179 and/or the mean of the second and third diastolic blood pressures is < 90-109, mark “Follow-up with primary health care provider for repeat BP within one month”. If the mean of the second and third systolic blood pressures is >= 180 and/or the mean of the second and third diastolic pressures is >= 110, mark “”Follow-up with primary health care provider or Urgent Care Center

for repeat BP within 24 hours". And, if another recommendation is made mark "Other" and indicate what the recommendation was.

If there are any notes or comments to be made, particularly if there are symptoms, record these in question 7.

The person who enters this form into the Data Entry System should record the HAPO staff ID in question 8. After the data have been entered, check the box at the top of the form to indicate that data entry is done.

## HAPO FOLLOW-UP STUDY
## BLOOD PRESSURE REPEAT MEASUREMENT FORM - MOTHER

**NOTE: This form should only be used if blood pressure measurements are repeated for the mother at the end of the Study Visit due to mean systolic ≥ 180 and/or mean diastolic ≥ 110.**

**1.** Time at which first blood pressure was measured after completion of study visit:

__ __ : __ __

**2.** Seated arm blood pressure reading 1:
[after sitting 5 minutes] __ __ __ / __ __ __ mmHg

**3.** Seated arm blood pressure reading 2:
[after sitting an additional 1-2 minutes] __ __ __ / __ __ __ mmHg

**4.** Seated arm blood pressure reading 3:
[after sitting an additional 1-2 minutes] __ __ __ / __ __ __ mmHg

**5.** HAPO staff ID of person measuring blood pressure: __ __ __ __ __ __

**6.** Recommendation for follow-up:
CHECK ONLY ONE BOX

- ☐ None
- ☐ Follow-up with primary health care provider for repeat BP within one month
- ☐ Follow-up with primary health care provider or Urgent Care Center for repeat BP **within 24 hours**
- ☐ Other

(If "Other", please specify: _____)

**7.** Notes/Comments: _____

**8.** HAPO staff ID of person entering data into Data Entry System: __ __ __ __ __ __

## Trutol Volume by Weight Chart

| Weight (kg) | Trutol (ml) |
|---|---|
| 20.0 – 20.2 | 140 |
| 20.3 – 20.5 | 142 |
| 20.6 – 20.8 | 144 |
| 20.9 – 21.1 | 146 |
| 21.2 – 21.4 | 148 |
| 21.5 – 21.7 | 150 |
| 21.8 – 22.0 | 152 |
| 22.1 – 22.2 | 154 |
| 22.3 – 22.5 | 156 |
| 22.6 – 22.8 | 158 |
| 22.9 – 23.1 | 160 |
| 23.2 – 23.4 | 162 |
| 23.5 – 23.7 | 164 |
| 23.8 – 24.0 | 166 |
| 24.1 – 24.3 | 168 |
| 24.4 – 24.6 | 170 |
| 24.7 – 24.9 | 171 |
| 25.0 – 25.1 | 174 |
| 25.2 – 25.4 | 176 |
| 25.5 – 25.7 | 178 |
| 25.8 – 26.0 | 180 |
| 26.1 – 26.3 | 182 |
| 26.4 – 26.6 | 184 |
| 26.7 – 26.9 | 186 |
| 27.0 – 27.2 | 188 |
| 27.3 – 27.5 | 190 |
| 27.6 – 27.7 | 192 |
| 27.8 – 28.0 | 194 |

| Weight (kg) | Trutol (ml) |
|---|---|
| 28.1 – 28.3 | 196 |
| 28.4 – 28.6 | 198 |
| 28.7 – 28.9 | 200 |
| 29.0 – 29.2 | 202 |
| 29.3 – 29.5 | 204 |
| 29.6 – 29.8 | 206 |
| 29.9 – 30.1 | 208 |
| 30.2 – 30.4 | 210 |
| 30.5 – 30.6 | 212 |
| 30.7 – 30.9 | 214 |
| 31.0 – 31.2 | 216 |
| 31.3 – 31.5 | 218 |
| 31.6 – 31.8 | 220 |
| 31.9 – 32.1 | 222 |
| 32.2 – 32.4 | 224 |
| 32.5 – 32.7 | 226 |
| 32.8 – 33.0 | 228 |
| 33.1 – 33.3 | 230 |
| 33.4 – 33.5 | 232 |
| 33.6 – 33.8 | 234 |
| 33.9 – 34.1 | 236 |
| 34.2 – 34.4 | 238 |
| 34.5 – 34.7 | 240 |
| 34.8 – 35.0 | 242 |

| Weight (kg) | Trutol (ml) |
|---|---|
| 35.1 – 35.3 | 244 |
| 35.4 – 35.6 | 246 |
| 35.7 – 35.9 | 248 |
| 36.0 – 36.1 | 250 |
| 36.2 – 36.4 | 252 |
| 36.5 – 36.7 | 254 |
| 36.8 – 37.0 | 256 |
| 37.1 – 37.3 | 258 |
| 37.4 – 37.6 | 260 |
| 37.7 – 37.9 | 262 |
| 38.0 – 38.2 | 264 |
| 38.3 – 38.5 | 266 |
| 38.6 – 38.8 | 268 |
| 38.9 – 39.0 | 270 |
| 39.1 – 39.3 | 272 |
| 39.4 – 39.6 | 274 |
| 39.7 – 39.9 | 276 |
| 40.0 – 40.2 | 278 |
| 40.3 – 40.5 | 280 |
| 40.6 – 40.8 | 282 |
| 40.9 – 41.1 | 284 |
| 41.2 – 41.4 | 286 |
| 41.5 – 41.6 | 288 |
| 41.7 – 41.9 | 290 |
| 42.0 – 42.2 | 292 |
| 42.3 – 42.5 | 294 |
| 42.6 + * | 296 * |

* whole bottle of Trutol