# 6. VISIT - CHILD

All children of mothers who provide consent will undergo a clinic visit that includes 3 components:

- Physical measurements (blood pressure, anthropometrics, and pubertal assessment)
- Blood sampling, either a 2-hour 75g oral glucose tolerance test (OGTT), non-fasting blood sampling if the child has diabetes and is on medication for it (Single Blood Draw), or a Single Blood Draw under special circumstances where the child might have been originally scheduled for an OGTT (see Section **6.4.1**)
- Questionnaire (administered to the mother)

Field center staff is 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.

## 6.1 Forms Used for the Visit – Child

The data forms used during the Visit – Child include:

- Test Qualification Form–Child – (see Section **6.4**)
- Physical Measurements–Child – (see Section **6.6**)
- OGTT Form–Child – (see Section **6.7.5**)
- OGTT Sample Processing Form–Child – (see Section **6.7.7**)
- Single Blood Draw Form–Child – (see Section **6.8.2**)
- Single Blood Draw Sample Processing Form–Child - (see Section **6.8.3**)

And, if not already completed during the Visit – Mother:

- Questionnaire – (see Section **6.10**)

Additional forms that may be needed during the visit include:

- Call Back Register – (see Section **6.3**)
- Phone Call Information
- *Study Visit Variation – (see Section **6.11**)*
- *Blood Pressure Repeat Measurement Form – Child (see Section **6.13.1**)*

**Note:** The Single Blood Draw forms will only be used for children 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-Child and Metformin Use Interview-Child which are only used in the special circumstance where the child takes Metformin but a diagnosis of diabetes was not reported (see Section **6.4.1** item 3).

### 6.2 Consent

Informed consent for the child's participation in the follow-up study 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-Child. 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 child during the study and the risks involved. Make certain that the mother 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-Child, 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-Child 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-Child is the opportunity to agree to or decline the long term storage of her child's 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-Child and this should also be noted on the OGTT Visit Form-Child or Single Blood Draw Form-Child by marking 'Yes' or 'No'.

Also included on the Consent Form-Child is the opportunity to have a child's sample for DNA collected. The mother's choice should be marked on the Consent Form-Child and this should also be noted on the OGTT Form-Child or Single Blood Draw Form-Child 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-Child also offers the opportunity to be recontacted for future research studies. The mother's choice for her child should be marked on the Consent Form-Child and this should also be noted on the Questionnaire by marking 'Yes' or 'No' for question 2b.

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

Each field center IRB or Ethics Panel will have its own policy as to whether it is necessary for the child to sign an Assent Form. Whether or not a signed Assent Form is required, the study should be explained to the child in language that the child can understand and which has been approved by the IRB or Ethics Panel. If a signed Assent Form is required, a copy of the signed form should be given to the mother and the original filed in her HAPO Follow-Up Study file.

**Note:** Whether or not the local IRB or Ethics Panel requires the child to sign an Assent Form, field center staff should make every effort to ensure that the child does not feel coerced to participate in the study, even though the mother has signed the Consent Form-Child.

### 6.3 Call Back Register

The Test Qualification Form-Child 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-Child or if the mother and child missed their scheduled study visit.

The Call Back Register includes space to copy the mother'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 mother is called back.

**Call Back Reason Codes**

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

'M-C': If the child is on metformin and the mother needs to confirm reason for use with the doctor's office, enter 'M-C' 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'.

### 6.4 Completing the Test Qualification Form-Child

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-Child. This set of questions, which are asked of the mother, will ensure that the child meets the requirements and conditions for the BOD POD portion of the physical measurements and, if applicable, the OGTT. The Test Qualification Form-Child contains questions on illnesses within the past 3 days; diet within the past 3 days; the time the child last exercised vigorously; use of a steroid inhaler within the past 8 hours; the time the participant last smoked; the time that the child last ate or drank anything other than water, including taking any medications; the time that the child

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 child'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 mother, enter the time using the 24-hour clock. For question 3, indicate whether the child 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 that her child has diabetes. If 'No', confirm that the child will do the OGTT and skip to question 6. If 'Yes', ask her if the child takes oral medication or insulin for treatment of diabetes. If 'Yes', confirm the child will do a single blood draw. If 'No' confirm the child will do the OGTT.

**HIV, Hepatitis B or Hepatitis C**

Questions 6-8:

Ask the mother if a medical person has ever told her that her child has HIV, hepatitis B or hepatitis C. If 'No', skip to question 9. If 'Yes', due to international shipping regulations her child's blood samples cannot be shipped. Complete questions 7 and 8 to evaluate the child's fasting status and readiness for the BOD POD. Then stop, cancel the OGTT or single blood draw. Enter your HAPO staff ID in question 25 and go to Physical Measurements - Child and Questionnaire.

**Medications, Questions 9-10**

'No' to Question 9:

If the child is not taking any medications regularly, skip to the directions preceding question 11.

'Yes' to Question 9:

Go to question 10 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 10a:

Go to question 10b.

'Yes' to Question 10a:

Skip to question 23. Even if the child was originally scheduled for an OGTT the child will now do a single blood draw.

Question 10a – forgot medications:

Enter 'FM-C' 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 10b:

Proceed to the instructions before question 11.

Question 10b – 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 23.

Question 10b – 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-C' on the Call Back Register with an Anticipated Call Back Date (yy/mm/dd) of two weeks from the visit date. Give her a Metformin Use–Child Form with her ID label attached.

Question 10b – Forgot medications:

Proceed to the instructions before question 11. An entry of 'FM-M' should already be entered for Call Back Reason in the Call Back Register from question 10a. After the call, send the Metformin Use–Child form if necessary. Correct the answer to question 10b once the answer is known.

Instructions before Question 11:

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

**Illnesses in the Past 3 Days**

Question 11:

Ask the mother if her child 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 12:

Ask the mother if her child has eaten his or her typical or usual diet for the last three days.

**Time of Last Vigorous Physical Activity**

Questions 13-14:

Ask the mother if her child 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 the child worked up a sweat. If 'No', skip to question 15. If 'Yes', ask her when the child finished exercising vigorously. Enter the time, using the 24-hour clock.

**Inhaler use**

Questions 15-16:

Ask the mother if her child 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 17. If 'Yes', ask her when her child last used the inhaler. Enter the time using the 24-hour clock.

**Time Last Smoked**

Questions 17-18:

Ask the mother if her child has smoked in the last two hours. If 'No', skip to question 19. If 'Yes', ask her what time her child 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 19-20:

Ask the mother if her child has had any water in the past 2 hours. If 'No', skip to question 21. If 'Yes' ask her when her child 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 21-22:

Ask the mother if her child 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 mother answers 'No', skip to question 25, since the child has fasted for at least 8 hours, the minimum fasting time required for the OGTT. If the mother indicates that her child ate or drank something after 11:00 PM (2300 hours), complete the follow-up question that asks for the time the child ate or drank something other than water. Enter the time using the 24-hour clock. If the time the child last ate or drank is after 2:00 AM (0200 hours), tell the mother

and child 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 child last ate or drank anything. Ask the mother if she can wait until that time to start the test. If the mother indicates that her child cannot wait, the visit needs to be rescheduled. Urinalysis and measurement of blood pressure, height, and weight can proceed while the child 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 child 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 child last ate or drank something.
- If the child 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 child does not have fasting blood samples drawn before that time. For example, if the child 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 the child hasn't fasted for 8 hours and the mother agrees to reschedule, go to Physical Measurements–Child and Questionnaire. Only the OGTT should be delayed until the rescheduled date. If the OGTT needs to be rescheduled because the child hasn't fasted for 8 hours, but the mother refuses to reschedule, the rest of the child visit should be completed and the samples that are collected at the fasting timepoint should be collected. Go to Physical Measurements–Child, Questionnaire, and Single Blood Draw–Child (collect the samples that are collected at the fasting draw).

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

Questions 23-24:

Ask the mother what time the child 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 the child

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 25:

Enter your HAPO Staff ID.

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

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

Question 26:

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 collecting blood samples, '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 27:

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.

### 6.4.1 Special *Situations Precluding the OGTT*

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

*1.* Mother reports that her child has a diagnosis of diabetes and is on treatment with oral medication or insulin. She will be asked to bring the medication to the visit. The child with diabetes on medication will be told not to fast and will have a single blood draw of the samples that are collected at the fasting draw will be drawn. 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, the child will be asked to fast and the usual visit will be conducted.

2. Mother reports the child is HIV positive or has Hepatitis B or C. In this *situation*, due to international shipping regulations, blood sampling will not be performed. The child will have the rest of the visit.

3. The child takes medication(s) regularly. The child 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. The mother should be given the Metformin Use-Child form, asked to contact the child's 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, the mother should be contacted by phone and questions 4-6 on the Metformin Use Interview-Child form should be asked. Whichever of the forms is completed should be entered into the REDCap Data Entry System.

## 6.5 Rescheduling the Visit

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

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

### 6.5.1 Procedures for Children Unable to Keep the Original Appointment

Some mothers and children will be unable to keep their original visit appointment and will need to reschedule the visit. If a mother 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.

### 6.5.2 Procedures for Children who Fail to Appear for the Visit

If a mother and her child are a “no show” at their original appointment, an entry of ‘NS’ should be made in the Call Back Register. Staff should attempt to contact the mother 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 and child who are a “no show” for a scheduled visit are 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.

### 6.5.3 Procedures for Children not Meeting OGTT *Requirements*

If the OGTT needs to be rescheduled because a child does not meet the *requirements* for the test, the OGTT should be scheduled for as soon as possible. **Note:** When the child comes for

the rescheduled OGTT, a **new** Test Qualification Form-Child, OGTT Form-Child, and OGTT Sample Processing Form-Child **must** be completed and entered.

If the mother does not wish to reschedule the OGTT, procedures for the Single Blood Draw should be followed (see Section **6.8**).

Physical Measurements-Child and the Questionnaire should be completed even if the OGTT is rescheduled or if the child is having the Single Blood Draw.

### 6.6 Physical Measurements - Child

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

#### 6.6.1 Urine Sample

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

A specimen cup should be labelled with the child's urine sample ID (ending with digits 606). Make sure that the first five digits of the ID affixed to the specimen cup match the ID on the child's Physical Measurements-Child form. The child should be instructed 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.

If the child is a girl, she and her mother 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

If the child is a boy, he and his mother should be given the following instructions:

- Wipe the tip of the penis 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 child's urine sample ID (ending with digits 606) 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-Child form. ***Note:*** *The urine sample should be sent for processing even if some or all of the blood samples are not collected.*

### 6.6.2 Measurement of Blood Pressure

For measurement of blood pressure, all of the procedures described in Sections **5.6.2.1 – 5.6.2.5** should be followed. **Note:** the Physical Measurements-Child form should be used to record answers for questions 4-11.

#### *6.6.2.1 Blood Pressure Remeasurement*

*If the mean of the second and third diastolic pressures is > 110 and/or the mean of the second and third diastolic pressures is >75, refer to the HAPO FUS Blood Pressure Alert Protocol for Children (see Section **6.13**).*

### 6.6.3 Measurement of Height

For measurement of height, the procedures described in Section **5.6.3** should be followed. **Note:** the Physical Measurements-Child form should be used to record answers for questions 12-15.

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

For BOD POD measurements of weight and percent fat, the procedures described in Section **5.6.4** should be followed. **Note:** the Physical Measurements-Child form should be used to record answers for questions 16-20.

### 6.6.5 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 child should be asked to remove the bathing suit, to put on his or her underclothing and to keep the robe or cover-up on.

Procedures described in Section **5.6.5** should be followed for measurements of waist circumference. **Note:** the Physical Measurements-Child form should be used to record answers to questions 21-22.

### 6.6.6 Mid-Arm Circumference Measurement

Arm circumference is measured at the midpoint of the upper arm, half way between the acromion process of the shoulder to the tip of the olecranon process of the mid-elbow. Arm circumference measurements should be done on the child's right arm and from the right side (rather than in front).

To determine the midpoint:

- Instruct the child to turn away from you and to stand upright with his/her weight evenly distributed on both feet, the right arm bent 90 degrees at the elbow, and the right palm facing up. Remove any clothes covering the child's arm.
- Hold the zero end of the tape at the acromion process and extend the tape down the posterior surface of the arm to the tip of the olecranon process. Using a non-permanent

marker, indicate the midpoint by making a horizontal mark at the midpoint and cross this mark with a perpendicular line. The vertical line must be centered on the posterior surface of the arm. (See Figure 1).

To measure the midarm circumference:

1. Keep the measuring tape horizontal at this level and record the measurement to the nearest 0.1 cm in question 23a on Physical Measurements–Child.
2. Repeat the measurement and record the second value to the nearest 0.1 cm in question 23b on Physical Measurements–Child.
3. 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–Child.

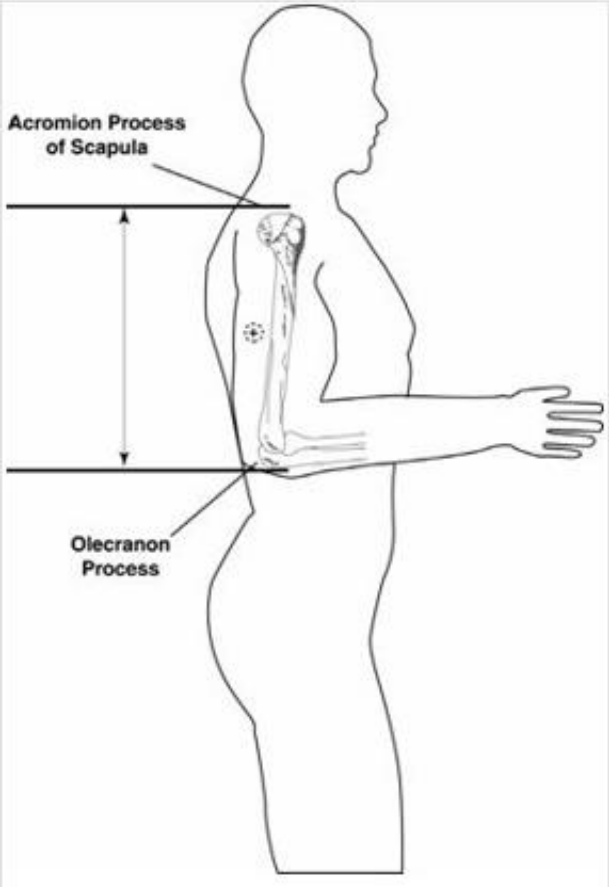


Figure 1. Mid-Arm Circumference (www.phenxtoolkit.org)

### 6.6.7 Skinfolds Measurements

Three sets of skinfolds measurements will be obtained: triceps, subscapular, and suprailiac. All of the skinfolds measurements are obtained with Harpenden skinfold calipers on the right side of the body and are recorded in millimeters (mm). **Note:** The sight of the caliper may make some children frightened and anxious. In order to make them feel more relaxed, explain the procedure and demonstrate the use of the caliper on the palm of the child and/or the mother.

The triceps skinfold is measured by taking the vertical fold over the triceps muscle half the distance between the acromion process and the olecranon (at the same location as mid arm circumference measurement). The subscapular skinfold is measured just below the lower angle of the scapula at about a 45° angle to the spine. The suprailiac skinfold is measured on the right side of the body and is taken on a diagonal fold on the midaxillary line just above the iliac crest. Once the specific landmark has been identified, it should be marked with washable ink. This will aid in identifying the correct location on subsequent measurements. (see Figures 2-3).

Skinfold measurements are obtained by lifting the top layer of the skin at the specific landmark with the thumb and the index finger. The caliper jaws should be placed perpendicular to the length of the fold. While holding onto the skin, the calipers are applied for 3-4 seconds, or until the reading has stabilized. Read the caliper dial at eye level and not at an angle.

Each set of skinfold measurements is obtained and recorded to the nearest 0.1 mm twice. The caliper dial shows 0.2mm increments. The caliper needle will commonly fall onto one of these increments. If you observe that the needle truly lies between two lines on the dial, e.g. between 10.6 and 10.8 mm, take the odd number in between the two, e.g., 10.7, as the measurement.

Sites should be rotated, i.e., obtain 1 measurement for the triceps, followed by the subscapular, followed by the suprailiac skinfold. Then, repeat this process. If the measurements at a specific skinfold site differ by more than 1.0 mm, a third measurement should be obtained and recorded. If two of the measurements at a specific skinfold site are within 1.0 mm, these two will be averaged by the data system. If there are three measurements and none are within 1.0 mm of each other, all three will be averaged by the data system.

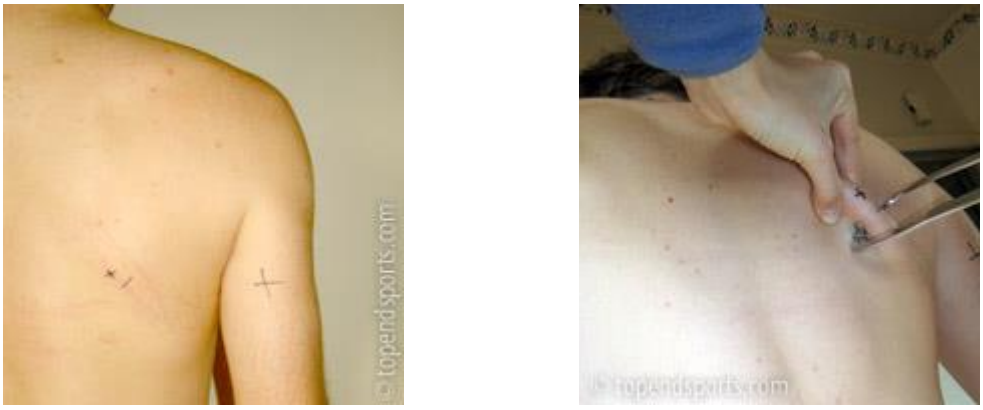

Figure 2. Subscapular and Tricep Skinfolds

Figure 3. Suprailiac Skinfold

To perform skinfolds measurements:

1. Measure the triceps skinfold and record the value to the nearest 0.1 mm in question 24a. on Physical Measurements–Child.
2. Measure the subscapular skinfold and record the value to the nearest 0.1 mm in question 25a. on Physical Measurements-Child.
3. Measure the suprailiac skinfold and record the value to the nearest 0.1 mm in question 26a. on Physical Measurements-Child.
4. Repeat the triceps skinfold measurement and record the value to the nearest 0.1 mm in question 24b. on Physical Measurements–Child.
5. Repeat the subscapular skinfold measurement and record the value to the nearest 0.1 mm in question 25b. on Physical Measurements-Child.
6. Repeat the suprailiac skinfold measurement and record the value to the nearest 0.1 mm in question 24b. on Physical Measurements-Child.

7. Compare the first and second measurement of each of the skinfold sites. If the first and second measurements differ by more than 1.0 mm, perform a third measurement and record the value to the nearest 0.1 mm on Physical Measurements–Child.
8. Record the HAPO staff ID number of the person performing the measurements in question 27 on Physical Measurements-Child.

### 6.6.8 Pubertal Assessment

The examiner should explain to the mother and child why pubertal assessment is necessary by saying something like: "it is important to assess puberty as it affects how your body reacts to insulin and the amount of muscle and fat that you have. Your body reacts less well to insulin during puberty and this affects your glucose (or sugar) levels in your blood."

**Note:** The child may be anxious about this part of the physical measurements. Explain to the child what you will be doing and that there will not be any pain.

**Note:** For more details concerning pubertal assessment, refer to the booklet "Assessment of Sexual Maturity Stages in Girls and Boys that was published by the American Academy of Pediatrics (this was distributed to each field center during the Central Training).

First, indicate whether the child is a male or female by marking the appropriate box for question 28 on Physical Measurements-Child.

Each child will complete a self-assessment of pubertal stage. Then a trained examiner will do a physical assessment of the Tanner stage. For boys, the examiner will do a physical examination to assess Tanner stage of genitalia, of pubic hair, and of testicular volume using the orchidometer. For girls, the examiner will do a physical examination of the breasts to determine the Tanner stage of breast development.

#### *6.6.8.1 Self Assessment*

To assess pubertal stage, children and their mothers will be given a set of pictograms depicting the 5 Tanner stages. Boys should be given the ToolKit – Boys to self-assess their genitalia and

pubic hair. Girls should be given the ToolKit – Girls to self-assess their breast development. The child will be asked to make a self-assessment either at the time they're changing into the bathing suit prior to the BOD POD, or while taking the bathing suit off, after the BOD POD. Read the paragraph at the top of the Tool-Kit to the child, and then ask them to read the descriptions next to the pictures. While changing the child should check his/her own private parts. He/she should decide which picture and description best matches his/her own private parts. They will be asked to check the box next to the diagram that they think most closely resembles their current stage.

#### *6.6.8.2 Pubertal Assessment – Boy*

1. Transcribe the Tanner stage for genitalia that the boy has checked on Page 1 of the ToolKit-Boys pictogram onto question 29 Physical Measurements-Child.
2. Transcribe the Tanner stage for pubic hair that the boy has checked on Page 2 of the ToolKit-Boys pictogram onto question 30 Physical Measurements-Child.
3. To perform the physical examination, ask the boy to stand. Sit in a chair in front of him. Warm your hands and apply gloves. Some boys may have an erection during this part of the exam and may be embarrassed. Reassure him that this is a normal response. Don't have him take down his underwear until you are ready to do the exam.
4. Bring out the orchidometer beads. Casually note that each testicle will be compared to several beads to see which one it matches. Let him examine them if he wishes. **Note:** Do not talk about measuring size.
5. As you start the exam for testicular volume, first check for the Tanner stage of the genitalia.
6. Next note the Tanner stage of the pubic hair.
7. Now start the testicular volume exam by gently grasping the right testicle between the thumb and third finger while holding the orchidometer beads in the opposite hand. Manipulate the testis to expose it. Opposing the fingertips just behind the testis should result in gentle stretching of the scrotal skin over the anterior surface of the testis, permitting more accurate assessment.
8. Start with a bead that is likely to be smaller than the testicle. While maintaining the grasp, maneuver to a bead with a higher number to assess for a better match.
9. Further check by comparing with the next larger bead. If the volume appears to be halfway between the volume of two beads, mark the box for the lower volume.

10. Repeat the procedure with the left testis. Again, if the volume appears to be halfway between the volume of two beads, mark the box for the lower volume.
11. Fill in the ToolKit-Boys-Trained by marking the Tanner stage of the genitalia, then the Tanner stage of the pubic hair, and then the volume of the right testis, and then volume of the left testis.
12. Transcribe the Tanner stage for genitalia that the examiner has marked on page 1 of the ToolKit-Boys-Trained pictogram onto question 31 on Physical Measurements–Child.
13. Transcribe the Tanner stage for pubic hair that the examiner has marked on page 1 of the ToolKit-Boys-Trained pictogram onto question 32 on Physical Measurements–Child.
14. Transcribe the volume of the right testis onto question 33a on Physical Measurements-Child.
15. Transcribe the volume of the left testis onto question 33b on Physical Measurements-Child.

***Note:*** *If either of the testes is known to be absent, mark the appropriate box for this.*

**Note:** If either of the testes is undescended, the mother should be instructed to contact the child's health care provider. *(The specific procedure to follow-up on this finding will be determined by each field center).*

#### *6.6.8.3 Pubertal Assessment – Girl*

Explain to the girl and her mother that you will be examining the girl's breasts to determine her stage of development and that this will involve looking at them and may involve feeling the breast tissue.

**Note:** Since it may affect the assessment of the examiner, it may be useful in girls with some breast development, to casually ask the girl whether she has started having periods and, if yes, for how long. Questions about menarche are also asked of the mother by the Questionnaire, but the information may not be available until after the pubertal assessment has been completed.

1. Transcribe the Tanner stage for breast development that the girl has checked on the ToolKit-Girls pictogram onto question 34 Physical Measurements-Child.

2. Explain to the girl and her mother that you will be examining the girl's breasts to determine her stage of development and that this will involve looking at them and may include feeling the breast tissue.
3. Warm your hands and apply gloves.
4. Ask the girl to lay down, as this may minimize misinterpretation of fat tissue as breast tissue. Assess the pigmentation and maturity of the areola, the width of the nipple, and width of the breast tissue.
5. If the girl is Tanner stage 1-3, or obese and Tanner staging is difficult, palpate the breast. If palpation is needed, use your 2nd, 3rd, and 4th finger and gently palpate for breast tissue around the areola and then over the areola. Repeat this examination on the other side.
6. The examiner should mark their assessment of right and left breast development on ToolKit-Girls-Trained form.
7. Transcribe the Tanner stage for right breast development that the examiner has checked on the ToolKit-Girls-Trained pictogram onto question 35a Physical Measurements-Child.
8. Transcribe the Tanner stage for left breast development that the examiner has checked on the ToolKit-Girls-Trained pictogram onto question 35b Physical Measurements-Child.

#### *6.6.8.4 Completion of Pubertal Assessment*

Record the HAPO staff ID number of the person performing the pubertal assessment in question 36 on Physical Measurements-Child.

Record the HAPO staff ID of person transcribing pubertal assessments from Pubertal Assessment ToolKit to Physical Measurements-Child form in question 37 on Physical Measurements-Child.

### 6.6.9 Data Entry Completion – Physical Measurements - Child

The person who enters the form into the REDCap Data Entry System should enter his/her HAPO staff ID in question 38 on Physical Measurements–Child 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.

### 6.6.10 Training

Field center staff will be trained in methods of measurement of height, weight/BOD POD, waist and midarm circumference, skinfolds and pubertal assessment 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.

### 6.6.11 Measurement Results

Results from the measurements of blood pressure, height, weight, waist and midarm circumference and skinfolds should be recorded on Physical Measurements-Child. These measurements should also be provided to the mother. To determine what blood pressure values should result in referral to a medical person refer to the HAPO-FUS Blood Pressure Alert Protocol for Children at the end of this Chapter (see Section **6.13**).

*If the mean of the second and third systolic pressures is > 110 and/or the mean of the second and third diastolic pressures is >75, refer to the HAPO FUS Blood Pressure Alert Protocol for Children (see Section **6.13**).*

## 6.7 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 collection of samples at 30-minutes and 1- and 2-hours after the start of consumption of the drink.

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 ≥ 3 times. Seasonal allergies would not be considered an illness.

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

### 6.7.1 75g Glucose Drink

Each child will be given a dose of glucose as a drink (Trutol). The pre-bottled drink contains a flavored solution which should be chilled prior to serving. **Note:** If the child weighs less than 42.6 kg, refer to the Trutol Volume by Weight Chart (see end of chapter) to determine the volume the child 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:** If the child weighs 42.6 kg or more, the entire contents of the bottle (10 oz or 296 ml) should be emptied into a cup and served to the child. ***Note:*** *If the child is to have the full volume but it appears that there is < 296 ml in the bottle, do NOT top off*. **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 child during the OGTT because this could change the rate of gastric flow and thereby affect OGTT results. **Note:** The Trutol should be consumed in 5 minutes or less.

### 6.7.2 Blood Drawing Procedure

Blood samples for glucose analysis will each be collected in 2 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 mother has specifically provided consent for this sample. Samples for glucose and insulin/C-peptide will be collected at 30-minutes and at 1- and 2-hours after the start of Trutol consumption.

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-Child.
3. Serve the glucose drink; instruct the child to consume it in its entirety within 5 minutes. Record the time the child begins to consume the glucose drink and the time he/she finishes the drink on the OGTT Form-Child (using a 24-hour clock, e.g. 09:15).
4. Draw the 30-minute blood glucose and insulin/C-peptide samples 30 minutes after the glucose drink is started. These samples should be drawn as close to the 30-minute time as possible and within 10 minutes of the 30-minute interval. Record the clock time the 30-minute sample is drawn, e.g. 09:45.
5. Draw the 1-hour blood glucose and insulin/C-peptide samples one hour after the glucose drink is started. These samples should be drawn as close to the 1-hour time as possible and within 10 minutes of the 1-hour interval. Record the clock time the 1-hour sample is drawn, e.g. 10:15.
6. Draw the 2-hour blood glucose and insulin/C-peptide 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.

**Note:** If there are problems obtaining the 30-minute and/or the 1-hour glucose sample, the 2-hour sample should be collected, if possible.

### 6.7.3 OGTT Stopped – Not Rescheduled

- If the test is stopped and not completed because the child vomited or fell ill, the test should be stopped and not rescheduled. Return to question 26 on the Test

Qualification Form-Child 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 of the OGTT Form-Child to indicate that the OGTT was incomplete. Then go to the Questionnaire. The forms from the incomplete OGTT should be entered.

### 6.7.4 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-Child. Make sure that the correct HAPO ID label has been affixed to each page of this form prior to sending it for processing.

#### *6.7.4.1 Fasting Blood Samples:*

Blood sample tubes required:

- Fasting Glucose – 1 x 2 ml Grey top(sodium fluoride) tube
- Insulin/C-peptide – 1 x 2 ml Red top tube
- hsCRP and Lipids – 1 x 4 ml Red top tube
- Storage – 1 x 4 ml Red top tube
- DNA – 1 x 3 ml Purple top tube (if consented)
- A1c – 1 x 3 ml Purple top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 600 (Fasting Glucose tube)
- 1 x Bar-code label ending with digits 601 (Insulin/C-peptide tube)
- 1 x Bar-code label ending with digits 602 (hsCRP and Lipids tube)
- 1 x Bar-code label ending with digits 603 (Storage tube)
- 1 x Bar-code label ending with digits 604 (DNA tube) (if consented)
- 1 x Bar-code label ending with digits 605 (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, hsCRP and 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-Child. **Note:** Samples must be processed within 2 ½ - 3 hours of when the fasting samples were drawn.

#### *6.7.4.2 30-Minute Blood Samples*:

Blood sample tubes required:

- Glucose – 1 x 2 ml Grey Top tube
- Insulin/C-peptide – 1 x 2 ml Red top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 650 (30-minute glucose)
- 1 x Bar-code label ending with digits 651 (30-minute insulin/C-peptide)

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.

***6.7.4.3 1-Hour Blood Samples***:

Blood sample tubes required:

- Glucose – 1 x 2 ml Grey Top tube
- Insulin/C-peptide – 1 x 2 ml Red top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 610 (1-hour glucose)
- 1 x Bar-code label ending with digits 611 (1-hour insulin/C-peptide)

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.

***6.7.4.4 2-Hour Blood Samples***:

Blood sample tubes required:

- Glucose – 1 x 2 ml Grey Top tube
- Insulin/C-peptide – 1 x 2 ml Red top tube

Blood tube labels required:

- 1 x Bar-code label ending with digits 620 (1-hour glucose)
- 1 x Bar-code label ending with digits 621 (1-hour insulin/C-peptide)

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.

### 6.7.5 OGTT Form Completion

The OGTT Form-Child is used to record the drawing of OGTT, insulin/C-peptide, hsCRP and 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. Transcribe the weight measurement from the BOD POD scale (copy it from Physical Measures-Child question 19).
3. Indicate whether the participant's mother consented to having her child's blood and urine samples stored at the NIH.
4. Indicate whether the participant's mother consented to having a DNA sample drawn for her child.
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 of the form indicating that the test was incomplete. Return to and complete question 26 on the Test Qualification Form-Child.
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 hsCRP and 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 in milliliters for the child's OGTT and record it.
13. Enter the time the child began to consume the glucose drink, using 24-hour clock format.

14. Enter the time the child completed consuming the glucose drink, using 24-hour clock format. **Note:** If the child vomited or fell ill prior to thse 2-hour blood draw, stop the OGTT, and leave the remaining items on the form blank, except the checkbox at the top of the form indicating the test was cancelled/incomplete. Return to and complete question 26 on the Test Qualification Form-Child.
15. Indicate in the checkbox whether the 30-minute glucose sample was drawn.
16. Indicate in the checkbox whether the 30-minute insulin/C-peptide sample was drawn.
17. Enter the time the 30-minute samples were drawn, using 24-hour clock format.
18. Indicate in the checkbox whether the 1-hour glucose sample was drawn.
19. Indicate in the checkbox whether the 1-hour insulin/C-peptide sample was drawn.
20. Enter the time the 1-hour samples were drawn, using 24-hour clock format.
21. Indicate in the checkbox whether the 2-hour glucose sample was drawn.
22. Indicate in the checkbox whether the 2-hour insulin/C-peptide sample was drawn.
23. Enter the time the 2-hour samples were drawn, using 24-hour clock format.
24. 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.
25. Indicate in the checkbox if all samples were sent for processing.
26. 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.
27. 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.

### 6.7.6 Aliquotting/Labeling

#### *6.7.6.1 General Instructions:*

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

***6.7.6.2 Urine Sample:***

Specimen vials required:

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

Specimen vial labels required:

- 4 x Bar-code labels ending with digits 606 (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 (606) without white insert in a "Urine-Analysis" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 606 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (606) with white insert in a "Urine-Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 606 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

### *6.7.6.3 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 600 (Glucose)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 600, 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 (600) without white insert in a “Blood Analysis” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 600 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

- Store the vial (600) with white insert in a “Blood Backup” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 600 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

#### *6.7.6.4 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 601 (insulin/C-peptide)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 601, 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 (601) without white insert in a “Blood Analysis” plastic freezer box at -20° C or colder, and attach one Bar-code label ending in digits 601 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.
- Store the vial (601) with white insert in a “Blood Backup” plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 601 to the box’s Shipping Grid, corresponding to the vial’s location in the freezer box.

#### *6.7.6.5 Fasting hsCRP and 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 602 (lipids)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 602, 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 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 (602) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach one Bar-code label ending in digits 602 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (602) with white insert in a "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 602 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

***6.7.6.6 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 603 (Storage)

Procedure:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Centrifuge the blood tube labeled with digits 603, 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 (603) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 603 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (603) with white insert in a "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 603 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

#### *6.7.6.7 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 604 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 604 attached to the box's Shipping Grid, corresponding to the tube's location in the cardboard box (see Section **7.1.6 Storage of DNA Samples**). Place the tube in the box so that only every other slot in the box is filled.

#### *6.7.6.8 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 605 (A1c)

Procedures:

- Follow General Instructions described above for aliquotting/labeling blood specimens.
- Do **not** centrifuge blood tube labeled with digits 605.
- 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 red 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 (605) without white insert in a "Blood Analysis" plastic freezer box at -20° C or colder, and attach a Bar-code label ending with digits 605 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.
- Store the vial (605) with white insert in "Blood Backup" plastic freezer box at -20° C or colder, and attach one Bar-code label ending with digits 605 to the box's Shipping Grid, corresponding to the vial's location in the freezer box.

#### *6.7.6.9 30-Minute and 1- and 2-hour Blood Glucose and Insulin/C-peptide Samples:*

Procedures described above in **6.7.6.3** and **6.7.6.4** should be followed for aliquotting of 30-minute and 1- and 2-hour glucose and insulin/C-peptide samples, respectively, making sure that the correct barcode is used for each of the samples and timepoints.

### 6.7.7 OGTT Sample Processing Form Completion

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

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, 30-minute, 1-hour, 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 top 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 top cryovials) made from the 30-minute glucose sample.
    c. Check the appropriate box to indicate the number of aliquots (yellow top cryovials) made from the 1-hour glucose sample.
    d. Check the appropriate box to indicate the number of aliquots (yellow top cryovials) made from the 2-hour glucose sample.
5. Check the box to indicate if the insulin/C-peptide, hsCRP and 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/C-peptide sample.
    b. Check the appropriate box to indicate the number of aliquots (green top cryovials) made from the 30-minute insulin/C-peptide sample.
    c. Check the appropriate box to indicate the number of aliquots (green top cryovials) made from the 1-hour insulin/C-peptide sample.
    d. Check the appropriate box to indicate the number of aliquots (green top cryovials) made from the 2-hour insulin/C-peptide sample.
    e. Check the appropriate box to indicate the number of aliquots (clear top cryovials) made from the fasting hsCRP and lipids sample.

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

### 6.7.8 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*)

### 6.7.9 Quality Control

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

## 6.8 Single Blood Draw Procedures

Children 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 treatment with medications that alter glucose metabolism (interfering medications), or unacceptable fasting status (originally scheduled for an OGTT but period of fasting insufficient and mother will not reschedule).

A blood sample for glucose analysis will be collected in a 2 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/C-peptide, hsCRP and lipids, storage, and A1c will also be obtained. A sample for DNA will also be obtained, if the mother 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.

### 6.8.1 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 **6.7.4.1**. Aliquotting and labeling should be performed as described in Section **6.7.6.**

### 6.8.2 Single Blood Draw Form Completion

The Single Blood Draw Form–Child is used to record the drawing of the glucose, insulin, HACRP and 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's mother consented to having her child's blood and urine samples stored at the NIH.
3. Indicate whether the participant's mother consented to having a DNA sample drawn for her child.
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 hsCRP and 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 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.
12. Indicate in the checkbox if all samples were sent for processing. **Note:** If blood drawing was not completed for any reason, answer question 26 of Test Qualification Form-Child.
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.

### 6.8.3 Single Blood Draw Sample Processing Form Completion

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

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 hsCRP and 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.

### 6.9 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 7.15**.

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.

### 6.10 Questionnaire

The Questionnaire should be administered to the mother, even if she is not having her visit at the time her child is having the visit. Procedures that should be followed for administration and completion of the Questionnaire are described in Section **5.10**.

### *6.11 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.*

### 6.12 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.*

### 6.13 Blood Pressure Alert Protocol for Children

If there is concern that the child's blood pressure may be abnormally elevated, refer to the HAPO-FUS Blood Pressure Alert Protocol for Children (see next page).

## HAPO-FUS BLOOD PRESSURE ALERT PROTOCOL for CHILDREN

Measure blood pressure 3 times per standard protocol (details in MOO).

- Exclude the first blood pressure reading.
- Take the average of the 2nd and 3rd systolic readings; this average will be used to determine whether the child needs referral for possible hypertension.
- Take the average of the 2nd and 3rd diastolic readings; this average will be used to determine whether the child needs referral for possible hypertension.

**STEP 1:** Is the child's average systolic or average diastolic value at or above the threshold in Table 1A (boys) or 1B (girls)?

***If NO*** → **Stop. The child's blood pressure is normal. No referral needed.**

***If YES*** → **Continue to STEP 2 (on page 2 for boys, page 3 for girls).**

**Table 1A.** 95th percentile BP thresholds for the shortest boys in each age group.

| BOYS: Age | Systolic BP Average (mmHg) | Diastolic BP Average (mmHg) |
|---|---|---|
| 8 | 111 | 75 |
| 9 | 113 | 76 |
| 10 | 115 | 77 |
| 11 | 117 | 78 |
| 12 | 119 | 78 |
| 13 | 121 | 79 |

**Table 1B.** 95th percentile thresholds for the shortest girls in each age group.

| GIRLS: Age | Systolic BP Average (mmHg) | Diastolic BP Average (mmHg) |
|---|---|---|
| 8 | 112 | 75 |
| 9 | 114 | 76 |
| 10 | 116 | 77 |
| 11 | 118 | 78 |
| 12 | 119 | 79 |
| 13 | 121 | 80 |

**STEP 2 (Boys):**

- Find the child's age and height-specific BP thresholds on tables 2A (systolic) and 2B (diastolic).
- Is the child's average systolic or average diastolic value at or above the threshold for Stage 1 or Stage 2 HTN?

***If NO*** → **Stop. The child's blood pressure is normal. No referral needed.**

***If YES*** → **Repeat 3 BP measurements at the end of the study visit. Go to STEP 3 (on page 4).**

**Table 2A. Systolic BP thresholds for Stage 1 and Stage 2 HTN for boys by age and height.**

| Age ↓ | BP Classification | Systolic BP (mm Hg) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | ***Height (cm)*** | ***121*** | ***123*** | ***127*** | ***131*** | ***135*** | ***139*** | ***141*** |
| | Stage 1 HTN | 111 | 112 | 114 | 116 | 118 | 119 | 120 |
| | Stage 2 HTN | 124 | 125 | 127 | 128 | 130 | 132 | 132 |
| 9 | ***Height (cm)*** | ***126*** | ***128*** | ***132*** | ***136*** | ***141*** | ***145*** | ***147*** |
| | Stage 1 HTN | 113 | 114 | 116 | 118 | 119 | 121 | 121 |
| | Stage 2 HTN | 125 | 126 | 128 | 130 | 132 | 133 | 134 |
| 10 | ***Height (cm)*** | ***130*** | ***133*** | ***137*** | ***141*** | ***146*** | ***150*** | ***153*** |
| | Stage 1 HTN | 115 | 116 | 117 | 119 | 121 | 122 | 123 |
| | Stage 2 HTN | 127 | 128 | 130 | 132 | 133 | 135 | 135 |
| 11 | ***Height (cm)*** | ***135*** | ***137*** | ***142*** | ***146*** | ***151*** | ***156*** | ***159*** |
| | Stage 1 HTN | 117 | 118 | 119 | 121 | 123 | 124 | 125 |
| | Stage 2 HTN | 129 | 130 | 132 | 134 | 135 | 137 | 137 |
| 12 | ***Height (cm)*** | ***140*** | ***143*** | ***148*** | ***153*** | ***158*** | ***163*** | ***166*** |
| | Stage 1 HTN | 119 | 120 | 122 | 123 | 125 | 127 | 127 |
| | Stage 2 HTN | 131 | 132 | 134 | 136 | 138 | 139 | 140 |
| 13 | ***Height (cm)*** | ***147*** | ***150*** | ***155*** | ***160*** | ***166*** | ***171*** | ***173*** |
| | Stage 1 HTN | 121 | 122 | 124 | 126 | 128 | 129 | 130 |
| | Stage 2 HTN | 133 | 135 | 136 | 138 | 140 | 141 | 142 |

**Table 2B. Diastolic BP thresholds for Stage 1 and Stage 2 HTN for boys by age and height.**

| Age ↓ | BP Classification | Diastolic BP (mm Hg) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | ***Height (cm)*** | ***121*** | ***123*** | ***127*** | ***131*** | ***135*** | ***139*** | ***141*** |
| | Stage 1 HTN | 75 | 76 | 77 | 78 | 79 | 79 | 80 |
| | Stage 2 HTN | 88 | 89 | 90 | 91 | 92 | 92 | 93 |
| 9 | ***Height (cm)*** | ***126*** | ***128*** | ***132*** | ***136*** | ***141*** | ***145*** | ***147*** |
| | Stage 1 HTN | 76 | 77 | 78 | 79 | 80 | 81 | 81 |
| | Stage 2 HTN | 89 | 90 | 91 | 92 | 93 | 93 | 94 |
| 10 | ***Height (cm)*** | ***130*** | ***133*** | ***137*** | ***141*** | ***146*** | ***150*** | ***153*** |
| | Stage 1 HTN | 77 | 78 | 79 | 80 | 81 | 81 | 82 |
| | Stage 2 HTN | 90 | 91 | 91 | 93 | 93 | 94 | 95 |
| 11 | ***Height (cm)*** | ***135*** | ***137*** | ***142*** | ***146*** | ***151*** | ***156*** | ***159*** |
| | Stage 1 HTN | 78 | 78 | 79 | 80 | 81 | 82 | 82 |
| | Stage 2 HTN | 91 | 91 | 92 | 93 | 94 | 95 | 95 |
| 12 | ***Height (cm)*** | ***140*** | ***143*** | ***148*** | ***153*** | ***158*** | ***163*** | ***166*** |
| | Stage 1 HTN | 78 | 79 | 80 | 81 | 82 | 82 | 83 |
| | Stage 2 HTN | 91 | 92 | 93 | 94 | 95 | 95 | 96 |
| 13 | ***Height (cm)*** | ***147*** | ***150*** | ***155*** | ***160*** | ***166*** | ***171*** | ***173*** |
| | Stage 1 HTN | 79 | 79 | 80 | 81 | 82 | 83 | 83 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Stage 2 HTN | 92 | 92 | 93 | 94 | 95 | 96 | 96 |

**STEP 2 (Girls):**

- Find the child's age and height-specific BP thresholds on tables 3A (systolic) and 3B (diastolic).
- Is the child's average systolic or average diastolic value at or above the threshold for Stage 1 or Stage 2 HTN?

  ***If NO*** → **Stop. The child's blood pressure is normal. No referral needed.**

  ***If YES*** → **Repeat 3 BP measurements at the end of the study visit. Go to STEP 3 (on page 4).**

**Table 3A. Systolic BP thresholds for Stage 1 and Stage 2 HTN for girls by age and height.**

| Age ↓ | BP Classification | Systolic BP (mm Hg) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | ***Height (cm)*** | ***121*** | ***123*** | ***127*** | ***131*** | ***135*** | ***139*** | ***141*** |
| | Stage 1 HTN | 112 | 112 | 114 | 115 | 116 | 118 | 118 |
| | Stage 2 HTN | 124 | 125 | 126 | 127 | 128 | 130 | 130 |
| 9 | ***Height (cm)*** | ***125*** | ***128*** | ***131*** | ***136*** | ***140*** | ***144*** | ***147*** |
| | Stage 1 HTN | 114 | 114 | 115 | 117 | 118 | 119 | 120 |
| | Stage 2 HTN | 126 | 126 | 128 | 129 | 130 | 132 | 132 |
| 10 | ***Height (cm)*** | ***130*** | ***132*** | ***136*** | ***141*** | ***146*** | ***150*** | ***153*** |
| | Stage 1 HTN | 116 | 116 | 117 | 119 | 120 | 121 | 122 |
| | Stage 2 HTN | 128 | 128 | 130 | 131 | 132 | 134 | 134 |
| 11 | ***Height (cm)*** | ***136*** | ***138*** | ***143*** | ***148*** | ***153*** | ***157*** | ***160*** |
| | Stage 1 HTN | 118 | 118 | 119 | 121 | 122 | 123 | 124 |
| | Stage 2 HTN | 130 | 130 | 131 | 133 | 134 | 135 | 136 |
| 12 | ***Height (cm)*** | ***143*** | ***146*** | ***150*** | ***155*** | ***160*** | ***164*** | ***166*** |
| | Stage 1 HTN | 119 | 120 | 121 | 123 | 124 | 125 | 126 |
| | Stage 2 HTN | 132 | 132 | 133 | 135 | 136 | 137 | 138 |
| 13 | ***Height (cm)*** | ***148*** | ***151*** | ***155*** | ***159*** | ***164*** | ***168*** | ***170*** |
| | Stage 1 HTN | 121 | 122 | 123 | 124 | 126 | 127 | 128 |
| | Stage 2 HTN | 133 | 134 | 135 | 137 | 138 | 139 | 140 |

**Table 3A. Diastolic BP thresholds for Stage 1 and Stage 2 HTN for girls by age and height.**

| Age ↓ | BP Classification | Diastolic BP (mm Hg) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | ***Height (cm)*** | ***121*** | ***123*** | ***127*** | ***131*** | ***135*** | ***139*** | ***141*** |
| | Stage 1 HTN | 75 | 75 | 75 | 76 | 77 | 78 | 78 |
| | Stage 2 HTN | 87 | 87 | 88 | 88 | 89 | 90 | 91 |
| 9 | ***Height (cm)*** | ***125*** | ***128*** | ***131*** | ***136*** | ***140*** | ***144*** | ***147*** |
| | Stage 1 HTN | 76 | 76 | 76 | 77 | 78 | 79 | 79 |
| | Stage 2 HTN | 88 | 88 | 89 | 89 | 90 | 91 | 92 |
| 10 | ***Height (cm)*** | ***130*** | ***132*** | ***136*** | ***141*** | ***146*** | ***150*** | ***153*** |
| | Stage 1 HTN | 77 | 77 | 77 | 78 | 79 | 80 | 80 |
| | Stage 2 HTN | 89 | 89 | 90 | 91 | 91 | 92 | 93 |
| 11 | ***Height (cm)*** | ***136*** | ***138*** | ***143*** | ***148*** | ***153*** | ***157*** | ***160*** |
| | Stage 1 HTN | 78 | 78 | 78 | 79 | 80 | 81 | 81 |
| | Stage 2 HTN | 90 | 90 | 91 | 92 | 92 | 93 | 94 |
| 12 | ***Height (cm)*** | ***143*** | ***146*** | ***150*** | ***155*** | ***160*** | ***164*** | ***166*** |
| | Stage 1 HTN | 79 | 79 | 79 | 80 | 81 | 82 | 82 |
| | Stage 2 HTN | 91 | 91 | 92 | 93 | 93 | 94 | 95 |
| 13 | ***Height (cm)*** | ***148*** | ***151*** | ***155*** | ***159*** | ***164*** | ***168*** | ***170*** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Stage 1 HTN | 80 | 80 | 80 | 81 | 82 | 83 | 83 |
| | Stage 2 HTN | 92 | 92 | 93 | 94 | 94 | 95 | 96 |

**STEP 3:**

- Exclude the first BP reading from the 3 obtained at the end of the study visit. Use the average of the 2nd and 3rd systolic and diastolic BP readings from the end of study visit.
- Find the child's age and height-specific BP thresholds on tables 2A (systolic) and 2B (diastolic) on page 2 for boys or tables 3A (systolic) and 3B (diastolic) on page 3 for girls.
- Is the child's average systolic or average diastolic value from the end of the study visit at or above the threshold for Stage 1 or Stage 2 HTN?

  ***If NO* → Stop. The child's blood pressure is normal. No referral needed.**

  ***If YES* → Referral is needed for possible HTN.**

  - If BP is at or above the threshold for stage 2 HTN, then recommend repeat BP within 24 hours to be done by primary health care provider, or Urgent Care Center.
  - If BP is at or above the threshold for stage 1 HTN but below the threshold for stage 2 HTN, then recommend repeat BP within one month to be done by primary health care provider.

**NOTE:** The BP values obtained at the beginning of the study visit will be used for data analysis. The repeat BP values obtained at the end of the study visit will be documented separately and will not be used in analysis of BP outcomes.

**REFERENCE:** The Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents. National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents. Pediatrics 2004;114(sup 2):555-576.

### *6.13.1 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. The recommendation will be determined by the HAPO FUS Blood Pressure Alert Protocol for Children. If the mean of the second and third systolic blood pressures and/or the mean of the second and third diastolic blood pressures is at or above the threshold for stage 1 HTN but below the threshold for stage 2 HTN, then 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 and/or the mean of the second and third diastolic pressures is at or above the threshold for stage 2 HTN, then 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 - CHILD

***NOTE: This form should only be used if blood pressure measurements are repeated for the child at the end of the Study Visit as directed by the HAPO-FUS Blood Pressure Alert Protocol for Children.***

**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 | 172 |
| 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